DEMOGRAPHICS, GROWTH, AND SURVIVORSHIP OF *SPIRANTHES PARKSII* CORRELL AND *SPIRANTHES CERNUA* (L.) RICH ARE INFLUENCED BY VERTEBRATE AND INVERTEBRATE HERBIVORY AND SUPPLEMENTAL WATER DURING SUMMER DORMANCY

A Thesis

by

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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

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December 2019

Major Subject: Ecosystem Science & Management

ABSTRACT

Spiranthes parksii Correll is a rare terrestrial orchid endemic to only thirteen Texas counties, and is a federally listed endangered species in the United States due to habitat loss and fragmentation. Individual plants of *S. parksii* show irregular patterns of appearance aboveground and may exhibit vegetative dormancy for one or more seasons, even several years. Their transient behavior poses research difficulties and to determine effective conservation practices, the biology and ecology of the species must be assessed further. This thesis presents data for a population of *S. parksii* and its sympatric congener, *Spiranthes cernua* (L.) Rich, in eastern Grimes County that was monitored from 2014 to 2018 to determine if (1) population survival is affected by annual weather and consistency in aboveground presence as rosettes and flower stalks, (2) growth and survival are affected by the type and timing of herbivory, and (3) scheduled summertime watering events will decrease the prevalence of summer rosette dormancy and increase survival and inflorescence growth in the fall.

A detailed analysis of demographics on these two species was conducted and results suggest the sample population presence is declining over time. Summer temperatures appeared to be negatively correlated with reproductive presence proportions and *S. parksii* flower stalk size, which indicated potential threats by climate change. Precipitation in previous and current years largely accounted for variations in rosette and reproductive proportions, and high and low precipitation thresholds possibly dictated stalk height and number of flowers. Contrary to previous research and regardless of the overall sample population decline, over 50% of the sample population flowered in three or more years during the study period, and most plants that flowered returned as rosettes each spring. Variability in seedling presence was perhaps also caused by variability of weather conditions and its effects on germination and soil moisture.

Experimentally mesh-protected plants that allowed only minute invertebrate access had the greatest presence proportions at all life stages and frequently exhibited the lowest herbivory rates while plants exposed to both vertebrates and invertebrates consistently sustained the greatest herbivory. Rosette herbivory did not affect flower stalk growth except in 2015 when the study site received unusually low precipitation, which indicated negative interactive effects of weather and herbivory on plant vigor. In general, greater rosette herbivory led to a greater probability of reproductive absence. Minimum fall season herbivory rates by treatment did not coincide with maximum flower stalk growth, therefore, the timing rather than type of herbivory appeared to have a greater impact on growth.

Historically small and large plants that received supplemental water in 2017 both showed reduced summer dormancy when compared to controls but reproductive growth was not affected. Results suggested that water-treated individuals were also less likely to forgo reproductive season dormancy and instead return aboveground as a fall rosette. Reproduction is considered a costly process that can diminish subsequent growth, but data here indicated that large flower stalks generally became large rosettes. Counter to expectations, there were no differences in dormancy by species yet soil around *S. parksii* presented lower volumetric water content, deeper claypans, and less slope than that of *S. cernua*. It is expected that microhabitat parameters have some influence on dormancy as large plants in this study resided on steeper slopes and exhibited a reduced tendency toward summer and fall dormancy than small plants, but results will benefit from more detailed soil analyses and the inclusion of genetic factors.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Fred E. Smeins, for his guidance, support, and patience throughout the course of this research. Thank you to my committee members, Dr. William E. Rogers and Dr. Cristine Morgan, who provided invaluable knowledge, ideas, equipment, and tools for experiment design and data acquisition.

To Dr. Derya G. Akleman of the Texas A&M Statistics Department, thank you for your direction and advice regarding data analysis methods and for assisting with my continued access to imperative statistical programs.

Thanks also goes to Samantha Best, Preston Ruley, and Howard Stough of Brazos Valley Solid Waste Management Agency for their assistance with site transportation, access to the study area, and weather records.

Finally, thanks to my husband who never doubted me and Deseri D. Nally for her friendship, knowledge, experience, countless hours in the field, and the positivity she never ceased to bring.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised and supported by a thesis committee consisting of Professors Fred E. Smeins and William E. Rogers of the Department of Ecosystem Science and Management and Professor Cristine Morgan of the Department of Soil and Crop Sciences.

The spring 2014 through summer 2016 data collected for Chapters III through V were provided by Professor Fred E. Smeins and his former student Deseri D. Nally.

All other work conducted for the thesis was completed by the student independently.

Funding Sources

This work was made possible in part by a research grant from Brazos Valley Solid Waste Management Agency, Inc., a joint effort between the cities of College Station and Bryan.

Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the cities nor Brazos Valley Solid Waste Management Agency, Inc.

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CHAPTER I[1]

INTRODUCTION

Spiranthes is a terrestrial orchid genus found across the globe, but is most diverse in North America (Correll 1950; Dueck et al. 2014). *Spiranthes parksii* Correll (Navasota Ladies'-Tresses, NLT) is a federally listed endangered species (Liggio & Liggio 1999) limited in distribution to thirteen counties within Texas where they occur at grassland-shrub interfaces within Post Oak Savanna communities (Wonkka et al. 2012; Wang et al. 2015). Agriculture, urban and exurban development, and oil and gas development are several contributing impacts affecting habitat loss and alteration. Despite the thousands of seeds dispersed by a single plant, seed germination and seedling survival rates are remarkably low (Ariza 2013). Their unique life history traits and narrow habitat niche (Ariza 2013; Wang et al. 2015) generally limit their abundance and distribution.

A congener species of *S. parksii, Spiranthes cernua* (L.) Rich. is better adapted to a variety of habitats and may be even more widespread today than in the past (Liggio & Liggio 1999). There is an ongoing debate as to the genetic distinction between *S. parksii* and *S. cernua* that is yet to be resolved (Pace & Cameron 2017). Given conservation concerns that *S. parksii* is a distinct and endangered species, the U.S. Fish and Wildlife Service secured land for several known extant populations but habitat quality is degrading in many of these areas as woody encroachment replaces herbaceous cover in the Post Oak Savanna ecosystems where *S. parksii* is most common (USFWS 2009). Habitat dynamics that lead to *S. parksii* establishment and persistence must be identified and assessed to maintain the species' existence, genetic diversification, and migration (Moritz 2002; Dueck et al. 2014).

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[1] This thesis follows the format of Conservation Biology

Various research projects have been conducted on *S. parksii* and the genetically-similar *S. cernua* congener (Sheviak 1992) including life history monitoring, demographic assessments, identifying mycorrhizal associations, transplant efforts, habitat characterization, and vertebrate herbivory manipulations (Hammons 2008; Wonkka et al. 2012; Ariza 2013; Bruton 2014). However, recent findings on the impact of insect herbivory on *S. parksii* survival would benefit from additional temporal data and further assessment of the causes and consequences of this damage (Nally 2016). Invertebrate herbivory is a threat faced by all plants, but if the long-term severity of the effects is known, conservation efforts of endangered plants can be improved (Crawley 1989; Züst & Agrawal 2017). Also, the *S. parksii* life cycle is characterized by a long summer period (two to three months) of belowground dormancy on the very droughty soil conditions where it resides (Wonkka et al. 2012). Thus, the effect of supplemental water treatments during the summer drought may contribute to improved growth and reproductive success and may hold insights for species protection.

Three goals of this study are to 1) document growth cycle, presence and absence, and morphological characteristics of individual *S. parksii* and *S. cernua* plants, 2) quantify invertebrate and vertebrate herbivory and determine their influence on *S. parksii* growth and survivability, and 3) determine how these plants react to supplemental water during their usual summer dormancy period and resultant growth during the fall flowering season. It is hypothesized (1) that the *S. parksii* and *S. cernua* sample population survival is affected by annual weather and consistency in aboveground presence as rosettes and flower stalks. Specific objectives include:

(i) Monitor previously located individuals and document the physical state and presence status each season.

(ii) Assess influences of precipitation and temperature on 2014 to 2018 presence and morphological data.

(iii) Determine and analyze population trends to assist conservation goals.

It is also hypothesized (2) that *S. parksii* growth and survival are affected by the type and timing of herbivory. Specific objectives include:

(i) Monitor previously located flowering individuals and apply and maintain treatments (uncaged, caged, uncaged+insecticide, caged+insecticide, meshed) implemented by Nally (2016) to build upon existing data to assess herbivory effects on survival and reproduction.

(ii) Estimate observed invertebrate and vertebrate herbivory on winter rosette and spring inflorescence structures of permanently located individuals to determine the extent of herbivory in comparison to whole-plant growth measurements with no herbivory.

(iii) Assess indirect influences of precipitation on plant-herbivore interactions by analyzing precipitation and herbivory variations since 2014.

It is hypothesized (3) that scheduled summertime watering events will decrease the prevalence and duration of summer rosette dormancy and increase survival and inflorescence growth in the fall. Objectives include:

(i) Locate and monitor flowering individuals with a recorded history of inflorescence and rosette emergence and apply treatments (control, watered) to assess growth variations.

(ii) Assess biophysical factors of soil texture, surface sandy loam depth to claypan, and slope position to determine how habitat relates to *S. parksii* growth variations.

CHAPTER II

STUDY AREA

All studies will be conducted at the Brazos Valley Solid Waste Management Agency (BVSWMA) Twin Oaks Landfill site (96°8’51.86’’W, 30°35’47.25’’N) located in central Grimes Country adjacent to Highway 30, two miles east of the Navasota River (Fig. 2.1). The Twin Oaks property of 246.5 ha has been documented as the location of the largest protected *S. parksii* population (USFWS 2009). In 2002, HDR Environmental Consulting Firm reported over 700 *S. parksii* individuals at BVSWMA (SWANA 2010), however, surveys since 2006 have indicated much lower counts (Hammons 2008). To protect the habitat, in 2007 the United States Fish and Wildlife Services (USFWS) deed-restricted 56 ha within the property into 13 deed-restricted areas (DRAs) with limited entry, but only about 45 ha of this are believed to be *S. parksii* habitat (Hammons 2008; SWANA 2010). Most *S. parksii* research has been conducted within the DRAs (Hammons 2008).

DRA 11 (Fig. 2.1) was selected as the study site after finding the largest *S. parksii* and *S. cernua* populations during surveys of the 13 DRAs in 2013 (Nally 2016). BVSWMA is characterized by Post Oak Savanna vegetation that was originally predominantly open grassland but now is largely transitional between prairie and woodland (Wilson 2002). Grass species such as little bluestem (*Schizachyrium scoparium*[2]), Indiangrass (*Sorghastrum nutans*), purpletop tridens (*Tridens flavus*), and longleaf woodoats (*Chasmanthium sessiliflorum*) dominate grasslands (Wilson 2002; Nally 2016; Skaalure, personal observation). Common trees are post oak (*Quercus stellata*), black jack oak (*Quercus marilandica*), and winged elm (*Ulmus alata*). Shrubs include

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[2] Taxonomic nomenclature follows Diggs et al. 1999 (plants)

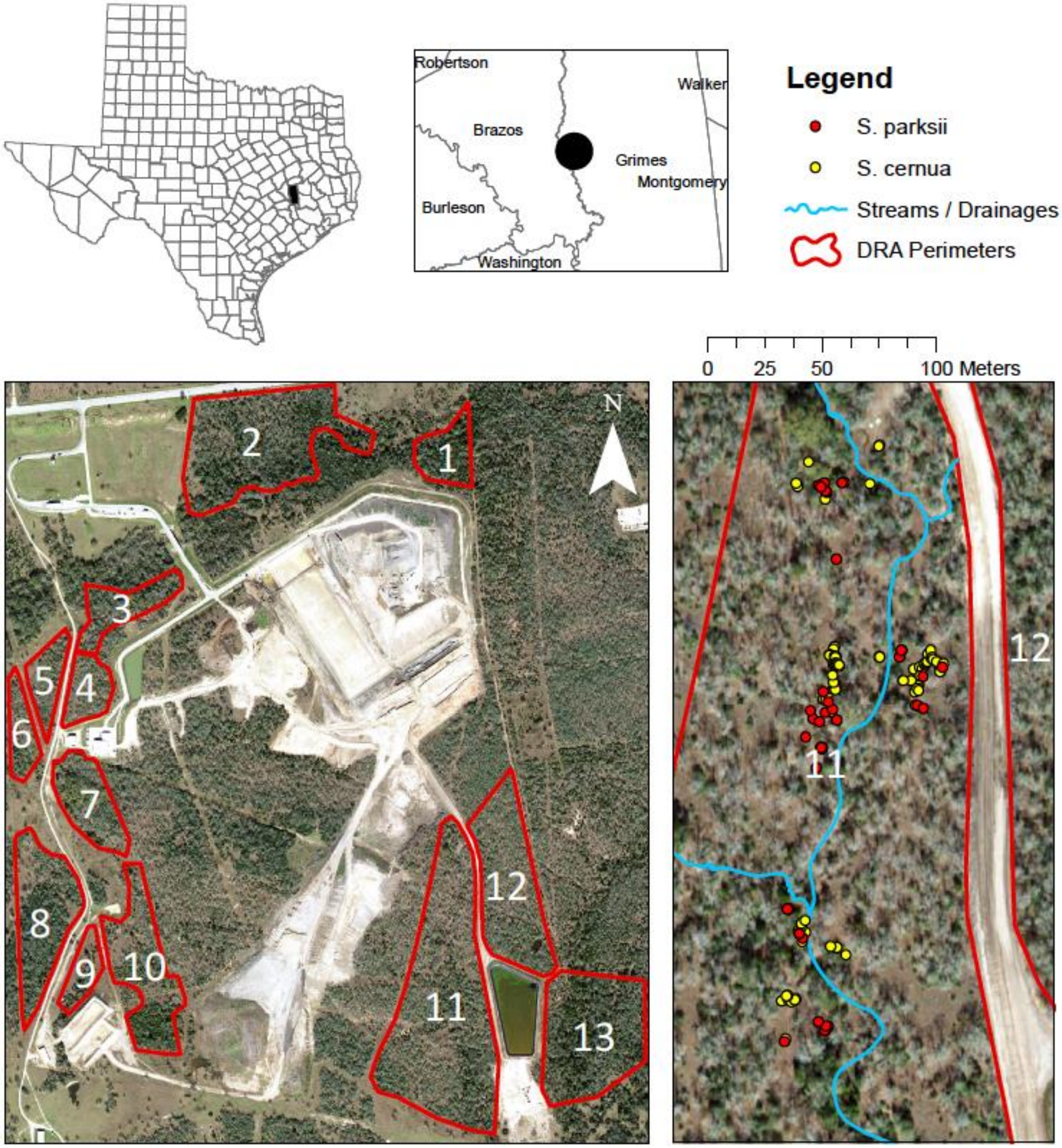

Figure 2.1. Deed-restricted areas within BVSWMA in Grimes County, Texas. Deed-restricted area 11 study site is enlarged with the herbivory study (Chapter IV) sample population of *S. parksii* and *S. cernua*.

yaupon (*Ilex vomitoria*), farkle-berry (*Vaccinium arboreum*), beautyberry (*Callicarpa americana*), and deciduous holly (*Ilex decidua*) (Wilson 2002; Hammons 2008; Skaalure, personal observation). All woody species are increasing their canopy density throughout the area, which is generally agreed to reduce *S. parksii* growth and reproduction. Species associated with *S. parksii* are mostly herbaceous and include little bluestem, split-beard bluestem (*Andropogon ternarius*), rayless-goldenrod (*Bigelowia nuttallii*), rough buttonweed (*Diodia teres*), sundew (*Drosera annua*), small-head boltonia (*Boltonia diffusa*) (Skaalure, personal observation), along with yaupon and beautyberry (Hammons 2008; Nally 2016). The interspersed grasses, shrubs, and trees create canopies that range between dense to relatively open, and generally leaf litter depth is medium to heavy (Nally 2016). *S. parksii* are most often found within canopy gaps of the tall grass and shrub vegetation where they can receive direct sunlight for part of the day (USFWS 2009).

BVSWMA lies within the subtropical humid zone of Texas and the nearby city of College Station, Texas receives approximately 100 cm of precipitation annually. The majority of the rainfall occurs in the spring and fall, and July is typically the driest month. Temperatures are regularly coldest in January (5 °C) and hottest in June, July, and August (36 °C). Snow and ice are not typical, but do occur occasionally (SRCC 2018).

At the landscape level, Post Oak Savanna ecoregions consist of gently rolling to hilly topography. Within DRA 11 at BVSWMA, elevation ranges from roughly 70 to 82 m. The DRA contains various streams and drainages which are typically shallow at the north side and deeply cut into the terrain toward the south. *S. parksii* is generally found along the naturally-eroded margins of small ephemeral drainages with both low-gradient and steep slopes into the waterway, and plant moisture is likely sustained by seepage along the claypan subsurface (USFWS 2009).

The property lies upon the geological Wellborn Formation, characterized primarily by indurated sandstone (USGS 2015). Soils in the region are mostly Burlewash fine sandy loam, 1 to 5 and 5 to 12 percent slopes (fine, smectitic, thermic Ultic Paleustalfs[3]), and other interspersed soils include Burlewash-Gullied land complex and Robco-Tanglewood complex (loamy, siliceous, active, thermic Aquic Arenic Paleustalfs) (NCSS 2017). Burlewash is the main series documented for the geographic location of DRA 11, and is characterized as well-drained and slowly permeable claypan soil (NCSS 2017). Burlewash is comprised of clayey residuum from weathered sandstone that forms an argillic horizon underlying the fine sandy loam surface, beginning around 15 cm in depth (NCSS 2017).

The majority of *S. parksii* individuals are found on fine sandy loam topsoil typical of the Burlewash soil series, however, a small percentage of the DRA 11 population has been seen to thrive (or survive) in severely eroded areas with clayey surface soil. Exposed claypan is typical along backslopes and footslopes where slope is significant, and fluvial deposits create small clayey mounds in waterways. Generally, clayey soils that support *S. parksii* and *S. cernua* tend to have high moisture contents, whereas sandy loam soils tend to have low to moderate moisture. However, some patches of sandy loam soil exhibit water contents closer to that of clay. These soils tend to be located at gradual transitions between foot- and toeslopes with gently declining to nearly level topography. Soil moisture variations may also influence *S. parksii* and *S. cernua* growth heights, as reproductive season growth documentation of plants found on clayey soils typically exhibit average to tall whole plant heights comparative to the population demographics.

Land use history of the BVSWMA property has been described by Hammons (2008) from communication with the previous landowner. In the 1920s a portion of the land was likely utilized

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[3] Taxonomic nomenclature follows Soil Survey Staff 2014 (soils)

for cotton farming, but that did not include the study site which did have a history of cattle grazing but no cultivation. In the last 50 to 60 years before purchase by BVWSMA, grazing and hunting were the primary uses. Stocking rates were estimated to have been at moderate levels, about 1 animal unit per 3.25 to 3.6 ha, and presumed hunting rates were 1 to 2 white-tail deer and around 20 feral hogs per year. Tree ringing was implemented in the 1940s and 1950s for pasture management, and prescribed burning followed once every three years during this time to clear dead trees and excess brush. Additionally, some areas of the site were bull-dozed and seeded with bahiagrass (*Paspalum notatum*).

Records of fauna at BVSWMA are based on compilations of observations and identifiable footprints. Mammals observed include white-tail deer (*Odocoileus virginianus*[4]), eastern cottontail rabbits (*Sylvilagus floridanus*), field mice (*Peromyscus* spp.), and feral hogs (*Sus scrofa*). Based on footprints impressed in clayey stream banks, the habitat is also home to common raccoons (*Procyon lotor*) and coyotes (*Canis latrans*). There is an extensive list of invertebrates that also occupy DRA 11 of BVSWMA, but those most commonly seen on or around *S. parksii* include leaf hoppers (family Cicadellidae[5]), the Post Oak Savanna grasshopper (*Dendrotettix quercus*) and other grasshopper species (family Acrididae), armyworms (family Noctuidae), paper wasps (family Vespidae), and various ants (family Formicidae) including infrequent sightings of cow killers (*Dasymutilla occidentalis*). Amongst the leaf litter are many mites (family Tetranychidae) and woodlice (family Armadillidiidae) (Wonkka 2010; Nally 2016; Skaalure, personal observation).

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[4] Taxonomic nomenclature follows Schmidly 1994 (vertebrates)

[5] Taxonomic nomenclature follows Triplehorn and Johnson 2005 (invertebrates)

CHAPTER III

DEMOGRAPHICS

Literature Review

Life history traits amongst species have been shaped by natural selection and measure demographic processes by timing, intensity, frequency, and duration (Salguero-Gómez 2017). Examples of these traits include longevity, generation time, and iteroparity, which influence survival and reproduction of a species. The idea of trade-offs, whereby fitness must be balanced by an allocation of limited resources toward survival, growth, or reproduction, means that not all possible values of trait combinations can exist (Shefferson et al. 2003). For example, rapid growth, high reproduction rates and output, and short lifespan are opposed by slow growth, infrequent reproduction with low output, and long lifespan (Salguero-Gómez 2017). The number of seeds and seed mass is another trade-off (Leishman et al. 2000); small seeds are more easily produced based on resource availability, yet seedlings from small seeds have lower survival rates (McCormick & Jacquemyn 2014).

The theory behind life history traits is based on the balance of costs and risks. In particular, costs and risks of reproduction in one season can present a cost to survival in the next (Shefferson et al. 2003; Salguero-Gómez 2017). Geophytes are plants characterized by underground meristems (Lesica & Steele 1994), and many species, particularly orchids, exhibit extended dormancy characterized by aboveground absence beyond a typical dormancy period while root function remains uninterrupted (Miller et al. 2004). In these geophytes, reproduction via flowering can be energetically costly (Primack & Stacy 1998) but measurement of the incurred costs to survival is difficult due to dormancy (Lesica & Steele 1994; Shefferson et al. 2003).

Researchers continue to debate the role of dormancy in geophytes, but two leading hypotheses have formed. Dormancy has been proposed as a strategy of dealing with plant stress: a method of compensation for harsh environmental conditions or herbivory that occurred in the previous or current growing season. However, dormancy represents a lost photosynthetic opportunity and may lead to a greater chance of mortality (Hutchings 1987). On the other hand, dormancy may benefit plants by allowing energy conservation during harsh conditions, preventing population-wide misfortunes, and allowing plants to sample different temporal environments (Shefferson et al. 2003). Therefore, life history traits of dormancy-experiencing geophytes are not only affected by reproductive costs but also by trade-offs between dormancy and survival, sprouting and survival, and flowering and dormancy (Shefferson et al. 2003).

General Life Cycle

The life cycles of *Spiranthes parksii* and *Spiranthes cernua* in eastern and central Texas are parallel and involve three physical states: a flowering stalk, vegetative rosette, and aboveground dormancy (Fig. 3.1). The physical states change with the seasons, where flower stalks are present in the fall, rosettes in the spring, and dormancy occurs in summers. Reproductive growth appears from September to early October and flowering stalks exhibit anthesis from late October to late November; after anthesis, the stalks senesce. Rosettes begin to develop from mid-November to March and typically persist through May, but in cool and wet years can last to July. Summers bring the hottest and driest conditions, during which plants undergo dormancy until mid-August to late September, when aboveground growth begins again.

The flowering stalks can produce from 5 to 45 flowers along the spiraled spike inflorescence, however, many suffer from complete inflorescence removal by herbivory so

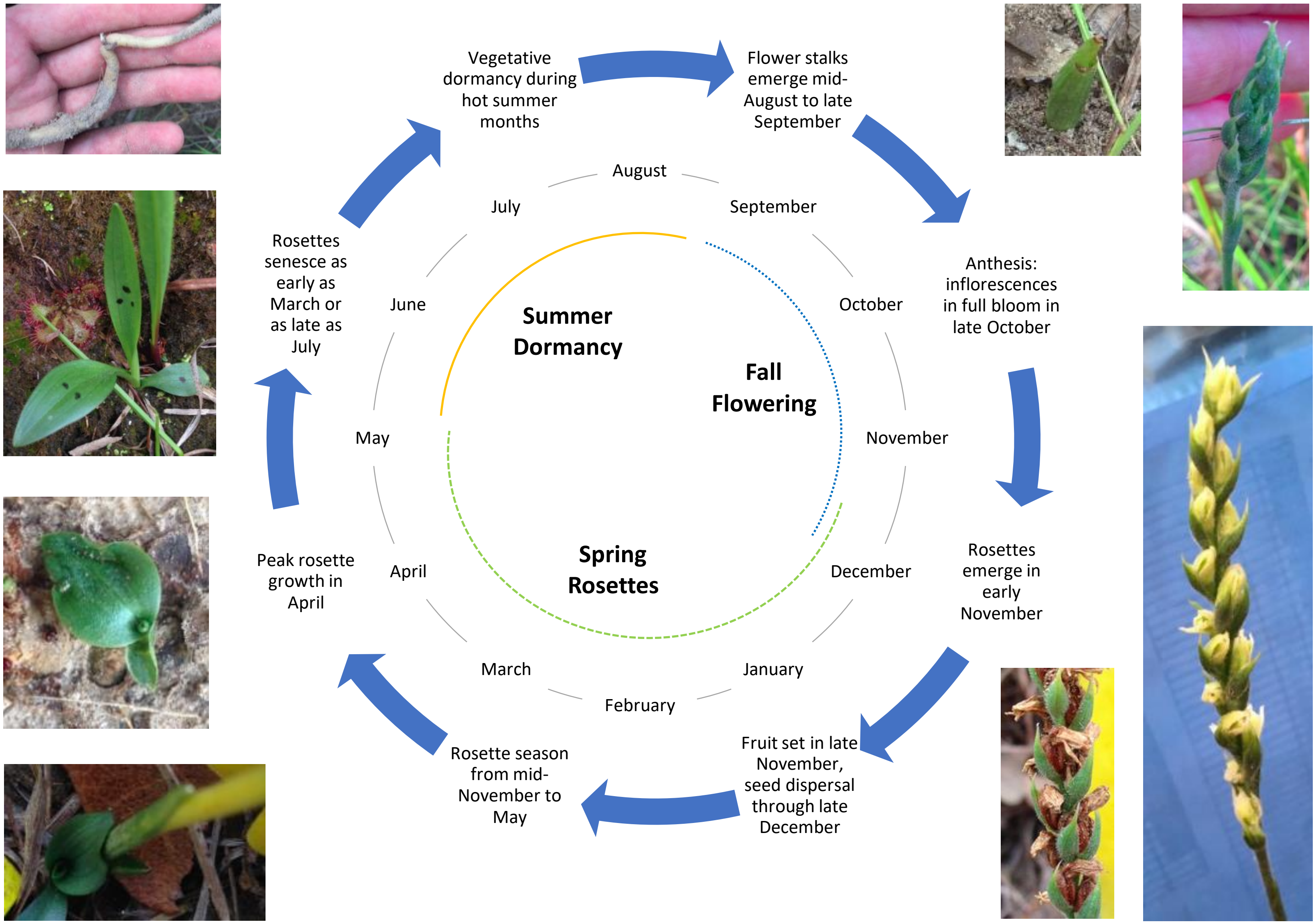

Figure 3.1. General annual life cycle of *S. parksii* and *S. cernua* (with minor changes adapted from Ariza 2013).

flower stalks are also referred to as reproductive structures. Inflorescences begin anthesis from the bottom to the top of the spiral; complete inflorescence anthesis can extend for up to one month in wet, cool years. Individual flowers generally remain open for several days but can last up to about two weeks (Skaalure, personal observation). Identification during anthesis is crucial as this is the only period that *S. parksii* and *S. cernua* can be confidently distinguished. However, some *S. cernua* never open their flowers and are labeled as “closed form,” or the cleistogamous peloric *S. cernua* ecotype (Ariza 2013) (Fig. 3.2). Seed dispersal begins once fruits have dehisced and can continue well after the flower stalk senesces, from late November through late December. Many plants begin to develop their evergreen rosettes at the time of or just before fruit set and seed dispersal, with either consistent or inconsistent aboveground presence through March, when rosettes reach peak abundance. Ariza (2013) found that maximum rosette growth was attained from late February to early March, but this study’s data from 2014 to 2018 indicated peak growth ranges from late March to late April.

It is not common for individuals to forgo summer dormancy but not impossible. Oftentimes in a few individuals each year, summer dormancy is sporadically broken by aboveground reemergence for a couple weeks, followed by absence again. Some individuals do not go dormant for entire summers, but this phenomenon is rare. When fall emergence initiates in mid-August to early September, growth as flower stalk or rosette leaves is indistinguishable for several weeks. Most individuals progress into reproductive structures and prepare for flower development, but some forgo reproduction and remain as rosettes through the fall with either consistent or sporadic presence. Individuals may also continue dormancy from the summer through fall seasons but often return aboveground later as a spring rosette. However, spring dormancy rates became more prevalent from 2014 to 2018.

Figure 3.2. Inflorescences of *S. parksii* (a) and *S. cernua* “open form” (b) and “closed form” ecotypes (c, d).

In addition, a defining characteristic of orchids is their seed size, the smallest in the plant kingdom (Swarts & Dixon 2009). Ariza (2013) suggested that *S. parksii* and *S. cernua* exhibit about 90% fruit set with approximately 3000 seeds per capsule. The large numbers of seeds produced by each plant relative to extant plants suggests that seed and seedling mortality is high. It has been estimated that 1% to 45% of orchid seeds germinate and even fewer develop into mature plants (Swarts 2007). A study on terrestrial *Caladenia arenicola* found that despite over 30,000 seeds per capsule, less than 1% germinated and survived summer dormancy (Batty et al. 2001). Orchid seeds lack endosperms, so germination only occurs when compatible mycorrhizal fungi penetrate the testa and access the embryo (Rasmussen 1995). Orchid seedlings then become completely dependent upon the mycorrhiza for development and growth until photosynthetically-capable tissue or true tubers develop, after which the orchid combines mycotrophy and phototrophy (termed mixotrophy) throughout its life as it continues to rely upon fungi as a carbon source to supplement or substitute photosynthetically-derived carbon (Rasmussen & Rasmussen 2009, Shefferson et al. 2018). *Spiranthes* seedling research will benefit from further investigations in an effort to conserve *S. parksii* at all life stages.

Conservation

Due to fluctuations in seedling survival and seasonal dormancy rates over time, *Spiranthes parksii* conservation efforts are imperative. Plant species exhibit ranges of specialization to the habitats in which they are found, and the specialization is regulated by trade-offs (Ariza 2013). Orchids are unable to occupy wide ranges of habitat, and instead exhibit various restrictions that confine their distributions. These restrictions are shaped by each species' degree of environmental tolerance and resource requirements (Liggio & Liggio 1999). In turn, these tolerances and requirements dictate each species' niche (Hutchinson 1957). For a population of plants to successfully exist within a habitat, individuals must also be able to withstand competition for resources between coexisting species. When multiple orchid species co-occur, resource availability from or competition between different mycorrhizal fungi may further contribute to orchid spatial distribution (McCormick & Jacquemyn 2014). Understanding species' limits of tolerances and requirements in natural environments is vital to determining how they function and therefore how their behavior may change in response to fragmented habitat or climate change.

In this study, the population dynamics of *S. parksii* and *S. cernua* were monitored from spring 2014 to fall 2018. Growth cycle, morphological characteristics of flowering, vegetative, and dormant states, and presence and absence data were assessed for 137 individuals. It is hypothesized that the *S. parksii* and *S. cernua* sample population survival is affected by annual weather and consistency in aboveground presence as rosettes and flower stalks. Specific objectives include:

(1) Monitor previously located individuals and document the physical state and presence status each season.

(2) Assess influences of precipitation and temperature on presence data by analyzing precipitation and presence proportion variations from 2014 to 2018.

(3) Determine and analyze population trends to assist conservation goals.

Methods

Presence-Absence Data

To examine plant phenology, seasonal variation, and population dynamics of *S. parksii* and *S. cernua*, 43 *S. parksii* and 94 *S. cernua* plants were permanently marked with aluminum identification tags and colored flags. To ensure complete recapture data each season and year regardless of aboveground presence, individual plants were mapped by GPS coordinates using a Trimble GeoTX system (see Chapter II Study Area, Fig. 2.1). The study was carried out from spring 2014 to fall 2018.

In spring 2014, the sample population consisted of 107 plants: 39 *S. parksii* and 68 *S. cernua*. Two observation dates recorded rosette presence and absence to determine how aboveground presence fluctuated; one in March documented presence at the time of peak abundance, and one in May observed presence near the end of the spring rosette season. Thirty plants were later added to the sample population in fall 2014, but considering they were all selected due to their presence as rosettes in the preceding spring, they were included in final spring 2014 presence-absence data. Beginning in September 2014, presence-absence data was recorded every 7 to 14 days through spring 2018. After this point, there was a single observation date on October 15, 2018 to determine fall presence status.

Spring rosette status was recorded as either present or absent while fall inflorescence status was categorized as present, absent, or present as a rosette. The goal of this study was to assess how presence as rosettes and flowering stalks affected aboveground occurrence in the next seasons, so plants present in the fall only as rosettes were considered absent because they failed to assume the reproductive state. Therefore, 'fall presence' hereafter refers to plants that exhibited flower stalk growth. Individuals were marked as present during an entire season if they had been aboveground in the expected form during at least one observation point. Summer presence was not included because plants generally senesced prior to the hottest months of July and August.

Growth Data

Fall plant area, height, inflorescence length, number of flowers and spring rosette area, number of leaves, and leaf length and width growth parameters (see Chapter IV Herbivory, page 73 for further details) were monitored and measured at the same time as presence-absence data collection, approximately every 7 to 14 days for the majority of the study period. Maximums of each measurement parameter were determined for every individual, then means were derived for each season in each year. Additionally, growth forms in previous seasons, such as flowering or non-flowering in the fall, were categorized and compared to mean growth parameters in the next season to determine whether the previous state had an influence on later growth. Spring presence and maximum levels of sustained herbivory were also compared to presence and growth in the subsequent fall.

Seedling Counts

With adequate rainfall, rosette seedlings are abundant from early December to late May, or when temperatures increase and rainfall decreases significantly. Beginning in spring 2017 and periodically through spring 2018, the absence, presence, and counts of first-year seedlings surrounding specimen locations at any life stage (rosettes, inflorescences, or dormant individuals) in the herbivory study (see Chapter IV Herbivory) were documented to attempt to relate how many seeds survive to photosynthetic seedlings. Potential seedlings and juveniles (those beyond their first rosette season) were differentiated according to seedling descriptions provided by Ariza (2013), and were recorded within a radius of 15 cm from the herbivory study plant, as most seeds land very close to the mother plant (Ariza 2013).

When multiple observations occurred in a single month, the maximum seedling and juvenile counts per mature plant were assessed. Monthly seedling maximums were obtained from April to September 2017 and December 2017 through May 2018; monthly juvenile maximum counts were collected during the same time points but ceased after March 2018 instead of May.

The designation of young plants as either seedlings or juveniles followed aboveground characteristics listed by Ariza (2013), where seedling features were acquired from germination to the second spring growing season and juvenile data from the second through fourth year of growth. While both seedlings and juveniles were observed with one to three leaves, seedlings presented total leaf surface areas of 3.5 ± 2.5 cm$_2$ while juveniles were larger, at 7.0 ± 3.9 cm$_2$. Similarly, the summed rosette leaf lengths of seedlings and juveniles averaged at 8.5 ± 1.3 cm and 11.5 ± 4.5 cm, respectively. Finally, summed rosette leaf widths of seedlings were 0.7 ± 0.4 cm and juveniles were 1.0 ± 0.5 cm (Ariza 2013). Rosette designation as a seedling was further supported if it offered no evidence of previous flowering. Juveniles may or may not have flowered yet but exhibited greater leaf areas than seedlings and smaller leaf areas than mature plants (Ariza 2013).

Seedling and juvenile distinctions were also acquired from observations noted during the studies presented here. Leaves on young individuals always exhibited a glossy surface that was not seen on mature plants, neither during yearly rosette initiation nor new leaf growth on mature individuals. Juveniles did not always maintain this sheen, but often displayed muted venation that was also typical of seedlings and contrasted the defined vascular tissue of mature leaves. While conservative visual discrimination between seedling rosettes versus juvenile or mature plant rosettes may provide novel observations, for more accurate age determination the root structure must be examined by exhuming the specimen (Ariza 2013). However, to avoid disrupting potential

S. parksii seedling survival, differentiation of seedlings from juveniles and adults were based on aboveground visual distinctions determined by Ariza (2013) and personal field observations.

Weather Data

Temperature and rainfall data were collected by BVSWMA's on-site weather station using a Davis Vantage Pro 2, located approximately 800 meters from DRA 11 (Nally 2016). Temperature maximums were averaged between weeks and months, as heat is thought to have a greater influence on growth than cooler weather. Rainfall records were summed between observation time points and monthly to infer weather-related responses.

Data Analysis

Seasonal presence data in 2014 through 2018 was assessed by the sample population as a whole, by date, and by individual. Using the entire sample population data by season, simple linear regression determined linear slopes and Pearson's correlation values amongst spring and fall presence percentages over time. Peak rosette count, growth, and flowering periods were determined by assessing presence and demographic data by date. Summed precipitation, mean temperatures, and seasonal presence data underwent multivariate analyses to examine multiple Pearson's correlations at a time. Presence and absence were also assessed by chronological spring and fall, and fall and spring seasons to determine what, if any, differences occurred when comparing a fall season to the preceding or succeeding spring. Least squares regression lines were utilized to predict future convergence between spring and absence lines, and yielded linear equations and Pearson's correlations.

Individuals were also assessed successively from 2014 to 2018 by only spring presence, only fall presence, and spring and fall presence; for example, the gains and losses indicated between fall of 2014 and 2015 represent the change in presence counts from fall 2014 to fall 2015. This method allowed visualization of how many plants returned aboveground after absence in the previous season, and how many became absent in a subsequent season. Additionally, individuals were assessed by the maximum number of cumulative and consecutive seasons they were present in from 2014 through 2018. Determination of any differences between species was performed using Wilcoxon Rank Sums. All analyses were run using JMP Pro 13 at significance levels of alpha = 0.05, but marginally significant *p*-values < 0.10 were also considered and noted when present.

Growth parameters from fall 2014 to spring 2018 were assessed by year to determine how each measurement changed over time. Kruskal-Wallis tests were utilized to determine any significant differences, and Wilcoxon Rank Sum tested means comparisons. To determine relationships between growth and weather, growth parameters, summed precipitation, and mean temperatures were assessed using multivariate analyses to yield Spearman correlation coefficients.

Seedling analyses utilized Wilcoxon Signed Rank tests to determine differences between mean counts of seedlings and juveniles around mature plants. Kruskal-Wallis tests evaluated differences in mean seedling and juvenile counts per plant by the herbivory study treatment the mature plants received, and Steel-Dwass All Pairs compared means. Seedling and juvenile counts were also assessed to determine whether counts were relative to which plants flowered or didn’t flower in falls 2014 through 2017 in an attempt to speculate ages of the immature rosettes. Wilcoxon Signed Rank was utilized to find differences in mean counts around flowering and non-flowering plants of each year. Correlations and simple linear regression analyses were attempted for comparisons of precipitation and seedling counts, but sample sizes were too low to produce results.

Results

Presence and Weather

Over the study years, the number of plants present each season varied greatly (Fig. 3.3). The overall trend in the sample population was downward, where both the percent of flower stalks and rosettes generally declined each year. Spring and fall 2015 are notable, however, for presence proportions higher and lower than expected, respectively. These seasons also exhibited the largest difference in presence between a spring and fall season, with a decrease of 38% in individuals aboveground from rosettes to reproductive structures. Year 2017 was very similar, with a 35.8% reduction in presence from spring to fall. Comparatively, 2014 and 2016 only experienced minimal presence differences of 13.1% and 16.8%, respectively.

The number of reproductive structures amongst the sample population fluctuated greatly and mostly in a downward trend. Fall 2015 exhibited a deviation from the other years, probably due to minimal rainfall during the summer and early fall. Without considering the 2015 data point, reproductive structure presence declined by 14.2% each year from 2014 to 2018 ($R_2 = 0.89$, $p = 0.0556$). However, fall 2018 presence data was obtained from a single observation date during anthesis so the presence proportion presented is possibly lower than what would have been obtained from several observation dates prior to anthesis.

Anthesis events began as early as September 28th and ended as late as November 30th in 2017, although the typical onset in each fall was approximately October 15th to 22nd with fruit set by November 20th to 27th. Each of the dates of first flowering were preceded by at least one week

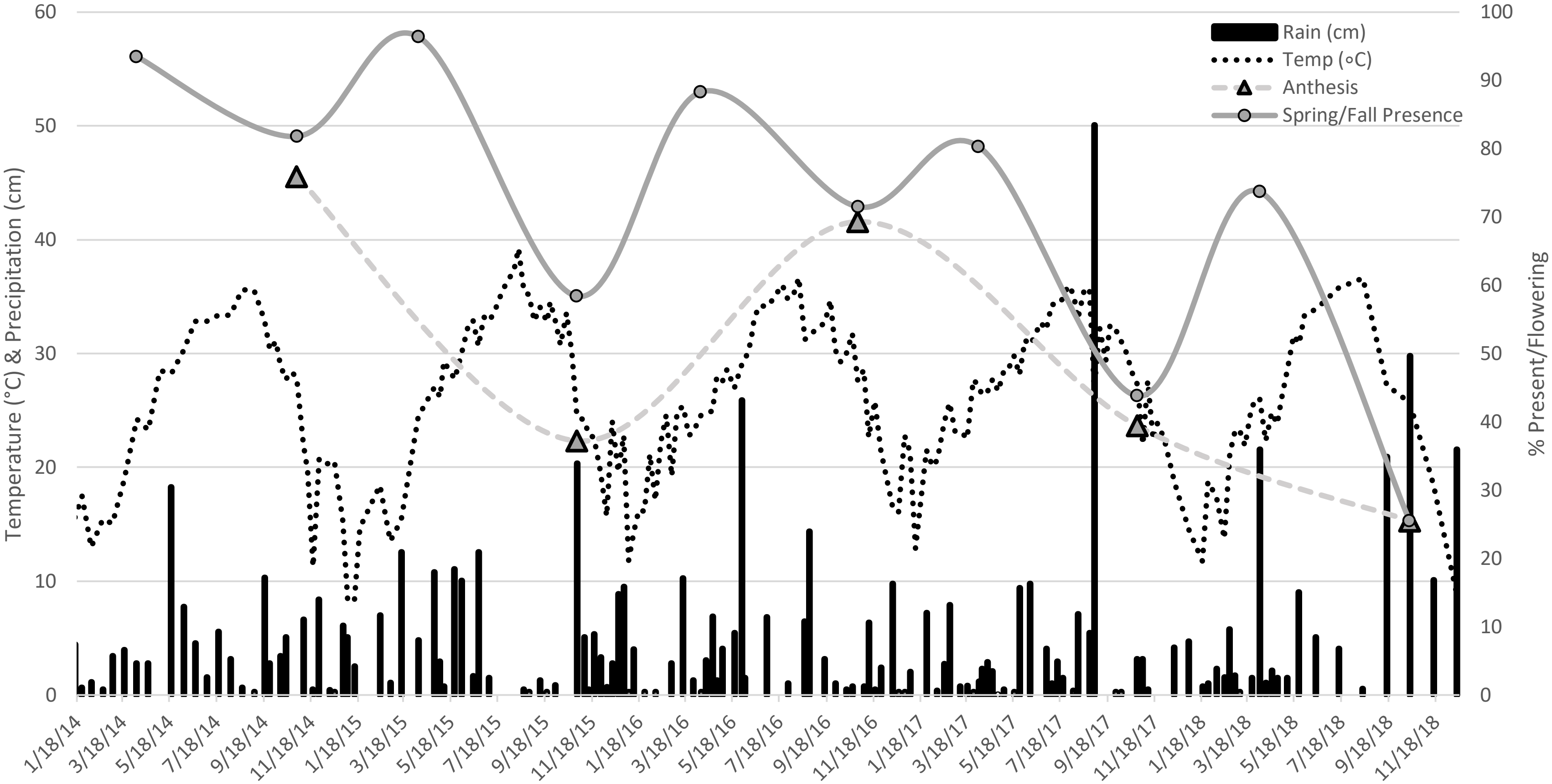

Figure 3.3. Weather data and percent presence of the sample population from springs 2014 to 2018 (*n* = 137). Spring presence refers to vegetative rosettes and fall presence refers to flower stalks. Percent of individuals that reached anthesis (triangles) are noted separately from percent of all plants with reproductive (flower stalk) growth.

of rainfall exceeding 1.5 cm within the three weeks prior, except 2017 which only received 0.25 cm of precipitation in three weeks but instead experienced over 50 cm five weeks prior due to Hurricane Harvey. It was therefore suspected that the reduced presence of flower stalks in 2015 was due to a very dry summer and early fall with minimal rainfall from July to late October, when anthesis would normally have already begun; only 4.70 cm of rainfall was received during this time while the 30-year average precipitation from July to September is 20.32 cm. Flowering in 2015 did not initiate until October 29th, only days after 20.32 cm of cumulative rainfall finally came from October 23rd to 25th. Additionally, fall 2015 exhibited the latest date of peak flowering amongst the years. Peak flowering was defined as the date at which 1) the greatest number of individual plants had open flowers, and 2) the greatest number of flowers across the sample population were open at one time. Years 2014, 2016, and 2017 exhibited peak flowering dates from October 26th through 30th whereas fall 2015 was November 13th.

Fall reproductive structure presence proportions were tested for correlations with summed monthly rainfalls in an effort to determine whether any relationships existed, as has been suggested by Hammons (2008) and Ariza (2013). Surprisingly, none of the presence proportions correlated significantly with fall rainfall in the same year, but instead with fall months from the year prior. Precipitation in September to October and October alone from the previous fall correlated significantly and positively with reproductive presence the next year (*t*-1 September to October: $R_2 = 0.95$, $p = 0.0481$; *t*-1 October: $R_2 = 0.99$, $p = 0.0101$) (Table 3.1). The only months of precipitation from the same year as the reproductive season in question that exhibited strong correlations were January through March, which had a negative correlation to presence proportions (January to March: $R_2 = -0.87$, $p = 0.0557$) and indicated that greater amounts of precipitation in these months led to reduced presence as reproductive structures in the fall. Additionally, flower stalk presence was negatively correlated with mean summer temperatures from the same year (May: R_2 = -0.88, p = 0.0497; May to July: R_2 = -0.91, p = 0.0345). There were no significant differences in fall presence proportions between species ($F_{3,1}$ = 36.9280, p = 0.1202).

Spring rosette presence proportions also exhibited a decrease over the years (Fig. 3.3). While 2015 was the only deviation from the reduction in presence proportions, it also appeared to be a peak year in which conditions were possibly highly favorable so plants were encouraged to come up aboveground. From 2015 on, the decline in spring appearance had a steady slope of -7.61% (R_2 = 0.99, p = 0.0011). Rosette appearances were consistently higher than reproductive presence in each preceding and subsequent fall. However, by spring 2017, rosette presence had declined below the highest reproductive presence proportion, recorded in fall 2014, which indicated that the sample population was diminishing.

Regular data observations throughout the years also indicated that the timing of peak rosette counts and growth fluctuated slightly. Only one observation date for spring 2014 was available, so data from 2015 through 2018 was assessed. Individuals appeared and disappeared

Table 3.1. Pearson's correlations between fall flower stalk and spring rosette presence proportions and summed rainfall and mean temperatures in given months. Only significant correlations are presented. Significance was tested at ∝ = 0.05, and * denotes p < 0.10.

	∑ Rainfall					Mean Temperature	
Fall Presence	*t*-1 Sep-Oct	*t*-1 Oct	Jan-Mar			May	May-Jul
	0.95	0.99	-0.87*			-0.88	-0.91
Spring Presence	*t*-1 Aug	*t*-1 Sep	*t*-1 Sep-Oct	*t*-1 Nov	Feb	*t*-1 Nov	Mar
	-0.90	0.89	0.86*	0.82*	-0.89	-0.95	-0.83*

during all periods of peak counts as some senesced and others were late arrivals, but the peak number of rosettes was always more or less sustained for a few weeks. Peak counts of spring rosettes in 2015 began in early March all the way through the end of April. This timeframe shifted and shortened as the years progressed, from mid-February to mid-April in 2016, mid-February to late March in 2017, and mid-February to mid-March in 2018. Peak growth, when the average leaf area amongst present individuals was highest, adjusted similarly over time. In 2015, maximum growth was achieved in late April; by 2016, it was mid-April; and in 2017 and 2018, peak growth occurred in early April.

Spring presence exhibited several correlations to precipitation, the majority of which occurred in the previous fall. Spring proportions were negatively correlated with late summer and early spring precipitation (t-1 August: $R_2 = -0.90$, $p = 0.0362$; February: $R_2 = -0.89$, $p = 0.0406$), as well as with fall and spring temperatures (t-1 November: $R_2 = -0.95$, $p = 0.0143$; March: $R_2 = -0.83$, $p = 0.0803$) (Table 3.1). The result for February rainfall was surprising as peak presence usually occurred from mid-February to mid-March, and it was presumed that additional rainfall leading up to and during peak counts would encourage aboveground presence. Correlations to the preceding August must be taken lightly, however, as in 2017 Hurricane Harvey deposited 63.02 cm of rainfall whereas the 30-year average August precipitation is 6.81 cm. Precipitation in fall months after August, however, yielded positive correlations with rosette presence (t-1 September: $R_2 = 0.89$, $p = 0.0402$; t-1 September to October: $R_2 = 0.86$, $p = 0.0603$; November: $R_2 = 0.82$, $p = 0.0884$). There were no significant differences in spring presence proportions between species ($F_{3,1} = 6.8825$, $p = 0.2715$).

Presence and Absence

Presence of the sample population from 2014 to 2018 was also examined for slopes, correlations, how presence in one season affected presence in the next, and how presence related to the percent of absence. There were no significant differences in season parameters between *S. parksii* and *S. cernua* (one-way ANOVA, $p > 0.80$), so presence and absence percentages for the entire sample population were assessed. In Fig. 3.4a, multi-season parameters (combined spring and fall presence, percent absent) were focused on values within a standard year t (i.e. the absence parameter encompassed individuals that had remained belowground in spring of year t and either belowground or without reproductive growth in fall of year t). Likewise, multi-season parameters in Fig. 3.4b referred to values in fall of year t and spring of year $t + 1$ to determine whether any distinctions arose by examining the data in a different consecutive sequence that is commonly utilized when discussing the mature *Spiranthes* life cycle (Ariza 2013; Nally 2016).

The percent of plants present in both spring and fall of year t (*Spring+Fall*) closely mirrored fall presence percent, except in 2014 when more plants were present in the fall than in *Spring+Fall* (Fig. 3.4a). As joint spring and fall absence in 2014 was 0%, plants dormant in the spring all came up as reproductive structures in the fall while 18.2% of plants present in the spring forewent reproduction in the fall. In 2015, 2017, and 2018, all plants dormant in the spring were also belowground or reproductively dormant in the fall, so fall and *Spring+Fall* presence percentages were equal. Year 2016 deviated slightly, however, with fall presence 1.5% ($n = 2$) greater than that of *Spring+Fall* presence, which indicated that two plants absent in the spring came up as inflorescence structures in the fall instead of remaining vegetative or dormant. Overall, it was more common for plants to have spring and fall or spring but not fall growth, rather than spring absence and fall growth.

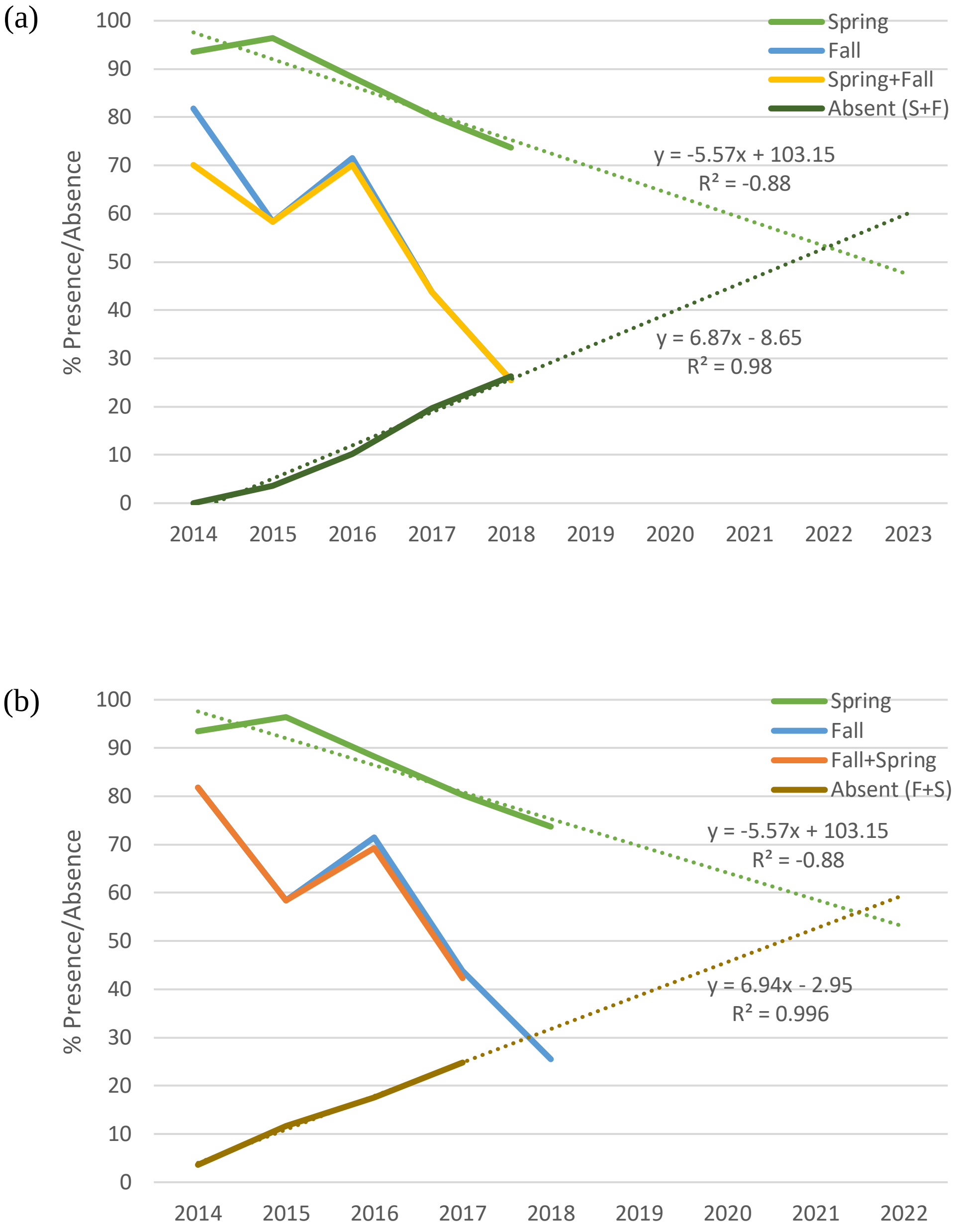

Figure 3.4. Percent presence and absence by (a) spring and fall (year *t*) where *Spring+Fall* and *Absent (S+F)* are the percent of individuals present or absent, respectively, in the two consecutive seasons, and (b) fall (year *t*) and spring (year *t*+1), where *Fall+Spring* and *Absent (F+S)* are the percent of individuals present or absent, respectively, in the two consecutive seasons. Least squares regression lines were utilized to predict line convergence.

Spring presence and percent spring and fall absence were always the highest and lowest values, respectively, so bivariate linear regression least squares lines of fit were compared between parameters to obtain an estimation of when consecutive spring and fall absence might overcome spring rosette presence (Fig. 3.4a). The percent of plants present in spring of each year steadily declined over time (slope = -5.57, R_2 = -0.88, $F_{1,3}$ = 22.0227, p = 0.0183) while the percent absent in both spring and fall increased slightly faster over time (slope = 6.87, R_2 = 0.98, $F_{1,3}$ = 147.4442, p = 0.0012). If the trendlines were to continue at the pace set from 2014 to 2018, total annual absence will overcome rosette presence in 2022 and the sample population will disappear. Additionally, when 2014 data was disregarded due to the irregular increase in spring presence to 2015, both slopes became steeper (spring: slope = -7.61, R_2 = -0.998, $F_{1,2}$ = 895.0866, p = 0.0011; absent: slope = 7.76, R_2 = 0.99, $F_{1,2}$ = 358.0119, p = 0.0028) and the least squares line predicted population demise in 2021. Due to uncertainty of percent presence in fall 2018, no trendlines were utilized for fall and *Spring+Fall* data. Likewise, fall absence in 2018 was also not definite, but considering that 2017 saw fall and *Spring+Fall* values decrease to almost 40% presence, it was possible that absence estimates from the observation data were more accurate than presence estimates.

Fall reproductive structure presence may have a different effect on subsequent spring rosette production than rosettes have on inflorescence prevalence, so consistent presence (*Fall+Spring*) and absence in fall of year t and spring of year $t + 1$ were also assessed (Fig. 3.4b). As before, fall and *Fall+Spring* presence percentages were very similar overall. In 2014 and 2015, the two parameters were equivalent and plants were only dormant in spring $t + 1$ if they had also been reproductively dormant in the previous fall. In 2016 and 2017, fall presence was 2.2% and 1.5% (respectively) greater than *Fall+Spring* presence, which indicated that three plants in 2016 and two plants in 2017 were present in the fall but became dormant in the following spring. Generally, it was more common for plants to forgo fall growth yet come up in the spring or have both fall and spring growth, rather than fall presence but spring absence.

Combined fall and spring absence began higher at 3.6% in 2014 and reached 24.8% in 2017 (Fig. 3.4b) and had a steeper slope (slope = 6.94, $R_2 = 0.996$, $F_{1,3} = 546.0726$, $p = 0.0018$) than spring and fall absence rates, so population longevity was calculated separately. The spring presence least squares line remained the same as before, with a slope of -5.57. If these trendlines continued, absence would overcome spring presence at year 2021.5; as absence here accounts for spring in year $t + 1$, the estimated year becomes spring 2022. When 2014 was removed from the least squares lines, the slope of the absence trendline became less steep (slope = 6.55, $R_2 = 0.996$, $F_{1,2} = 228.8133$, $p = 0.0420$), but line convergence and population disappearance was still predicted to be in 2021.

The number of individuals present for consecutive stages fluctuated by season and just as presence percentage, overall numbers declined with time (Fig. 3.5). By assessing fall seasons in pairs, it was discovered that plants that developed reproductive growth in one fall tended to be reproductively active in the next and more plants than expected were reproductively absent in two assessed years (Fig. 3.5a) as opposed to mostly different plants flowering or being dormant year to year. In both 2014 and 2015, 70 of the same plants came up as inflorescences; in 2015 and 2016, 73 plants were consistent; as were 59 in 2016 and 2017, and 28 in 2017 and 2018. In each of these pairs, the probability of reproductive presence or absence in both years was significantly greater than the probability of presence in one and absence in the other (Fisher's Exact Test, $p < 0.05$). Likewise, plants were more likely to be absent or develop reproductive growth in one fall if they had also been absent or reproductively active, respectively, two (Fisher's Exact Test, $p <$

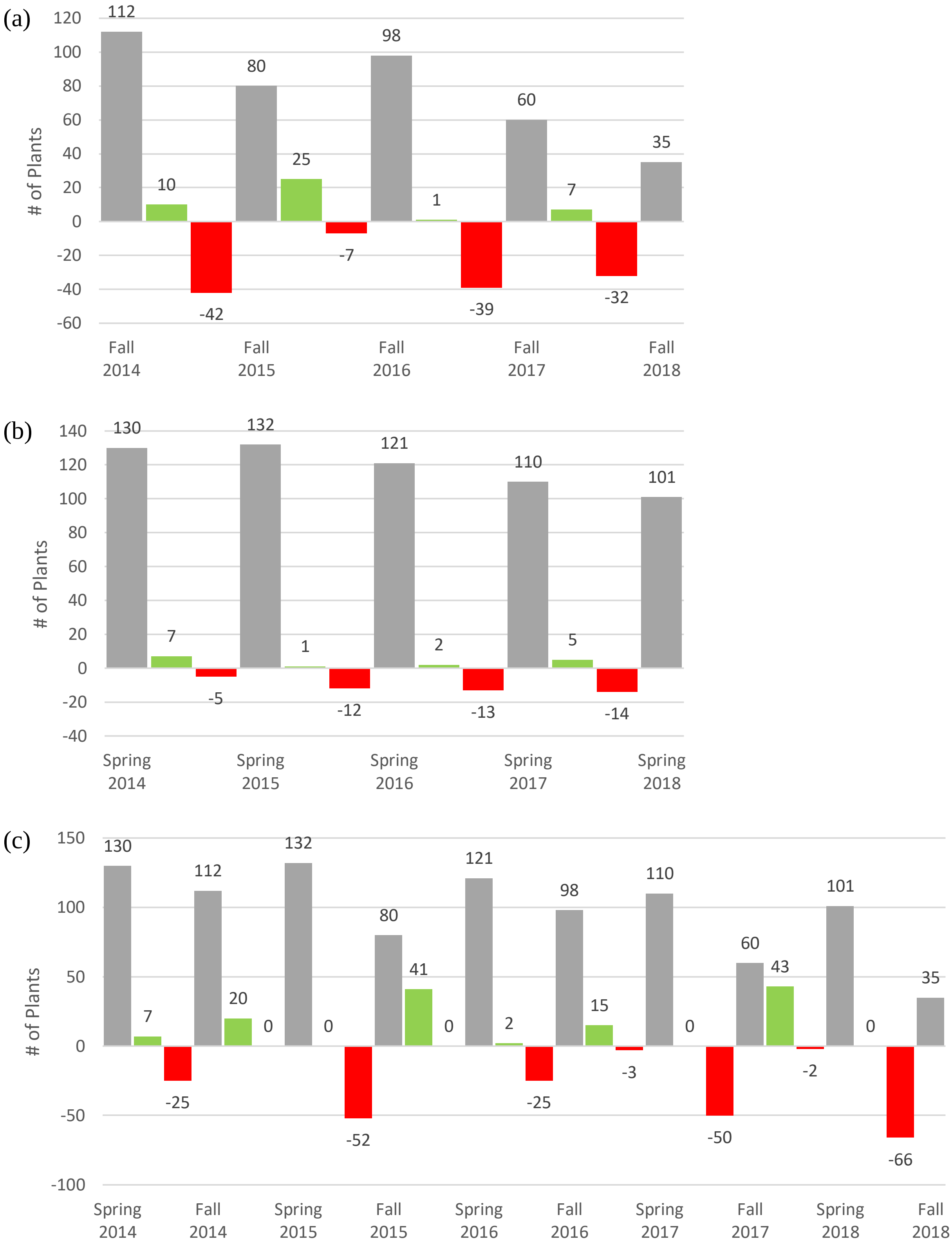

Figure 3.5. Presence by consecutive season and individual. Consecutive falls (a), springs (b) and spring and fall seasons (c). Green bars represent the reemergence of previously absent or reproductively inactive plants; red bars represent the loss in previously present or reproductively active plants.

0.007) and three (Fisher's Exact Test, $p < 0.009$) falls prior. For example, 86 of the 98 plants in fall 2016 were also reproductively present in 2014, and more plants were absent in both falls than were expected by the contingency table (Fisher's Exact Test, $p = 0.0065$). However, there was no significant association between fall presence in 2014 and reproductive growth in fall 2018 (Fisher's Exact Test, $p = 0.6152$), which indicated that the behavior of plants in a given fall season was influenced by their presence or absence in the fall of one to three years prior. In addition, fall 2017 was the first year in which the amount of absent plants was greater than the amount of reproductive plants.

Spring rosette presence was even more consistent year to year than fall growth, which was expected due to spring counts close to the sample population size. Annual gain in spring rosette presence was fairly low and losses in two-year consecutive presence increased over time (Fig. 3.5b), but counts remained well above those of fall presence in the same years. Similar to the fall seasons, plants were more likely to come up or be absent in the spring if they had been present or absent, respectively, in one (Fisher's Exact Test, $p < 0.0007$), two (Fisher's Exact Test, $p < 0.04$), or three (Fisher's Exact Test, $p = 0.0010$) springs prior. However, most associations involving spring 2014 were not significant and any suspected relationships were likely due to chance. Springs 2014 and 2015 were unique in that presence counts were extremely high and zero plants shared spring absence in both years, which led to a complete lack of association (Fisher's Exact Test, $p = 1.0000$). Relatedly, there were no significant associations between spring presence in 2014 and 2017 nor 2018 (Fisher's Exact Test, $p = 1.0000$) because most often, absent plants in spring 2014 were not absent again in later springs.

By assessing the gains and losses season to season, it is apparent that consecutive spring and fall presences became more dependent on the prior season's status as the years progressed

(Fig. 3.5c). From spring to fall 2014, there was no significant association (Fisher's Exact Test, $p = 0.3495$) and all plants that had been absent in spring 2014 were reproductively present in fall 2014. In fall 2014 and spring 2015, a significant relation became apparent (Fisher's Exact Test, $p = 0.0001$); all plants present in fall 2014 were also present in spring 2015 and most significantly, fewer plants were absent in both fall 2014 and spring 2015 than was expected from chance. This was the beginning of a trend in which most or all of reproductively active fall plants in 2014 to 2017 were also present the next spring, and of individuals absent in the fall, more were present in the spring than expected (Fisher's Exact Test, $p < 0.0001$). A separate trend developed in spring to fall seasons. Beginning in 2015, it became standard for all plants absent in spring to also be absent that fall (although 2016 was a slight exception in which two absent spring plants developed reproductive growth in the fall); and presence in the spring led to a greater probability of presence in the fall (Fisher's Exact Test, $p < 0.02$).

Presence and physical status at the time of anthesis was also assessed to determine if spring presence was affected. Each fall, plants were noted as either reproductively absent, flowering, or non-flowering due to desiccation or herbivory prior to anthesis. Presence in the subsequent spring was then determined for each category. From 2015 to 2017, the number of fall absences that remained absent the next spring increased from 20.0% to 61.5%, then fell to 44.2% in spring 2018 (Table 3.2). Generally, if a plant grew or started to grow a flower stalk in the fall, it was more likely to also be present the next spring. A maximum 3.7% of fall flowering plants were absent the next spring from 2014 to 2018, while all plants that had begun to develop a flower stalk but could not reach anthesis due to either desiccation or herbivory continued their aboveground presence the next spring.

Table 3.2. Fall presence status by resultant spring counts from fall 2014 to spring 2018. “Present, Desiccated” and “Present, Herbivory” indicates initiation of reproductive growth but failure to reach anthesis due to the respective ailment.

		Spring 2015	
		Absent	Present
Fall 2014	Absent	5	20
	Present, Flowered	0	104
	Present, Desiccated	0	4
	Present, Herbivory	0	4

		Spring 2016	
		Absent	Present
Fall 2015	Absent	16	41
	Present, Flowered	0	51
	Present, Desiccated	0	17
	Present, Herbivory	0	12

		Spring 2017	
		Absent	Present
Fall 2016	Absent	24	15
	Present, Flowered	3	92
	Present, Desiccated	0	0
	Present, Herbivory	0	3

		Spring 2018	
		Absent	Present
Fall 2017	Absent	34	43
	Present, Flowered	2	52
	Present, Desiccated	0	2
	Present, Herbivory	0	4

Cumulative and Consecutive Presence

During the observation period, seasonal individual plant presence was also examined. In each type of seasonal analysis, there were no significant differences in presence by species (Wilcoxon, $p > 0.70$) so the sample population was assessed as one unit. Analysis of cumulative fall season reproductive presence from 2014 through 2018 indicated that reproductive activity in a single season (15.3%, 21 plants) was about as common as that in all five seasons (16.8%, 23 plants). Of those present in one fall, 19 plants were only reproductively active in 2014 and one plant each were active in 2015 and 2016. Plants present in only two falls (19.0%, 26 plants) were mostly reproductively active during 2014 to 2016. These plants were mostly active in consecutive falls or skipped one year, and although skipping two or three falls was observed, it was very rare. Those present in three falls (19.7%, 27 plants) were not limited to specific years, but presence tended to be in consecutive falls beginning in 2014, or in 2014, 2016, and 2017; in one individual, presence occurred in every other fall. Reproductive presence in four of the five seasons was most common, at 21.2% (29 plants) of the sample population (Fig. 3.6a). Of these, absence was most common in 2018 (22 plants), while 3 plants were absent in 2017 and 2 plants each were absent in falls 2014 and 2015. Also of note, 8.0% (11) of plants did not come up as reproductive structures at all during the 2014 to 2018 observation period. Of these, one plant was present in four springs, three were present in both two and three springs, and four plants were present in only one spring, always the spring of 2014.

Assessments of individual plant presence in consecutive falls revealed more insight; most of the time, the amount of cumulative and consecutive reproductive seasons were the same. The percent present in non-consecutive falls was the greatest, where 26.3% (36 plants) of the sample population were reproductively active for one or more seasons that were each separated by a minimum of one year of reproductive absence (Fig. 3.6b); 21 of these had only been present in one fall season from 2014 to 2018, while 14 were present in two falls, and 1 was present in three. Plants present for two consecutive seasons made up 14.6% (20 plants), of which 12 were present in two cumulative falls and 8 were present in three. Plants present for three consecutive seasons made up 16.8% (23 plants), of which 18 were present in three cumulative falls and 5 were present in four. Plants present for four consecutive seasons made up 17.5% (24 plants), all of which were present in only four cumulative falls. Finally, plants present for five consecutive seasons made up 16.8% (23 plants). As the number of consecutive fall seasons increased, the percent of plants that were also present in the same number of cumulative seasons increased as well, from 58.3% in one consecutive season to 100% in both four and five consecutive seasons.

The distributions of spring season presence were very different from those of fall and had greater distribution means, medians, and modes. Unlike cumulative fall season presence, there was an exponential increase in the number of spring plants by increasing number of cumulative spring

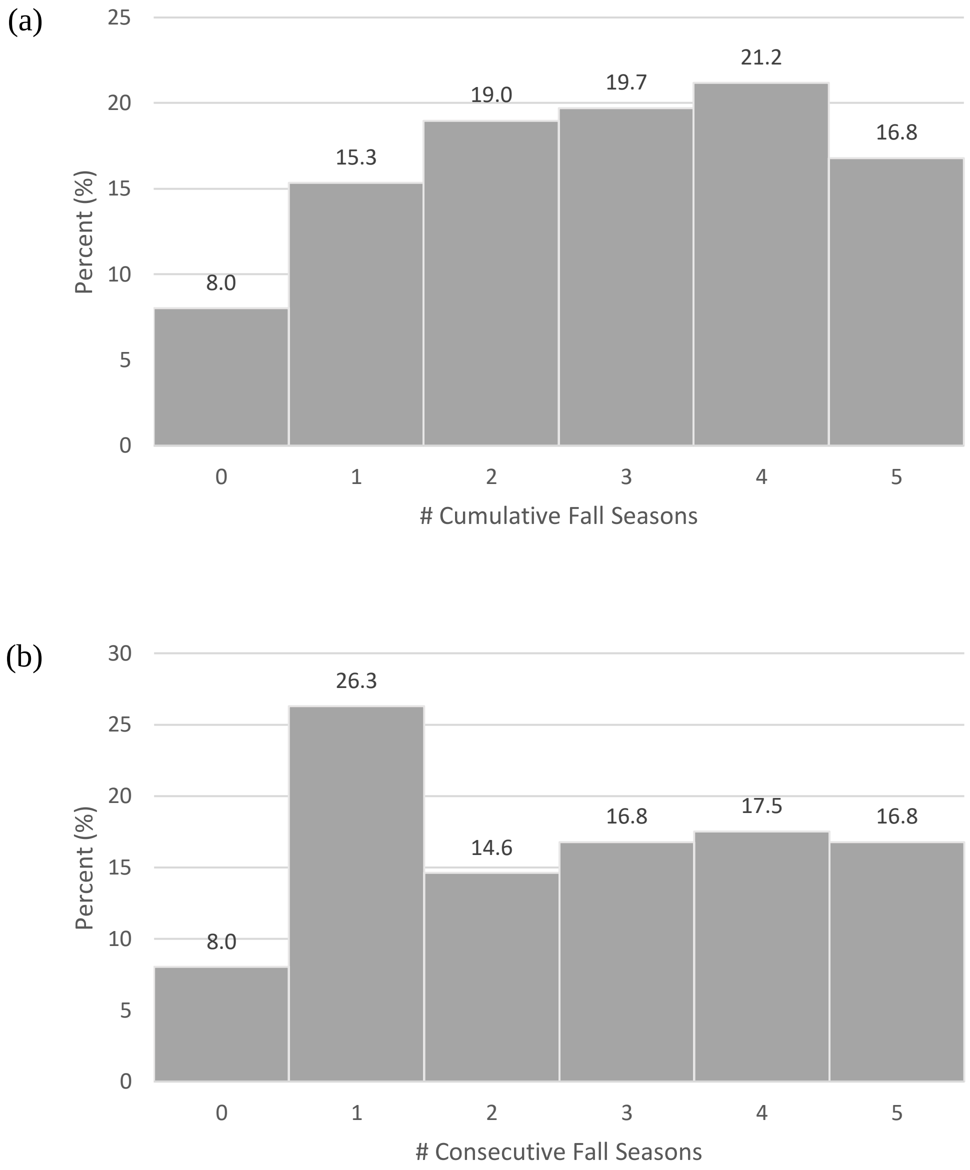

Figure 3.6. Percent of the sample population present for *x* number of (a) cumulative fall seasons (mean = 2.79, median = 3) and (b) maximum consecutive fall seasons (mean = 2.58, median = 3) from 2014 to 2018 (*n* = 137).

presence. Of the 3.6% present in one cumulative spring, 4 of the 5 plants were only aboveground in 2014 and the remainder was only active in 2015 (Fig. 3.7a). Perhaps not surprisingly, those that only appeared in spring 2014 were absent for every fall and spring season thereafter, while the individual from spring 2015 had only otherwise been present in the preceding fall. Those present in only two springs (5.8%, 8 plants) were mostly active during 2014 and 2015 but two individuals had skipped one spring between presence years. Those present in three springs (10.2%, 14 plants) were most often seen from 2014 to 2016 but also sometimes skipped up to two springs in a row between presence years. Rosette presence in four of the springs was seen in 13.9% (19 plants) of the sample population. Of these, absence was most common in 2018 or 2014, but 2 individuals were absent in spring 2017. About two-thirds (66.4%, 91 plants) of the sample population were aboveground as rosettes in all five springs from 2014 to 2018. No plants were absent from all springs.

The spread of consecutive spring seasons was very similar to the distribution of cumulative spring seasons and like the consecutive fall seasons, cumulative spring counts made up over half of each of the consecutive spring counts. The percent present in non-consecutive/single springs was the smallest, made up of 5.1% (7 plants) of the sample population (Fig. 3.7b); 5 of these were from the single-season cumulative group, while one had been present in springs 2014 and 2016 and the other in springs 2015 and 2017. Plants present for two consecutive seasons made up 7.3% (10 plants), of which 6 were present in two cumulative falls and 4 were present in three. Plants present for three consecutive seasons made up 8.8% (12 plants), of which 10 were present in three cumulative falls and 2 were present in four. Plants present for four consecutive seasons made up 12.4% (17 plants), all of which were present in only four cumulative falls. Finally, plants present for five consecutive seasons again made up 66.4% (91 plants).

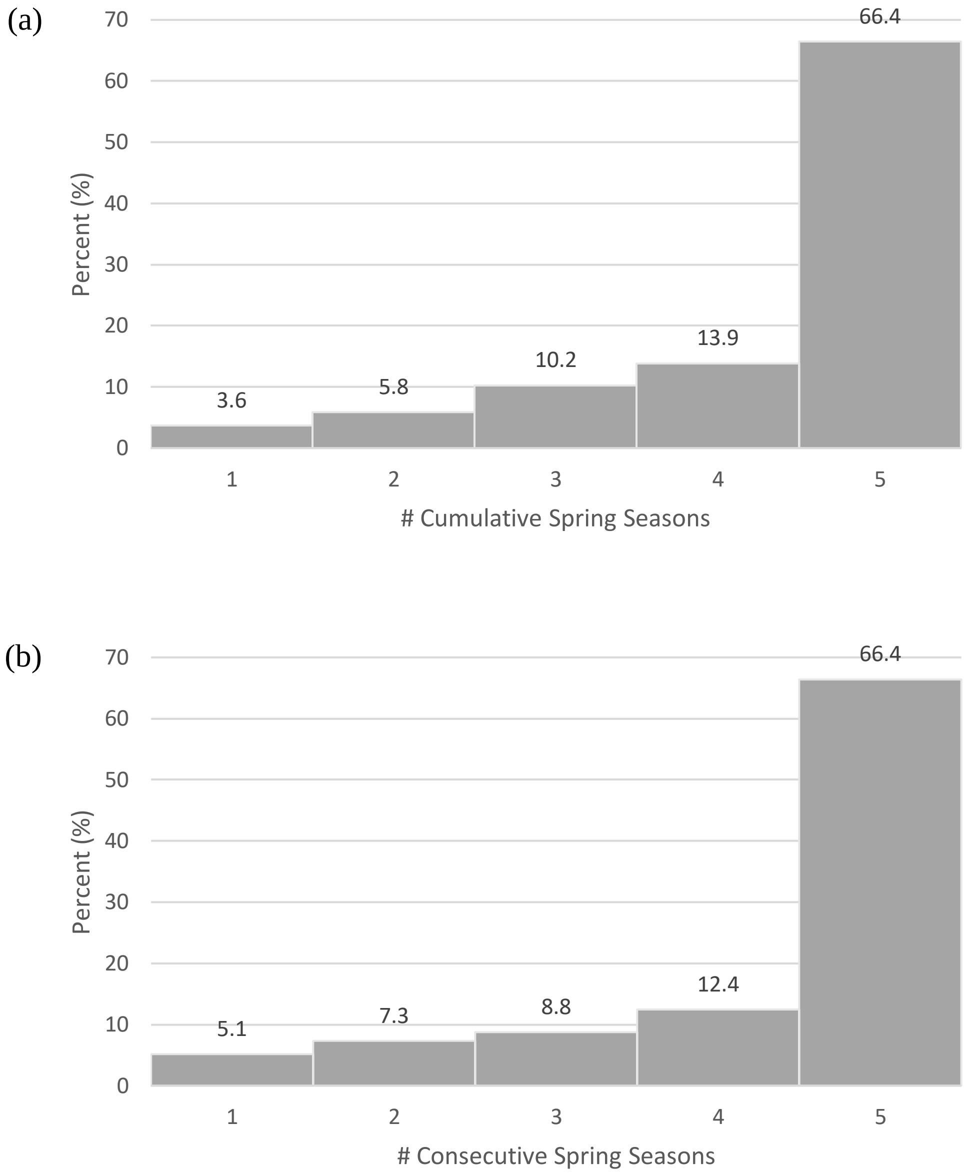

Figure 3.7. Percent of the sample population present for *x* number of (a) cumulative spring seasons (mean = 4.33, median = 5) and (b) maximum consecutive spring seasons (mean = 4.26, median = 5) from 2014 to 2018 (*n* = 137).

Cumulative presence statistics over the entire ten seasons from spring 2014 to fall 2018 indicated that overall, a greater percentage of plants were present in six or more seasons (78.1%) than in five or less (21.9%) (Fig. 3.8a; see Appendix A for more detail). Those present in one season (2.9%, 4 plants) were all only aboveground in spring 2014 and possibly experienced mortality shortly after. Of the 2.9% present in two seasons total, 3 were rosettes in springs 2014 and either 2015 or 2016, and the other had been active from fall 2014 to spring 2015. Seven plants (5.1%) were present in three cumulative seasons; four of these displayed consecutive presence from spring 2014 to spring 2015 while the others were only present in springs, most often consecutive springs 2014 through 2016, however one individual exhibited an unusual span, with presence in springs 2014, 2015, and 2018. Those present in four seasons (3.6%, 5 plants) had more diverse patterns. Two plants were present spring 2014 through spring 2015 and either spring 2016 or spring 2018; another was present spring 2014 and spring 2015 through spring 2016. Another plant forwent fall seasons completely, and was only present as rosettes in springs 2014 through 2017; and the last individual was present in fall 2014, spring 2015, and fall 2016, spring 2017. Of the 7.3% (10 plants) present in five seasons, 4 were aboveground consecutively from spring 2014 to spring 2016. Five others were present in three or four of these five consecutive seasons, in which fall 2015 or fall 2015 and spring 2016 were the most frequent absences; gaps between presence seasons ranged from one to five spring/fall seasons, where gaps almost always began with absence in a fall season. Those present in six seasons (13.1%, 18 plants) were spread across the board, with absence in falls 2015 through 2018 most common. Plants present in seven seasons (12.4%, 17 plants) exhibited various patterns, but about one-third of these had absences in falls 2015, 2017, and 2018. Plants present in eight seasons (15.3%, 21 plants) were most often absent in falls 2015, 2018 or 2017, 2018. Those present in nine seasons (21.2%, 29 plants) were absent mostly in fall

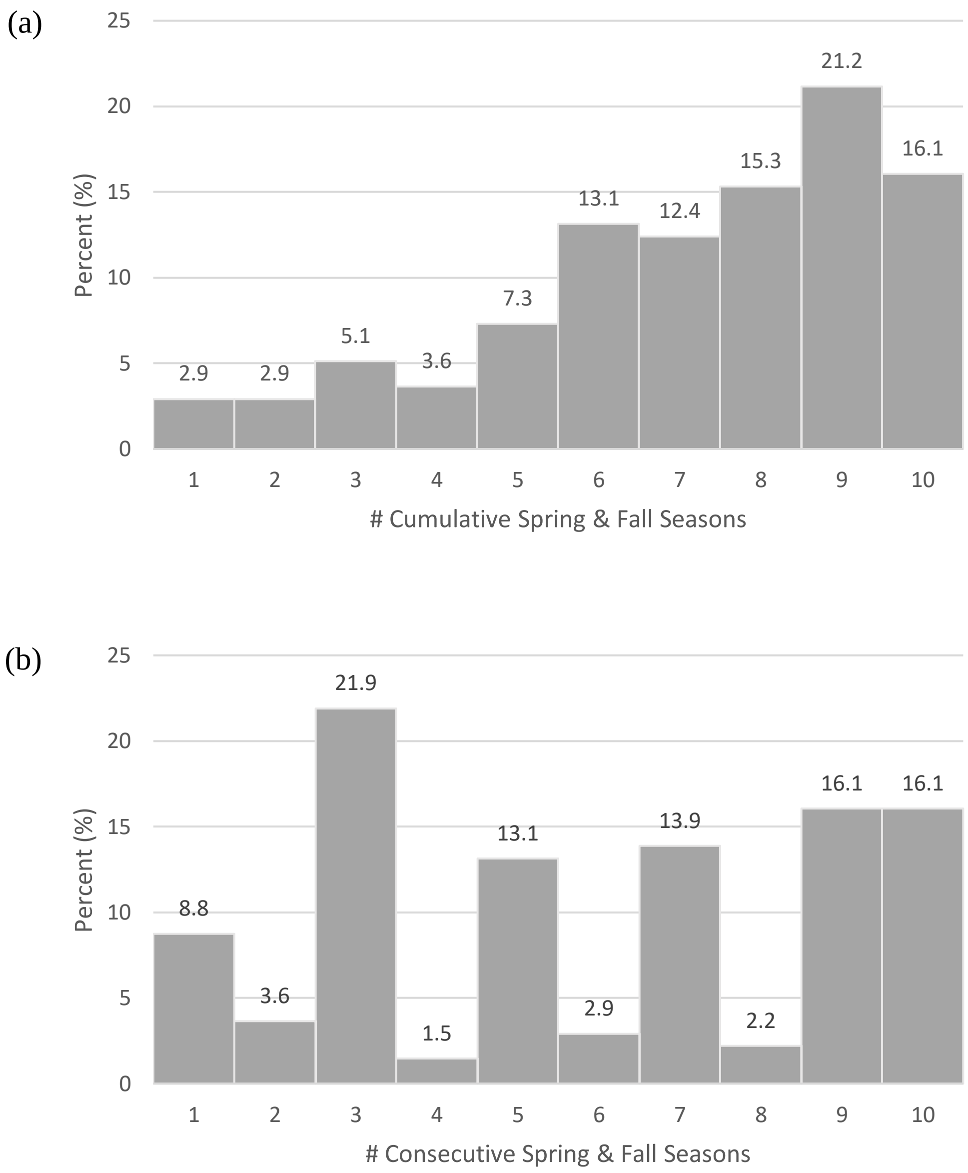

Figure 3.8. Percent of the sample population present for *x* number of (a) cumulative spring and fall seasons (mean = 7.15, median = 8) and (b) maximum consecutive spring and fall seasons (mean = 5.91, median = 6) from 2014 to 2018 (*n* = 137).

2018, but absences in falls 2014, 2015, and 2017 also occurred; surprisingly, 1 individual was only absent in spring 2014. Despite the poor turn-out in fall 2018, 16.1% of the sample population (22 plants) were aboveground as spring rosettes and inflorescence structures during the entire observation period.

Plants that had persisted from spring 2014 to fall 2018 were assessed more closely to determine whether or not any obvious effects had influenced their endurance. Four of the 22 plants were uncaged (18.2%) while 9 (40.9%) were caged and 9 (40.9%) were in the mesh treatment (caged with tulle fabric secured around), but these percentages were not significantly different ($LR\chi^2_2$ = 2.5235, p = 0.2832). A significantly greater number of plants from this group had not been treated with insecticide (17 plants) than those that had (5 plants; $LR\chi^2_1$ = 6.9162, p = 0.0085). Of the latter, 1 was uncaged and 4 were caged. Two of the 5 had generally been smaller in size compared to the rest, with shorter and more slender reproductive stalks. However, the other 3 insecticide-treated plants had been historically characterized by robust growth similar to the other 17 that had not been treated with insecticide. Additionally, habitat exposure of the 22 plants was marginally significant, by which 15 were located in open spaces, and 7 were moderately- to well-covered by shrub growth ($LR\chi^2_1$ = 2.9769, p = 0.0845). However, amongst all 137 plants, open habitat was significantly more common than covered habitat ($LR\chi^2_1$ = 19.4502, $p < 0.0001$).

Finally, assessment of consecutive presence patterns amongst both spring and fall seasons revealed a strong preference for plants to appear in odd numbers of sequential seasons (Fig. 3.8b). Single-season consecutive plants made up 8.8% of the sample population (12 plants) and presence ranged from 2014 to 2018 with 11 of the 12 plants only present in spring seasons; the twelfth individual was present every spring but only fall of 2018. Amongst these plants, the longest gap in presence followed by return aboveground was three years, from spring 2015 to spring 2018.

Three consecutive seasons of presence was the most prevalent, at 21.9% (30 plants); most of these plants experienced their consecutive seasons from spring 2014 to spring 2015, but several were also sequentially present from spring 2016 to spring 2017, often with presence again in spring 2018. Amongst this group, cumulative presence of six or seven seasons were most common but it ranged from three to eight seasons. Five-season consecutive presence was at 13.1% (18 plants); of these, cumulative seasons ranged from five to eight seasons, where seven and eight were most frequent. Six of these began their consecutive terms in spring 2014, 5 in spring 2015, and 7 in spring 2016; of the latter, 5 were also present from spring 2014 to spring 2015. Consecutive presence for seven seasons made up 13.9% (19 plants) and most often occurred from spring 2014 to spring 2017 with an additional spring 2018 presence. Nine-season consecutive presence was almost exclusively from spring 2014 to spring 2018 and made up 16.1% (22 plants) of the sample population; ten-season presence was also at 16.1%.

Growth and Weather

The demographic parameters such as plant height and leaf length changed annually, and it was suspected that precipitation and temperature contributed in part to the fluctuations. Annual fall and spring growth averages are presented in Table 3.3. For each fall parameter, there were significant differences between values and years (plant area, height, number of flowers: $\chi^2{}_3 > 54.8600$ and inflorescence length: $F_{3,346} = 28.0033$; $p < 0.0001$), but fall 2014 mean values were always statistically similar to those in either 2016 or 2017, depending on the variable (Steel-Dwass Z and Tukey-Kramer, $p > 0.1000$) (Table 3.3). Species values were only significantly different in fall 2016, where *S. cernua* mean plant height was greater than that of *S. parksii* (Wilcoxon $Z = -1.8001$, $p = 0.0719$). Spring parameters displayed similar variability ($\chi^2{}_3 > 36.6280$, $p < 0.0001$), but mean

Table 3.3. Fall and spring growth parameters means each year for all plants and each species. Values are means ± SE. Capital letters represent significant differences between years for all plant values, using Kruskal-Wallis tests. Lowercase letters indicate significant differences between means for species values, using Wilcoxon tests. * denotes $p < 0.10$.

Fall[a]

		PA (cm^2)	PH (cm)	IL (cm)	#F
2014	All	22.18 ± 0.97 **_A*_**	25.12 ± 0.65 **_A_**	6.48 ± 0.22 **_A_**	16.23 ± 0.71 **_A_**
	S. parksii	20.99 ± 1.74	24.35 ± 1.11	6.46 ± 0.37	16.27 ± 1.17
	S. cernua	22.67 ± 1.13	25.43 ± 0.72	6.48 ± 0.24	16.22 ± 0.75
2015	All	8.57 ± 2.24 **_B_**	11.44 ± 0.77 **_B_**	3.66 ± 0.26 **_B_**	9.93 ± 0.84 **_B_**
	S. parksii	8.14 ± 1.19	11.58 ± 1.37	3.00 ± 0.51	8.21 ± 1.75
	S. cernua	8.75 ± 0.78	11.37 ± 0.90	3.95 ± 0.33	10.66 ± 1.14
2016	All	26.43 ± 1.03 **_C*_**	26.67 ± 0.70 **_A_**	6.29 ± 0.24 **_A_**	19.27 ± 0.76 **_C_**
	S. parksii	23.87 ± 2.13	24.64 ± 1.23 **_a*_**	6.21 ± 0.40	19.67 ± 1.32
	S. cernua	27.56 ± 1.42	27.57 ± 0.81 **_b*_**	6.32 ± 0.27	19.09 ± 0.88
2017	All	20.56 ± 1.32 **_A_**	21.35 ± 0.89 **_C_**	4.94 ± 0.30 **_C_**	14.97 ± 0.97 **_A_**
	S. parksii	19.79 ± 2.86	19.72 ± 1.87	4.96 ± 0.62	15.32 ± 1.80
	S. cernua	20.92 ± 1.94	22.10 ± 1.27	4.93 ± 0.42	14.80 ± 1.23

Spring[b]

		LA (cm^2)	#L	LL (cm)	LW (cm)
2015	All	17.02 ± 1.12 **_A_**	3.83 ± 0.08 **_A*_**	12.71 ± 0.47 **_A_**	1.11 ± 0.03 **_AC_**
	S. parksii	14.93 ± 1.87	3.65 ± 0.13	11.40 ± 0.88	1.06 ± 0.05
	S. cernua	17.92 ± 1.24	3.90 ± 0.08	13.29 ± 0.58	1.13 ± 0.03
2016	All	21.55 ± 1.17 **_B_**	4.17 ± 0.08 **_B_**	12.68 ± 0.49 **_A_**	1.33 ± 0.03 **_B_**
	S. parksii	20.42 ± 2.42	4.15 ± 0.15	11.69 ± 0.86	1.35 ± 0.06
	S. cernua	22.09 ± 1.67	4.17 ± 0.10	13.15 ± 0.60	1.32 ± 0.04
2017	All	13.49 ± 1.23 **_C_**	3.54 ± 0.09 **_C*_**	8.57 ± 0.51 **_B_**	1.16 ± 0.03 **_A_**
	S. parksii	13.36 ± 2.04	3.50 ± 0.19	8.32 ± 0.86	1.24 ± 0.06
	S. cernua	13.55 ± 1.37	3.55 ± 0.12	8.68 ± 0.58	1.12 ± 0.04
2018	All	12.91 ± 1.28 **_C_**	3.42 ± 0.09 **_C_**	9.55 ± 0.53 **_B_**	1.01 ± 0.03 **_C_**
	S. parksii	15.99 ± 2.17 **_a_**	3.63 ± 0.15	11.16 ± 0.94 **_a_**	1.10 ± 0.05 **_a_**
	S. cernua	11.48 ± 1.48 **_b_**	3.32 ± 0.10	8.81 ± 0.64 **_b_**	0.97 ± 0.04 **_b_**

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers; *n* = 350.

[b] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width; *n* = 464.

values of leaf area, number of leaves, and leaf lengths showed insignificant differences between years 2017 and 2018 (Steel-Dwass Z, $p > 0.1000$). With the exception of the leaf length parameter, all mean values significantly increased from 2015 to 2016 (Steel-Dwass $|Z| > 2.5980$, $p < 0.0470$), then decreased in 2017 (Steel-Dwass $|Z| > 3.3840$, $p < 0.0050$) and continued to decrease to values below those of 2015 (Table 3.3). Species values only differed amongst 2018 spring parameters, where *S. parksii* mean leaf area, length, and width were all significantly greater than *S. cernua* (Wilcoxon $Z > 1.9700$, $p < 0.0500$).

Correlations between demographic parameters and weather variables were also assessed amongst the sample population and by species. According to Morrison et al. (2015), precipitation during previous life stages and growing seasons may produce a lag effect on growth, so rainfall from up to one year prior to each fall season was assessed. Precipitation sums and fall growth parameters exhibited mostly negative correlations, and there were no statistically significant positive correlations amongst all rainfall periods examined, nor significant correlations with rainfall from the year prior (Table 3.4). January rainfall exhibited strong negative correlations with all fall growth parameters, and January to March, June, and October rainfall sums displayed similar relationships, although not with all growth variables. Mean temperatures in August were significantly negatively correlated with plant area in the fall, so it is possible that hotter August temperatures may inhibit overall growth before or during emergence from summer dormancy.

In contrast, Spearman correlations between spring growth parameters and rainfall were mostly positive (Table 3.4). Rainfall from one spring prior (t-1 March) was positively correlated with rosette leaf width but no other growth parameters. Two time points within September through December prior to rosette season were positively related to all four growth parameters, but none were significant specifically to *S. parksii*. It is possible that *S. cernua* rosettes may respond more

Table 3.4. Spearman ρ correlations between fall and spring growth parameter means, summed precipitation, and mean temperatures. Only pairings with significant relations are shown. Bold numbers are statistically significant, and * indicates $p < 0.10$.

		∑ Rainfall				Mean Temperature
Fall[a]		Jan	Jan-Mar	Jun	Oct	Aug
PA	All	**-0.92***	-0.84	-0.70	**-0.95**	**-0.91***
	S. parksii	**-0.91***	-0.85	-0.69	**-0.96**	**-0.92***
	S. cernua	**-0.93***	-0.83	-0.70	**-0.95***	-0.90
PH	All	**-0.96**	**-0.91***	-0.79	**-0.90***	-0.85
	S. parksii	**-0.98**	**-0.94***	-0.84	-0.86	-0.80
	S. cernua	**-0.95**	-0.90	-0.77	**-0.92***	-0.87
IL	All	**-0.99**	**-0.97**	**-0.92***	-0.75	-0.68
	S. parksii	**-0.98**	**-0.97**	-0.87	-0.81	-0.75
	S. cernua	**-0.99**	**-0.97**	**-0.95***	-0.71	-0.63
#F	All	**-0.94***	-0.81	-0.73	**-0.91***	-0.86
	S. parksii	**-0.92***	-0.81	-0.70	**-0.94***	-0.90
	S. cernua	**-0.95***	-0.80	-0.75	-0.89	-0.83

Spring[b]		*t*-1 Mar	*t*-1 Sep-Oct	*t*-1 Nov-Dec	Jan-May	Feb	*t*-1 Sep	*t*-1 Nov	*t*-1 Dec
LA	All	0.70	**0.92***	**0.95**	0.80	-0.80	0.68	-0.70	0.84
	S. parksii	0.53	0.65	0.69	0.65	-0.53	0.60	-0.29	0.74
	S. cernua	0.71	**0.94***	**0.97**	0.79	-0.83	0.67	-0.77	0.82
#L	All	0.72	**0.93***	**0.97**	0.78	-0.81	0.69	-0.74	0.84
	S. parksii	0.65	0.75	0.82	0.69	-0.61	0.70	-0.41	0.83
	S. cernua	0.71	**0.93***	**0.97**	0.77	-0.83	0.65	-0.80	0.80
LL	All	0.26	**0.99**	0.72	**0.98**	**-0.99**	0.21	**-0.92***	0.44
	S. parksii	-0.14	0.71	0.31	0.89	-0.76	-0.10	-0.55	0.13
	S. cernua	0.35	**0.99**	0.79	**0.94***	**-0.99**	0.29	**-0.95**	0.50
LW	All	**0.97**	0.62	**0.95***	0.38	-0.42	**0.95**	-0.38	**0.99**
	S. parksii	**0.94***	0.22	0.70	-0.02	0.02	**0.98**	0.10	**0.92***
	S. cernua	**0.91***	0.75	**0.99**	0.52	-0.58	0.88	-0.56	**0.95**

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

[b] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width.

strongly to rainfall than *S. parksii*, or *S. parksii* rosette sizes varied too much over time and so did not yield significant correlations; the higher standard errors for all *S. parksii* measurements in Table 3.3 supports the latter. Correlations involving rainfall during the entire rosette season (January to May) were predicted, but only leaf length was positively associated and surprisingly, February rainfall was strongly and negatively correlated with leaf length. Similarly, warmer temperatures in September and December were positively related to greater leaf widths while cooler November weather was correlated with greater leaf length. Correlations with leaf width must be taken lightly, however, as mean leaf widths had narrow ranges.

Growth by Season

Correlations between demographic parameters of fall flowering plants and spring rosettes were examined to determine if rosette and flower stalk measurements were related and predictable. Only plants that flowered were included in fall parameters, as they were able to grow to their full potential. Rosette leaf area and fall plant area parameters are assessed in detail, as they exhibited the strongest correlations within each temporal comparison (see Appendix B for all results tables). Spring leaf area and fall plant area of the same years consistently exhibited moderately strong, positive correlations to each other (Spearman $\rho = 0.62 – 0.71$, $p < 0.0001$), in which 2016 and 2017 had the strongest and weakest correlations, respectively. Fall to spring areas were significantly correlated as well, although not as strongly (Spearman $\rho = 0.51 – 0.64$, $p \leq 0.0001$); fall 2016 to spring 2017 was the strongest, while fall 2015 to spring 2016 had the weakest correlation. Fall 2015 was characterized by few and stunted flower stalks, followed by plentiful and large growth all year in 2016, then small rosettes but moderately-sized flower stalks in 2017. Although the results are not pronounced, it may be suggested that rosette size has more influence on subsequent fall growth than vice versa. When fall parameters of consecutive years were assessed, correlations were weak (Spearman $\rho = 0.28 – 0.36$, $p < 0.06$) except for comparisons between 2016 and 2017 (Spearman $\rho = 0.64$, $p < 0.0001$), most likely because more of the same plants flowered in each of these years. Spring leaf areas of consecutive years displayed the strongest correlations (Spearman $\rho = 0.71 – 0.79$, $p < 0.0001$), which indicated that rosette size within individuals does not vary much from year to year.

In addition, comparisons were made to determine whether or not there were differences in rosette or flower stalk growth based on behavior in the previous season. In general, plants that had been reproductively absent in one fall had significantly smaller rosettes than those that had flowered, in terms of their leaf area, maximum number of leaves at one time, leaf length, and leaf width ($\chi^2{}_{2 \text{ or } 3} > 6.61$, $p < 0.09$; Steel-Dwass $|Z| > 2.42$, $p < 0.07$) (Table 3.5). Plants that had developed reproductive growth but did not reach anthesis due to desiccation or herbivory often exhibited rosette parameter means that were not significantly different from those that had flowered nor those absent. At times both the desiccated and herbivory groups had the greatest parameter means, but these were often non-significant due to small sample sizes with wide ranges in values.

Increments of herbivory were categorized based on the maximum percent damage inflicted upon individuals during the entire spring rosette season, and assessed to determine if any effects were passed on to fall growth capabilities (Table 3.6). Only flowering plants were included to ensure growth measurements were not skewed by herbivory or desiccation. Fall 2015 showed the only significant difference in means ($\chi^2{}_2 > 7.03$, $p < 0.03$), where less than or equal to 10% rosette herbivory yielded greater inflorescence lengths and numbers of flowers than rosettes that experienced between 51% and 100% herbivory (Steel-Dwass $|Z| > 2.34$, $p \leq 0.05$). Sample sizes

Table 3.5. Spring growth parameter means after controlling for physical status during the previous fall growing season. Previous fall categories of “Present, Desiccated” and “Present, Herbivory” indicate that reproductive growth was observed but plants were not able to develop mature flowers due to the respective ailments. Values are means ± SE. Different letters in each column represent significant differences in means, using Kruskal-Wallis and Steel-Dwass tests; * indicates $p < 0.10$, otherwise $\alpha = 0.05$.[a]

		Spring 2015				Spring 2016			
		LA (cm^2)	#L	LL (cm)	LW (cm)	LA (cm^2)	#L	LL (cm)	LW (cm)
Previous Fall	Absent	10.56 ± 2.62 ***A****	3.65 ± 0.18 ***A***	9.40 ± 1.23 ***A***	1.04 ± 0.07 ***A***	12.77 ± 2.14 ***A***	3.66 ± 0.13 ***A***	9.51 ± 0.75 ***A***	1.13 ± 0.05 ***A***
	Present, Flowered	18.13 ± 1.15 ***B***	3.84 ± 0.08 ***A***	13.33 ± 0.54 ***B***	1.11 ± 0.03 ***A***	27.64 ± 1.92 ***B***	4.43 ± 0.12 ***B***	15.39 ± 0.68 ***B***	1.40 ± 0.05 ***B***
	Present, Desiccated	18.52 ± 5.85 ***AB***	3.75 ± 0.41 ***A***	14.83 ± 2.74 ***AB***	1.18 ± 0.16 ***A***	22.72 ± 3.33 ***B***	4.24 ± 0.21 ***AB***	11.85 ± 1.17 ***A***	1.40 ± 0.08 ***B***
	Present, Herbivory	18.72 ± 5.85 ***AB***	4.50 ± 0.41 ***A***	11.30 ± 2.74 ***AB***	1.33 ± 0.16 ***A***	24.01 ± 3.96 ***B***	4.67 ± 0.25 ***B***	13.16 ± 1.39 ***AB***	1.62 ± 0.10 ***B***
		Spring 2017				Spring 2018			
		LA (cm^2)	#L	LL (cm)	LW (cm)	LA (cm^2)	#L	LL (cm)	LW (cm)
Previous Fall	Absent	2.85 ± 2.86 ***A***	2.47 ± 0.26 ***A***	2.54 ± 1.14 ***A***	0.74 ± 0.09 ***A***	7.49 ± 1.75 ***A***	3.05 ± 0.13 ***A***	7.00 ± 0.74 ***A***	0.84 ± 0.04 ***A***
	Present, Flowered	15.45 ± 1.15 ***B***	3.71 ± 0.10 ***B***	9.59 ± 0.46 ***B***	1.22 ± 0.04 ***B***	17.90 ± 1.59 ***B***	3.71 ± 0.11 ***B***	11.88 ± 0.68 ***B***	1.15 ± 0.04 ***B***
	Present, Desiccated					9.91 ± 8.09 ***AB***	4.00 ± 0.58 ***AB***	8.15 ± 3.45 ***AB***	1.10 ± 0.19 ***AB***
	Present, Herbivory	6.75 ± 6.39 ***AB***	3.67 ± 0.58 ***AB***	7.53 ± 2.55 ***AB***	1.30 ± 0.19 ***AB***	7.89 ± 5.72 ***AB***	3.25 ± 0.41 ***AB***	7.45 ± 2.44 ***AB***	1.03 ± 0.13 ***AB***

[a] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width.

Table 3.6. Fall growth parameter means after controlling for physical status during the previous spring growing season. Previous spring presence categories are followed by maximum percent of total herbivory observed across a rosette. Values are means ± SE. Different letters in each column represent significant differences in means, using Kruskal-Wallis and Steel-Dwass tests; * indicates $p < 0.10$, otherwise $\alpha = 0.05$.[a]

		Fall 2014				Fall 2015			
		PA (cm$_2$)	PH (cm)	IL (cm)	#F	PA (cm$_2$)	PH (cm)	IL (cm)	#F
Previous Spring	Absent	22.81 ± 3.63 ***A***	26.21 ± 1.80 ***A***	6.89 ± 0.61 ***A***	17.57 ± 1.92 ***A***				
	Present, ≤ 10%	19.06 ± 3.40 ***A***	23.83 ± 1.68 ***A***	6.28 ± 0.57 ***A***	16.00 ± 1.79 ***A***	12.42 ± 0.91 ***A***	16.09 ± 0.81 ***A***	5.79 ± 0.32 ***A***	17.08 ± 0.96 ***A***
	Present, ≤ 50%	28.06 ± 2.05 ***A***	28.59 ± 1.01 ***A***	7.48 ± 0.35 ***A***	19.27 ± 1.08 ***A***	11.28 ± 1.16 ***A***	14.69 ± 1.03 ***A***	4.88 ± 0.41 ***AB***	15.19 ± 1.22 ***AB***
	Present, ≤ 100%	22.64 ± 1.58 ***A***	25.82 ± 0.78 ***A***	6.64 ± 0.27 ***A***	17.00 ± 0.83 ***A***	9.90 ± 1.55 ***A***	13.29 ± 1.37 ***A***	4.07 ± 0.55 ***B***	11.89 ± 1.63 ***B***

		Fall 2016				Fall 2017			
		PA (cm$_2$)	PH (cm)	IL (cm)	#F	PA (cm$_2$)	PH (cm)	IL (cm)	#F
Previous Spring	Absent	27.38 ± 8.13 ***A***	28.55 ± 4.58 ***A***	6.80 ± 1.42 ***A***	22.00 ± 4.57 ***A***				
	Present, ≤ 10%	27.54 ± 1.56 ***A***	27.69 ± 0.88 ***A***	6.50 ± 0.27 ***A***	19.76 ± 0.88 ***A***	24.52 ± 2.71 ***A***	25.24 ± 1.44 ***A***	5.93 ± 0.54 ***A***	16.39 ± 1.51 ***A***
	Present, ≤ 50%	28.19 ± 2.35 ***A***	27.32 ± 1.32 ***A***	6.92 ± 0.41 ***A***	21.08 ± 1.32 ***A***	19.38 ± 2.71 ***A***	20.89 ± 1.44 ***A***	4.91 ± 0.54 ***A***	16.89 ± 1.51 ***A***
	Present, ≤ 100%	21.62 ± 2.97 ***A***	23.73 ± 1.67 ***A***	5.29 ± 0.52 ***A***	18.07 ± 1.67 ***A***	23.49 ± 2.71 ***A***	23.30 ± 1.44 ***A***	5.51 ± 0.54 ***A***	16.61 ± 1.51 ***A***

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

varied widely across Tables 3.5 and 3.6, however, and samples amongst categories in each season were rarely equivalent and often quite small (i.e. $n < 10$).

Seedlings and Juveniles

At the time the investigation was initiated in April 2017, seedling counts were the highest seen in this study, at 369 observed (Fig. 3.9a). Additionally at this time, the mean number of seedlings per mature plant was significantly greater than that of juveniles (Wilcoxon $Z = 6.3425$, $p < 0.0001$) (Fig. 3.9b). By May 2017, seedlings and juveniles were present in approximately the same amounts and persisted this way until September and October, when juveniles were more abundant and had greater mean counts per mature plant than seedlings (Wilcoxon $Z = -26.9980$, $p < 0.0001$). December 2017 counts were equivalent, but from January through March 2018 juveniles were again present in greater numbers and exhibited greater mean counts per plant (Wilcoxon $Z < -3.17$, $p < 0.002$). In 2017, seedling numbers tapered off during May and June before disappearing in early July, whereas in early May 2018 counts reached 91 before dropping to just 2 on May 31st.

Seedling and juvenile summed censuses and counts per mature plant were also assessed by the herbivory study treatment received by the established individual, to determine if cages or mesh tulle allowed more immature plants to prosper for longer. Total seedling counts in April 2017 were notably highest in the Uncaged Treated cohort at a peak of 138; Mesh exhibited 90 seedlings, and Uncaged Untreated had 85 at its greatest April observation, and both caged cohorts were equivalent with peaks of 36 and 38 seedlings (Fig. 3.9a). Despite what appears to be distinguished differences between seedling sums, Kruskal-Wallis tests did not yield statistically significant results for mean seedling counts per plant across treatments in April 2017 ($p > 0.16$). Mean maximum seedling counts per plant and treatment in May 2017 was marginally significant, but Steel-Dwass tests did

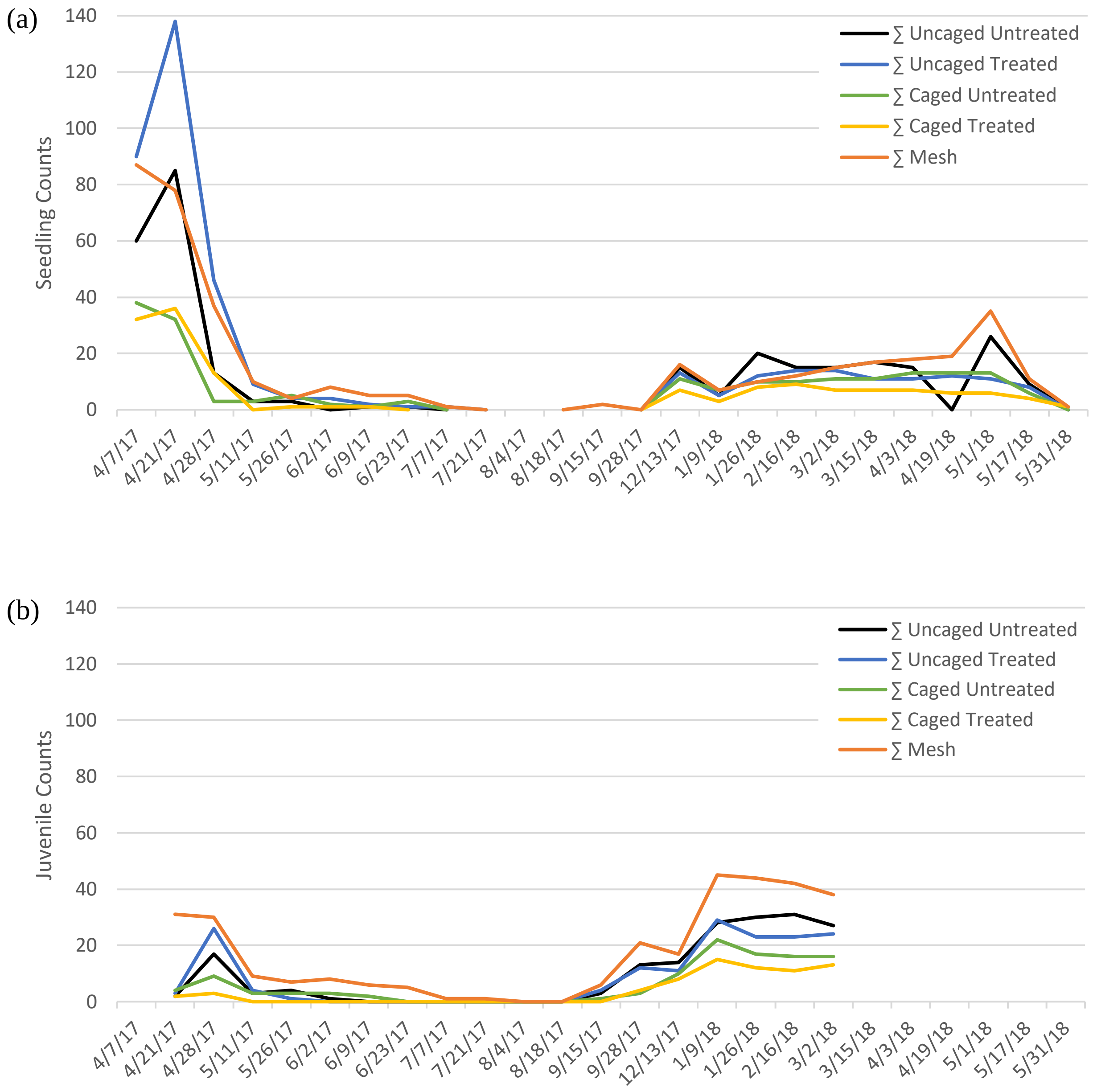

Figure 3.9. Total seedling (a) and juvenile (b) counts across all individuals from the herbivory study, summed by treatment and observation date.

not produce any significant means comparisons ($p > 0.10$). Mesh plants maintained the maximum seedling counts for almost the entire seedling investigation, and were only overtaken briefly in late January and early February 2018 by uncaged seedling counts.

Juveniles displayed the largest differences in summed counts per treatment from January through March 2018, when Mesh plants upheld the greatest numbers, followed by uncaged then caged treatments (Fig. 3.9b). This pattern of treatments appeared to be consistent all the way back to the investigation initiation. Interestingly, April 2017 procured the only statistically meaningful differences amongst mean counts of juveniles per mature plant ($\chi^2{}_4 = 16.6148$, $p = 0.0023$). At this time, plants in the Mesh cohort had significantly more seedlings per mature plant than both caged treatment groups (Steel-Dwass $Z > 2.88$, $p < 0.10$), and the Uncaged Treated cohort also had more juveniles per plant than Caged Treated (Steel-Dwass $Z = 2.6922$, $p = 0.0551$).

Analyses comparing precipitation and seedling appearance were limited by data from this study, but total seedling counts were available in both April and May of 2017 and 2018 and so precipitation effects were only assessed for these months. No statistical tests could be performed as maximum April and May counts each provided two samples. Despite the lack of tests, visual interpretation of the data suggests that summed monthly rainfall has an effect on maximum seedling counts in these months (Fig. 3.10). In April 2017, precipitation was just above the 30-year average of 6.81 cm and seedling counts were very high; in April 2018, precipitation was below the average and seedling presence was much lower. The 30-year average precipitation in May is 11.00 cm and while 2017 and 2018 rainfall values were both slightly lower, in May 2017 precipitation and seedling counts were lower than those seen in May 2018. More data points are required to determine any statistical significance here, but these findings present an avenue for further research.

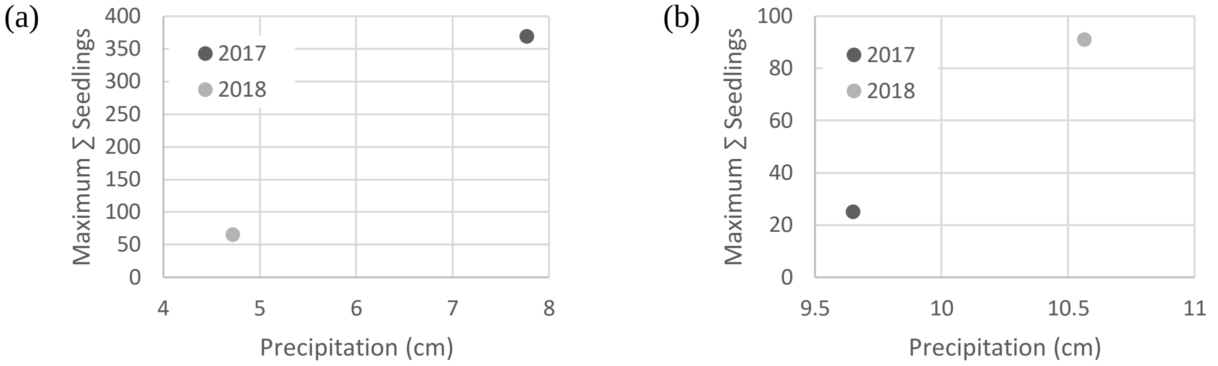

Figure 3.10. Summed April (a) and May (b) rainfall and maximum seedling counts in 2017 and 2018.

Seedling age determination is fairly straightforward shortly after germination when they become photosynthetic and consist of the protocorm and one to two minute leaves (each about 0.3 cm long), typically in late January and early February (Ariza 2013). Over the next three months, the mycorhizome grows down into the soil, the plant develops up to three leaves each averaging about 3.0 cm in length, and visual distinction between seedling, juvenile, and small but mature rosettes becomes difficult without seeing the root structure and number of tubers (Ariza 2013). Based on research by Ariza (2013), it was presumed that rosettes that fit the seedling description were likely borne from one to two flower seasons prior while those in the juvenile category were more likely to have been from two to four seasons prior. In addition, while most seeds land very close to the mother plant as evidenced by higher amounts of seedlings within only centimeters of mature plants, longer dispersal distances of up to several meters are possible (Ariza 2013). Therefore, when several mature plants with different flowering histories are clustered, determination of which mother plant bore specific seedlings or juveniles is impossible and can only be surmised.

To speculate the accuracy of rosette age estimations in this study, the numbers and dates of chronicled seedlings and juveniles at every plant in the herbivory study were compared with flowering records in the previous study years, 2014 through 2017. If plants flowered, it was presumed that they had set seed. Mean seedling counts in April 2017 were significantly higher for plants that had flowered in fall 2016 than those that had not flowered (Wilcoxon $Z = 2.1793$, $p = 0.0293$) (Table 3.7). Similarly, flowering plants in fall 2017 had significantly greater seedling count means in December 2017 through May 2018 (Wilcoxon $Z > 2.11$, $p < 0.04$; February 2018 was marginal: Wilcoxon $Z = 1.8591$, $p < 0.0630$). Seedling counts in December 2017 and January and May 2018 were also associated with fall 2016 flowering (Wilcoxon $Z > 1.64$, $p < 0.10$).

Analyses of juveniles' relations to flowering histories did not match as well with descriptions by Ariza (2013) (Table 3.7). April 2017 mean juvenile counts were marginally and significantly greater around plants that flowered in 2015 and 2016, respectively (2015: Wilcoxon $Z = 1.7681$, $p = 0.0770$; 2016: Wilcoxon $Z = 2.4148$, $p = 0.0157$), while May 2017 juvenile means were greater for plants that did not flower in 2015 (Wilcoxon $Z = -2.4659$, $p < 0.0137$). Juvenile counts from December 2017 through March 2018 had no significant relations to flowering in 2014 nor 2015, and instead presented evidence of associations to plants that flowered in 2016 and 2017 (Wilcoxon $Z > 2.14$, $p < 0.04$; March 2018 and 2016 flowering was marginal: Wilcoxon $Z = 1.9307$, $p = 0.0535$).

Discussion

Terrestrial orchid demographic studies are essential for better understanding behavior and requirements over time, especially in endangered species. Tamm (1972) pioneered such studies, and now Orchidaceae has attracted more demographic research than any other plant family

Table 3.7. Mean seedling and juvenile counts per mature plant, by flowering history (flowered, did not flower) in the respective year. Values represent means ± SE. Only pairings with at least one significant relation are shown. Wilcoxon Signed Rank was used to test for differences. Different letters in a column indicate significant differences, and * denotes $p < 0.10$. Fall 2017 flowering could not have produced seedlings/juveniles in April or May 2017, so means were not tested.

Seedlings		2016	2017
Apr 2017	Flowered	4.08 ± 0.49 **A**	
	Non-flowering	2.00 ± 0.74 **B**	
Dec 2017	Flowered	0.60 ± 0.12 **A**	0.85 ± 0.16 **A**
	Non-flowering	0.12 ± 0.18 **B**	0.19 ± 0.13 **B**
Jan 2018	Flowered	0.61 ± 0.12 **A***	0.81 ± 0.16 **A**
	Non-flowering	0.14 ± 0.19 **B***	0.24 ± 0.13 **B**
Feb 2018	Flowered	0.55 ± 0.13 **A**	0.78 ± 0.16 **A***
	Non-flowering	0.19 ± 0.19 **A**	0.22 ± 0.13 **B***
Mar 2018	Flowered	0.62 ± 0.14 **A**	0.87 ±0.18 **A**
	Non-flowering	0.19 ± 0.21 **A**	0.24 ± 0.15 **B**
Apr 2018	Flowered	0.65 ± 0.15 **A**	0.87 ± 0.20 **A**
	Non-flowering	0.17 ± 0.23 **A**	0.27 ± 0.16 **B**
May 2018	Flowered	0.88 ± 0.24 **A***	1.29 ± 0.31 **A**
	Non-flowering	0.19 ± 0.36 **B***	0.27 ± 0.25 **B**

Juveniles		2015	2016	2017
Apr 2017	Flowered	1.04 ± 0.21 **A***	1.00 ± 0.15 **A**	
	Non-flowering	0.65 ± 0.16 **B***	0.33 ± 0.23 **B**	
May 2017	Flowered	0.02 ± 0.08 **A**	0.16 ± 0.06 **A**	
	Non-flowering	0.27 ± 0.07 **B**	0.21 ± 0.09 **A**	
Dec 2017	Flowered	0.49 ± 0.13 **A**	0.56 ± 0.09 **A**	0.69 ± 0.13 **A**
	Non-flowering	0.41 ± 0.10 **A**	0.17 ± 0.14 **B**	0.28 ± 0.10 **B**
Jan 2018	Flowered	1.41 ± 0.25 **A**	1.49 ± 0.18 **A**	1.94 ± 0.23 **A**
	Non-flowering	1.06 ± 0.20 **A**	0.50 ± 0.27 **B**	0.70 ± 0.19 **B**
Feb 2018	Flowered	1.06 ± 0.23 **A**	1.13 ± 0.16 **A**	1.69 ± 0.20 **A**
	Non-flowering	0.80 ± 0.18 **A**	0.38 ± 0.25 **B**	0.39 ± 0.16 **B**
Mar 2018	Flowered	1.06 ± 0.22 **A**	1.07 ± 0.16 **A***	1.56 ± 0.20 **A**
	Non-flowering	0.74 ± 0.17 **A**	0.38 ± 0.24 **B***	0.41 ± 0.16 **B**

(Hutchings 2010). Despite countless studies and publications, many aspects of orchid population dynamics are still poorly understood.

Spiranthes parksii populations have declined in range and abundance due to habitat destruction and unsuitable land management, amongst other reasons (USFWS 2009), so appropriate management and an understanding of demographic responses to climatic and temporal variables are vital for their conservation. Like many terrestrial orchids, *S. parksii* population size is believed to be highly variable over time (Tamm 1972; Jacquemyn & Hutchings 2010), in part due to the phenomenon of periodic vegetative dormancy (Hutchings 1987; Shefferson et al. 2003). Relatively large proportions of annual populations may be either dormant or vegetative during the flowering seasons (Morrison et al. 2015), when census counts and identification of new recruits is most feasible. This creates a bias in population monitoring efforts, by which population size estimates are much lower than the true values (Ariza 2013). In addition, climate change may influence organism life history traits in a way that alters population dynamics, such as shifts in distributions, flowering time, and developmental schedules (Shefferson et al. 2017). A recommended solution is to frequently monitor populations throughout growing seasons over multiple years, to distinguish true absence from late emergence or prolonged vegetative dormancy (Hutchings 1991; Tremblay & Hutchings 2003) and to determine effects of climate. In this study, presence and absence data was assessed to determine the incidence of vegetative dormancy, demographic fluctuations, and influence of weather on a sample population of *S. parksii* and *S. cernua* over time.

The spring and fall census counts in the sample population declined steadily from spring 2014 to spring 2018 (Fig. 3.3), and agreed somewhat with the inconsistent presence seen in previous *S. parksii* observations (USFWS 1993; Hammons 2008). In a previous study, two *S.*

parksii cohorts were each followed for five years. Most plants flowered once or twice in nonconsecutive years during this time and spring rosette appearance declined progressively (USFWS 1993). Hammons (2008) found irregular *S. parskii* appearances across two years, where 18 individuals flowered in 2006 and 118 in 2007, with only one plant flowering in both years.

In addition, Hammons (2008) found that rainfall in August was highly correlated to flowering presence in the respective year (R_2 = 0.92, p = 0.0360), which agreed with previous suggestions that rainfall during the eight to ten weeks prior to flowering is critical for reproductive success in *S. parksii* (Parker 2001). While *S. parksii* presence in spring and fall is supposedly directly correlated with rainfall levels above or below seasonal averages (Wilson 2006), data analyzed here did not agree. Not only did August precipitation not correlate with reproductive presence, but spring and fall presence did not follow spring and fall precipitation. However, the occurrence of Hurricane Harvey in August 2017 may require treatment as an outlier deserving exclusion in analyses, but due to the brevity of this study and increasing unpredictability of weather, the data point was not excluded. It is possible that mortality caused the steady decline in annual presence rather than precipitation, and perhaps counts of new recruits each year would better reflect the effects of precipitation.

Spiranthes parksii fall presence and precipitation from up to one year prior was positively associated (Table 3.1), and this phenomenon has also been described in other species (Miller et al. 2004; Pfeifer et al. 2006; Morrison et al. 2015; Tenhumberg et al. 2018). Rainfall during the reproductive state may allow more plentiful root development after tuber exhaustion from inflorescence development and anthesis, when new tubers begin to the replace the old (Ariza 2013), and may allow flower stalks in the next season to be more efficiently equipped. Rainfall one fall prior may also have a greater effect on presence than precipitation during a given year as plants are crucially dependent upon their tubers and mycorrhizae at various time points of brief or prolonged dormancy throughout a year (McCormick et al. 2018). However, increased spring precipitation (January through March) showed negative relations to fall presence. Some plants experience decreased mycorrhization after heavy rain events waterlog soil (Walter 2018), and only January through March precipitation during 2015 and 2018 were above the 30-year average by 6.78 cm and 13.03 cm, respectively. While these years did procure lower than expected aboveground fall occurrences, they also exhibited lower than average rainfall in the summer and August, so it is possible that a combination of biotic and abiotic interactions led to the observed results. Only year 2018 exhibited both May and May through July mean temperatures above the 30-year average, but May through July means steadily increased from 2014 to 2018, contrasting with the steady decrease in fall presence proportions.

Spring presence proportions exhibited various unsurprising correlations with fall months of precipitation, but *t*-1 August yielded a negative correlation whilst *t*-1 September yielded a positive correlation (Table 3.1). The very low precipitation in August 2013, 2014, and 2015 (0.76 cm, 3.84 cm, and 0.76 cm, respectively) and very high precipitation in August 2016 and 2017 (24.0 cm and 63.02 cm, respectively) contrasted sharply with the decreasing spring rosette presence over the years, creating a negative correlation, and February precipitation followed a similar pattern. It was predicted that February rainfall would be positively correlated with spring presence as March typically presents the highest counts, so perhaps more years of data would provide different results. September, September through October, and November precipitation from 2013 to 2017 generally increased slightly into 2014 then gradually declined, almost perfectly mirroring percent rosette presence. These results coincide with tuber development described by Ariza (2013), in which new roots appear six to eight weeks before rosettes arise from nonflowering individuals in early

November, and appear just after anthesis in flowering plants. In *t*-1 November, mean temperatures rose from 18.7 ºC to 24.6 ºC (2013 to 2017) and in March from 19.8 ºC (2014) to a high of 24.9 ºC in 2017 before dropping to 24.5 ºC in 2018, each with high ranges more than 2.0 ºC greater than the 30-year averages. It is suggested that current and future annual recruits of *S. parksii* be followed closely for longer periods of time than presented here, as imminent climate change and warmer weather may begin to suppress seasonal rosette occurrence, such as has been observed in other species (Davis et al. 2019).

Knowledge of *S. parksii* lifespan is crucial for tracking and predicting population presence over time. If percent rosette and flowering plant presence continue at the current rates, the sample population will disappear by year 2022 (Fig. 3.4). These plants were selected with the presumption that they were all or mostly new recruits (Nally 2016), although age cannot be confidently inferred based on rosette or flower stalk size (Ariza 2013). By continuing the presumption, however, the data yields an aboveground lifespan of about 9 years and a total lifespan of 11 to 12 years, when accounting for germination to protocorm and mycorhizome stages (Hammons 2018), which agrees with suggestions of a 12 year maximum lifespan (Ariza 2013). Only a few individuals would reach the maximum span due to naturally-occurring population depletion, which, according to Ariza (2013), exhibits a constant mortality rate of 15% for *S. parksii* and 21% for *S. cernua* once plants reach maturity (after the mycorhizome stage). Ariza (2013) found no significant differences between mean half-lives of each species, but 50% of both *S. parksii* and *S. cernua* were expected to have shorter lifespans of six to seven years since establishment.

Earlier work by Willems and Dorland (2000) found in *Spiranthes spiranthes* that high costs of flowering commonly lead to a tendency for flowering plants to remain vegetative or become dormant in the following year (Shefferson et al. 2018), although results here contrast and indicate that 60.2% or more flowered at least two years in a row from 2014 to 2017 (Fig. 3.5a). This number dropped to 46.7% between 2017 and 2018, but flowering presence in 2018 was possibly higher than observed. These outcomes coincide with previous findings in other species that flowering in lady's slipper orchids *Cypripedium parviflorum*, *C. candidum*, and early spider orchid *Ophrys sphegodes* led to a greater likelihood of flowering in the following year than non-fruiting plants (Hutchings 1987; Shefferson et al. 2003; Shefferson & Simms 2007). Spring presence remained fairly consistent year to year, with a mean of 90.9% of plants returning each year from 2014 to 2018 despite the population decline witnessed in this study and others (USFWS 1993) (Fig. 3.5b). The percent of plants present as rosettes in a given spring that returned aboveground to flower in the fall varied greatly, ranging from 34.7% in 2018 to 80.7% in 2014 (mean of 62.0%), while almost all plants (mean of 98.1%) that flowered returned as rosettes the next spring (Fig. 3.5c). Additionally, despite the added visibility and vulnerability to herbivores that flower stalks impose, subsequent spring presence in all plants with reproductive growth, whether or not they reached anthesis, remained consistently high and provided no evidence of costs to demographics (Shefferson et al. 2003).

In contrast to the USFWS (1993) study that recorded individuals flowering only once or twice across a five-year period and findings by Ariza (2013) that three or more consecutive flowering events occurred in less than 5% of observed plants, over 50% of the *S. parksii* and *S. cernua* individuals in the present study flowered in three or more cumulative and consecutive years from 2014 to 2018 (Fig. 3.6). Similarly, Antlfinger and Wendel (1997) reported that almost 50% of studied *S. cernua* flowered in five of nine years. Ariza (2013) also suggested that *S. cernua* flowered more frequently than *S. parksii* individuals, but here there were no significant differences between species for fall presence each year, and more *S. parksii* (20.9%) flowered in all five years than *S. cernua* (14.9%), although results were not significantly different for this statistic nor any of the cumulative and consecutive seasonal presence analyses. As flowering is influenced by genetics and environmental conditions (García et al. 2010; Juárez et al. 2014), the number of plants that flower in a given year is subject to constraints by biotic and abiotic factors as well as life history stages (Kindlmann & Balounová 2001), so variations between study results do not negate the findings of another; rather, longer term data should be compiled and assessed to determine potential causes for variations (Knapp & Wiegand 2014).

Correlations between demographic parameters of different seasons were examined but showed no consistent results. Significant positive correlations were found between rosette and flower stalk characteristics and between fall behavior and subsequent spring measurements, although correlations were strongest for spring to fall parameters, similar to prior research (Ariza 2013). This suggests that rosette size may have more influence on fall growth than flower stalk size has on rosettes, or that greater spring growth allows more nutrient acquisition and tuber expansion for larger reproductive structures, while post-flowering rosette sizes decline as a cost of reproduction (Antlfinger & Wendel 1997). It is possible that stronger rosette to flowering correlations emulate the critical size threshold required for flowering that is described for several species of terrestrial orchids (Antlfinger & Wendel 1997; Willems & Dorland 2000; Kindlmann & Balounová 2001; Mróz & Kosiba 2011; Ariza 2013); a threshold rosette size may be required for flowering, and rosette size may diminish post-flowering as a cost of reproduction (Ariza 2013).

Besides the unquestionable distinction in flower morphology, differences in demographic characteristics and population dynamics between species were not common. *Spiranthes parksii* and *S. cernua* were present in similar ratios in each season of each year, and the number of seasons each were present from 2014 to 2018 were not significantly different, which disagrees with Ariza's

(2013) suggestion that *S. cernua* individuals flowered more frequently. Morphologies were assessed by year, and dissimilarities were not common. While *S. parksii* exhibited shorter flower stalks in 2016, there were no differences in plant height means nor any other fall parameters amongst the other years (Table 3.3). Likewise, *S. parksii* mean rosette leaf area, length, and width were greater, longer, and wider in 2018 when compared with *S. cernua*, but no other time points or characteristics were significantly different (Table 3.3). On the other hand, Ariza (2013) found that *S. parksii* had longer mean rosette leaf lengths and fewer leaves per rosette. It is curious that *S. parksii* in this study only exhibited greater rosette morphology means in 2018 rather than over the years, but *S. spiralis* has been documented as having larger rosettes (more leaves and surface area) as the population size decreased (Jacquemyn et al. 2007). Additionally, environmental influences and herbivory can yield wide variations in sizes each season (Méndez & Karlsson 2004; Mróz & Kosiba 2011).

Like plant presence behavior, growth parameters are also governed by an individual's genetics and responses to weather, amongst other factors (Pfeifer et al. 2006; Jacquemyn & Hutchings 2010). In 2013, Ariza (2013) recorded average plant heights of 25.1 cm with August and September precipitation at 12.6 cm; in 2014 and 2016, plant heights ranged from 25.1 to 26.7 cm with precipitation of 17.2 and 25.0 cm. Heights varied significantly amongst 2015 and 2017 but remained significantly lower than those previously stated, yet rainfall varied from 3.2 to 63.3 cm, respectively. Nally (2016) suggested the existence of a low precipitation threshold between 3.2 and 12.6 cm that influenced plant heights, but the addition of 2017 data indicates that there is also a high precipitation threshold somewhere above 25.0 cm that also limits height and number of flowers (Table 3.3). With increasingly erratic weather behavior of drought and hurricanes, *Spiranthes* are expected to encounter these thresholds more frequently and thus may produce less flowers.

In this study, both *S. parksii* and *S. cernua* flowering morphologies displayed negative associations with rainfall in the spring, June, and October of year *t* (Table 3.4). In contrast, Wells and Willems (1991) and Ariza (2013) found that inflorescence lengths and number of flowers were directly related to abundant precipitation. Associations between greater flower stalk morphologies and drier weather may reflect adaptations to drier habitats, but rainfall is possibly also correlated with a number of influences on and factors that regulate plant growth, such as nutrient supply, soil moisture, humidity, and light intensity (Longino 1986). However, rosette characteristics exhibited mostly positive relations to past and current rainfall positive correlations (Table 3.4), which supports the idea that environmental factors strongly influence growth in the phenological stage that acquires and stores nutrients for reproduction (Ariza 2013). Warmer August and November temperatures resulted in smaller flowering plant areas in *S. parksii* and shorter rosette leaf lengths, respectively, indicating a potential threat by climate change that may influence increased herbivory and senescence with rising temperatures. Increasing temperatures have also been reported to decrease the number of flowers and fruits in orchids *Tipularia discolor* and *Dendrobium* 'Jaquelyn Thomas,' and slow rosette growth rates by about 60% (Paull et al. 1995; Marchin et al. 2014).

Understanding plant behavior from one season to the next is important for predicting population behavior, especially in rare endemic species that are vulnerable to changes in their unique habitats (Swarts & Dixon 2009). Spring growth in *S. parksii* and *S. cernua* was strongly affected by plant behavior in the fall, while spring behavior and herbivory had little effect on subsequent flower stalk morphologies except in years of extreme heat and little precipitation (Tables 3.5, 3.6). Similarly, Hammons (2008) suggested that rosettes are larger when a plant is preparing to flower in the subsequent fall. Kindlmann and Balounová (2001) state that plants' decision to flower, be sterile (vegetative), or dormant is made late in the preceding fall or just before emergence in the current fall, with reserve size or tuber development and environmental conditions as major factors. If only energy budget is considered, then it would be presumed that plants absent in the spring will not flower in the fall. While several plants assessed here did return to flower in the fall after spring dormancy, the majority did not, so energy partitioning in these species is possibly an important predictor in individual behavior.

The endangered status of *S. parksii* and the constant change to its environment in the form of uncontrolled woody growth highlight the need to investigate seedling behavior so that re-introduction programs can be more efficient. In addition, seedling tracking is important for determining variations in annual recruitment rates and thus population trends over time, knowledge that can be useful for restoration and conservation strategies. Ariza (2013) determined that seeds that germinate promptly after dispersal have the greatest chances of developing a photosynthetic rosette and perennial rhizome before summer, when dormancy is standard. The window for seed viability is brief, as well. Peak viability (90% or greater) lasts from dispersal to approximately May, less than 50% are viable by July, and almost none are viable by the following January (Ariza 2013). Furthermore, less than 2% and 6% of seeds followed in *in situ* and *ex situ* experiments, respectively, were able to develop fully into seedling rosettes (Ariza 2013).

This study assessed seedlings and juveniles that had already germinated and established themselves photosynthetically by April 2017. The peak in seedling counts immediately after the study initiation could be attributed to the steady rainfall that occurred from January to May 2017, which supports previously discovered correlations between germination and soil moisture (Ariza 2013). Temperature may also explain why seedlings counts tapered off slowly before summer

2017 whereas counts dropped abruptly in May 2018; May 2017 high temperatures averaged at 29.7 ºC while one year later, the average was 31.7 ºC. Ariza (2013) found no significant correlations between germination and temperature, but did not assess temperature relations to established seedlings. At only one point in the study were mean counts around mature plants significantly different by treatment, but within both seedling and juvenile observations, those within Mesh cages were the most abundant while caged and Uncaged Treated plants tended to have the least amount of recruits. It is possible that the mesh fabric created a favorable microclimate for young plants while insecticide spray and cages had inhibitory effects.

Based on estimations of possible seedling and juvenile ages from records of past flowering, it appeared that the youngest recruits were less than one year old while juveniles seemed to have been dispersed about 1.5 years prior to their observations. These analyses are possibly fairly accurate as Ariza (2013) determined that leaf primordium can be visible from protocorms as early as one month after seed dispersal, and leaves can become photosynthetic by the second month. Ames (1921) stated that in New England, *S. cernua* seedlings typically appear in the fall but the annual life cycle of *S. cernua* in this area is also shorter than that of the species in Texas. Here, *S. parksii* and *S. cernua* have longer annual life cycles due to the hot, dry summer months that halt vegetative growth. Therefore, rapid germination and quick development of rosettes and tubers appear to be adaptations to the region that allow establishment prior to summer dormancy (Ariza 2013). In this study, the estimated years from which seedlings and juveniles were presumed to have been borne from were perhaps correct as the mother plant flowering occurred within the timelines suggested by Ariza (2013). However, this study highlights the need to monitor seedlings by mark-recapture methods rather than just counts during each observation period, and to select solitary seed-bearing mature plants so that proposed seedling origins are less ambiguous.

Yearly seedling survival and recruitment into a population is an important aspect to population dynamics and conservation efforts, especially in endangered species such as *S. parksii*. While this study did not assess recruitment, it can be speculated from the observations that seedlings and juveniles would replace mature individuals that suffered mortality. In the data and predictions presented in Fig. 3.4, annual absence within the sample population increases by about 7% each year, which is approximately equivalent to a loss of 10 individuals in a given year. From our findings that spring-present plants are more likely to return in the fall (Fig. 3.5c) and the seedling and juvenile turnouts recorded in spring 2018 (Fig. 3.9), it can be presumed that recruitment rates easily surpassed the sample population mortality rates in 2018. However, the long-term survival of seedlings and juveniles at this study site is unknown, so the assumption that all or most recruits will endure long enough to reproduce is unsupported by empirical data.

Terrestrial orchids are characterized by high niche specificity, and conservation efforts for rare species are often contested by habitat loss, fragmentation, and changes to biotic facilitators such as pollinators and mycorrhiza (Swarts & Dixon 2009). Even slight fluctuations in ecosystem equilibria such as light, hydrology, nutrient acquisition, and competition for and between mycorrhiza can affect survivability in both mature and immature individuals (Swarts & Dixon 2009; McCormick & Jacquemyn 2014). While complex interactions exist between biotic and abiotic factors, knowledge of population dynamics is an essential step toward conservation, and while this study addressed natural variations in a sample population of *S. parksii* and its congener *S. cernua*, long-term observations of multiple populations should be assessed so that more effective monitoring and conservation strategies can be developed.

CHAPTER IV

HERBIVORY

Literature Review

Plants and herbivores have coexisted for millions of years, and their interactions have shaped ecological and evolutionary processes (Turcotte et al. 2014*b*). Evolutionary impacts of herbivory include effects on "plant fitness, local adaptation, the evolution of defenses, and the diversification of plants as well as natural enemies" (Turcotte et al. 2014*a*). Ecological processes affected involve plant productivity, population and community dynamics, energy flow, and nutrient cycling (Turcotte et al. 2014*b*; Genua et al. 2017).

Although herbivory is typically considered disadvantageous to plant performance, the timing and type of tissue damaged can yield different results (García & Ehrlén 2002; Puentes & Ågren 2012). García and Ehrlén (2002) found that simulated leaf herbivory (defoliation) on the herb *Primula veris* during anthesis had negative effects on seed production but leaf removal after fruiting had little to no consequence; flower removal of any intensity, however, had significant results on future population growth. Their findings indicated that "photosynthetic activity during fruit and seed maturation" in a given year guides growth and flowering for the following year (García & Ehrlén 2002). Similarly, Puentes and Ågren (2012) noted that significant leaf damage in *Arabidopsis lyrata* reduced flower production and the fruiting proportion in the following year. Conversely, leaf damage alone expressed far greater consequences on plant fitness than when combined with inflorescence damage (Puentes & Ågren 2012). Understanding plant fitness in relation to herbivory is therefore not straightforward, and long-term studies are needed to assess vegetative and floral herbivory consequences.

Schmitz (2008) divided herbivores into two general classes: those that remove tissue and those that feed on sap, the former of which includes mammals and tissue chewing invertebrates. Leaf tissue removal by invertebrate grazers and phloem extraction by sap-feeders has in some cases been seen to increase photosynthetic rates in remaining leaves and yield other advantageous traits, although the mechanisms behind these phenomena are unclear (Huntly 1991; Doak 1992; Trumble et al. 1993; Nabity et al. 2009). Mammalian grazers not only cause changes to physiology, but also physical form. Shoot and root herbivory can result in tissue proliferation, but if the amount of removed tissue crosses a certain threshold, plant survivability can decrease prodigiously (Huntly 1991). Rare plant populations are at risk from any herbivore, no matter the feeding class, if tissue consumption is significant (Doak 1992; Schemske et al. 1994).

Prior research on plant-herbivore interactions involving terrestrial orchids has documented various feeder effects. Hutchings (1987) found that the rare spider orchid *Ophrys sphegodes* was mostly utilized by gastropods whose effects on plant fitness were insignificant. However, sheep caused drastic outcomes by grazing entire flower spikes; after inflorescences were removed, stalk and rosette senescence quickly followed (Hutchings 1987). Knapp and Wiegand (2014) attributed 41 years of orchid population trends to white-tail deer densities in the Catoctin Mountains and found that 19 species declined more than 90% and 3 species disappeared. Several *Spiranthes* species were included in the study: *S. cernua* declined by about 10%, *S. lacera* var. *gracilis* declined more than 90%, and *S. ochroleuca* disappeared (Knapp & Wiegand 2014). Similarly, in Australia Bower et al. (2015) found that large herbivores had eliminated about 50% of an endangered Tuncurry Midge Orchid (*Genoplesium littorale*) population in one reproductive season by removing inflorescences or all above-ground organs.

Invertebrate communities also can be a threat to terrestrial orchid conservation efforts. Light and MacConaill (2011) studied 26 years of various terrestrial orchid species herbivory in Canada and found that "aphids, leafminers, moths, thrips, weevils, and whiteflies" were injurious either alone or in combination and that impacts varied between seed loss and entire plant desiccation and varied between hosts, habitat, and year. Grasshoppers and their nymphs have also been observed on leaves of Puttyroot (*Aplectrum hyemale*) and Cranefly (*Tipularia discolor*) wintergreen orchids (Menzies & Rossell 2017). Additionally, research on the rare terrestrial lady slipper orchid (*Cypripedium calceolus*) indicated that significant leaf herbivory (insect or mammalian) slowed overall growth and reduced the probability of flowering the following year (García et al. 2010).

Prior large herbivore research on *Spiranthes parksii* was conducted by Wonkka (2010) who found that deer and rabbit consumption of inflorescences and stalks reduced seed production and threatened population growth. Leaf removal by mammals was also suspected to limit plant fitness by reducing photosynthetic surface area and nutrient access (Wonkka 2010; Ariza 2013). Nally (2016) analyzed vertebrate and invertebrate utilization of and effects on *S. parksii*, finding that herbivory rates were relatively equal between the two groups of feeders. However, herbivory to individuals during the study ranged from zero to 100%, indicating that variations in local habitat access, topography, and environmental conditions could play a role in vulnerability (Nally 2016).

While orchid population variability is natural from year to year, endangered species tend to have restricted habitats so invertebrate and vertebrate herbivory can be a significant issue (Hutchings 1987; Knapp & Wiegand 2014; Bower et al. 2015). Furthermore, climate change may lead to changes in invertebrate abundance that can greatly affect conservation efforts (Light & MacConaill 2011). An extension of the research by Nally (2016) could provide more insight as to the variations in herbivory by timing, herbivore type, and weather, and thus improve our knowledge and understanding of *S. parksii* responses to plant-herbivore interactions to enhance conservation efforts.

It is hypothesized that *S. parksii* growth and survival are affected by the type and timing of herbivory. Specific objectives include:

(1) Monitor previously located flowering individuals and apply and maintain treatments (uncaged, caged, uncaged + insecticide, caged + insecticide, meshed) implemented by Nally (2016) to build upon existing data to assess herbivory effects on survival and reproduction.

(2) Estimate observed invertebrate and vertebrate herbivory on spring rosette and fall inflorescence structures of permanently located individuals to determine the extent of herbivory in comparison to whole-plant growth measurements with no herbivory.

(3) Assess indirect influences of precipitation and temperature on plant-herbivore interactions by analyzing precipitation and herbivory variations since 2014.

Methods

Treatments

This is a continuation of a study by Nally (2016). During the 2013 fall flower surveys at the Twin Oaks site, *S. parksii* and *S. cernua* were flagged throughout DRAs 1 through 13. DRA 11 was chosen as the study site in an effort to limit spatial variability, and had the highest population of *S. parksii* individuals. However, *S. parksii* counts were insufficient to provide the desired sample size so individuals of the congener species *S. cernua* were included due to their similar biology (Ariza

2013) (Fig. 4.1). In spring 2014, 39 *S. parksii* and 68 *S. cernua* (total of 107) were located and permanently marked with flags at points 15 cm north and south of each plant and given a unique identification label attached to the north flag.

One hundred and seven plants were randomly assigned to 2 *x* 2 factorial treatments: (a) uncaged, not insecticide-treated, (b) uncaged, insecticide-treated, (c) caged, not insecticide-treated and (d) caged, insecticide-treated (Figs. 4.2, 4.3). Cages were intended to keep out vertebrate predators of *Spiranthes* such as deer, feral hogs, rabbits, and rodents (Wonkka 2010) while the insecticide treatment would limit invertebrate herbivory. Therefore, treatments were designed to (a) allow free access to herbivores, (b) prevent invertebrate access, (c) allow only invertebrate access, and (d) prevent both invertebrate and vertebrate herbivores, respectively. Cages were constructed as cylinders 45 cm tall by 15 cm diameter out of galvanized wire cages with 1 cm$_2$ mesh openings and were anchored to the ground with bent stakes. The insecticide Sevin (GardenTech, carbaryl 0.126%) was sprayed until saturation on treated plants, while untreated plants received equal sprays of deionized water. Treatment applications were performed every 7 to 10 days and growth measurements were taken at estimated peak rosette growth in May.

A fifth treatment was added in spring 2014 after witnessing invertebrates of various sizes consuming plant tissue. To differentiate between small- and large-invertebrate herbivory, 30 unidentified plants from the 2014 spring rosette survey were given mesh treatments (Figs. 4.2, 4.3) in an effort to deter large insects like some grasshoppers (suborder Caelifera) that could fit through the galvanized cages. The new treatment included the same cage structure but was wrapped in nylon tulle fabric with 1 mm mesh. At this time, insecticide treatments were extended from every 7 to 10 days to every 14 to 17 days due to what appeared to be rosette stress from potential overtreatment, and growth measurements were performed weekly (Nally 2016). It was later

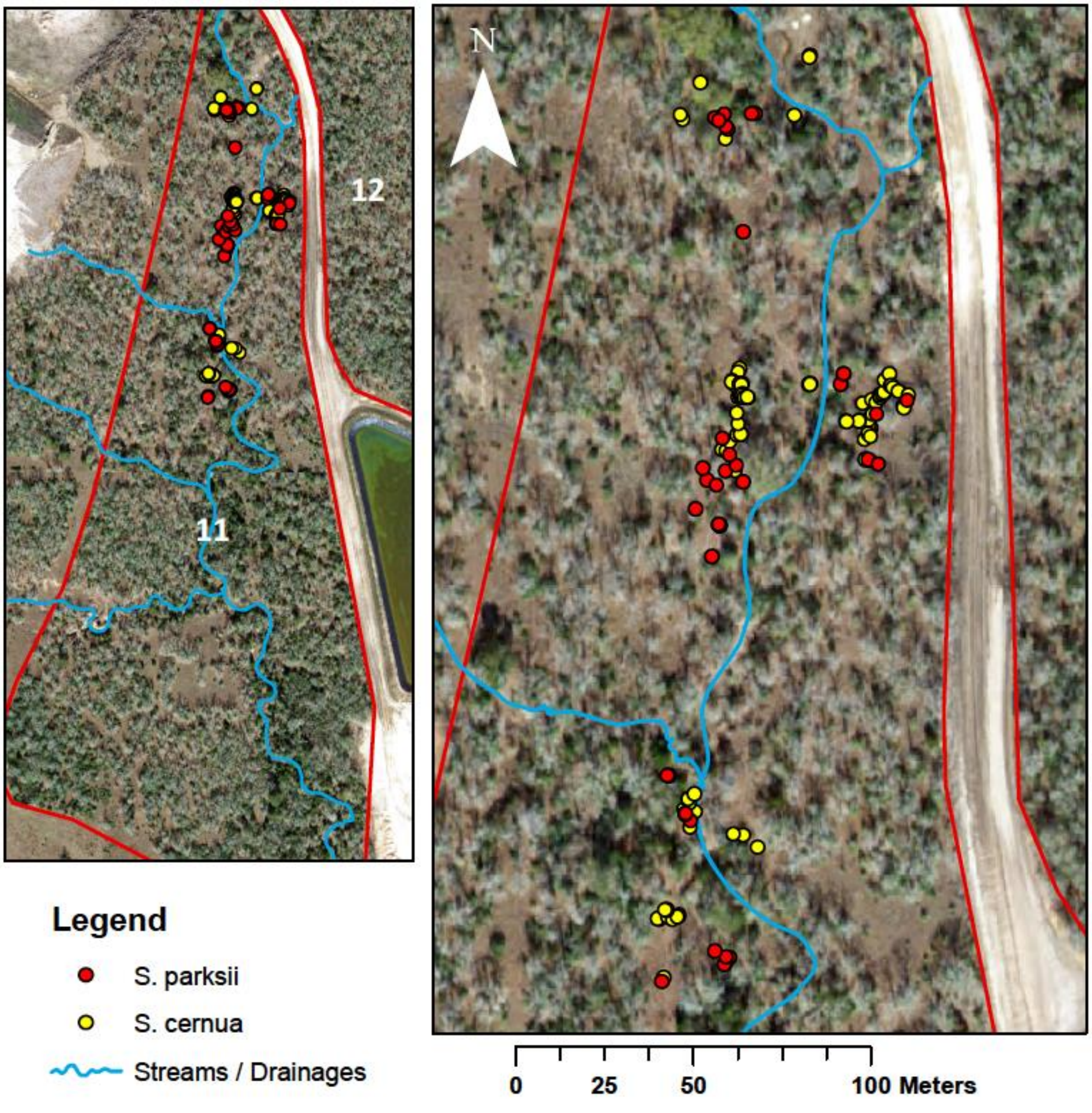

Figure 4.1. Deed-restricted area 11 with herbivory study sample population of *S. parksii* and *S. cernua* enlarged.

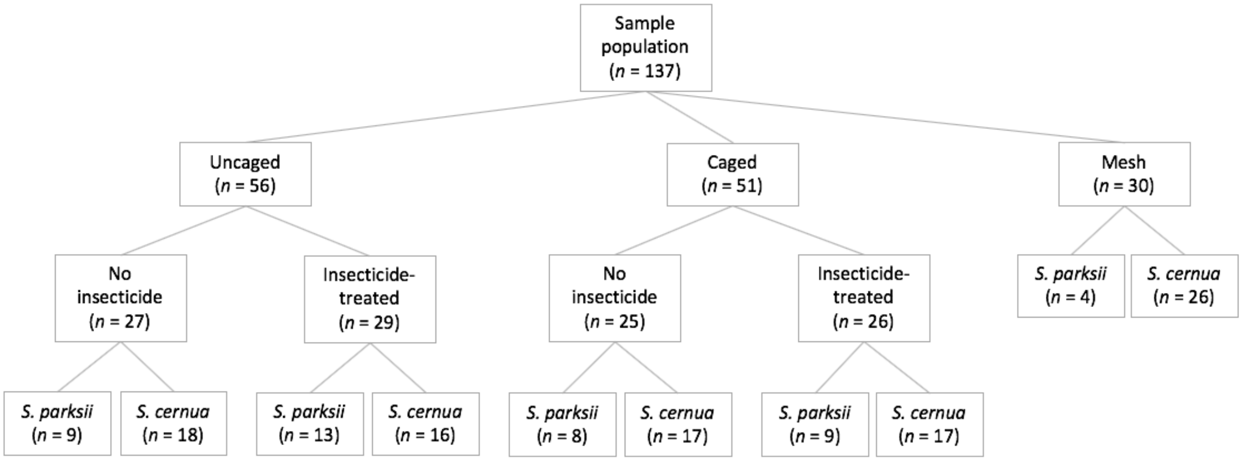

Figure 4.2. Herbivory treatments and sample size (*n*) per treatment.

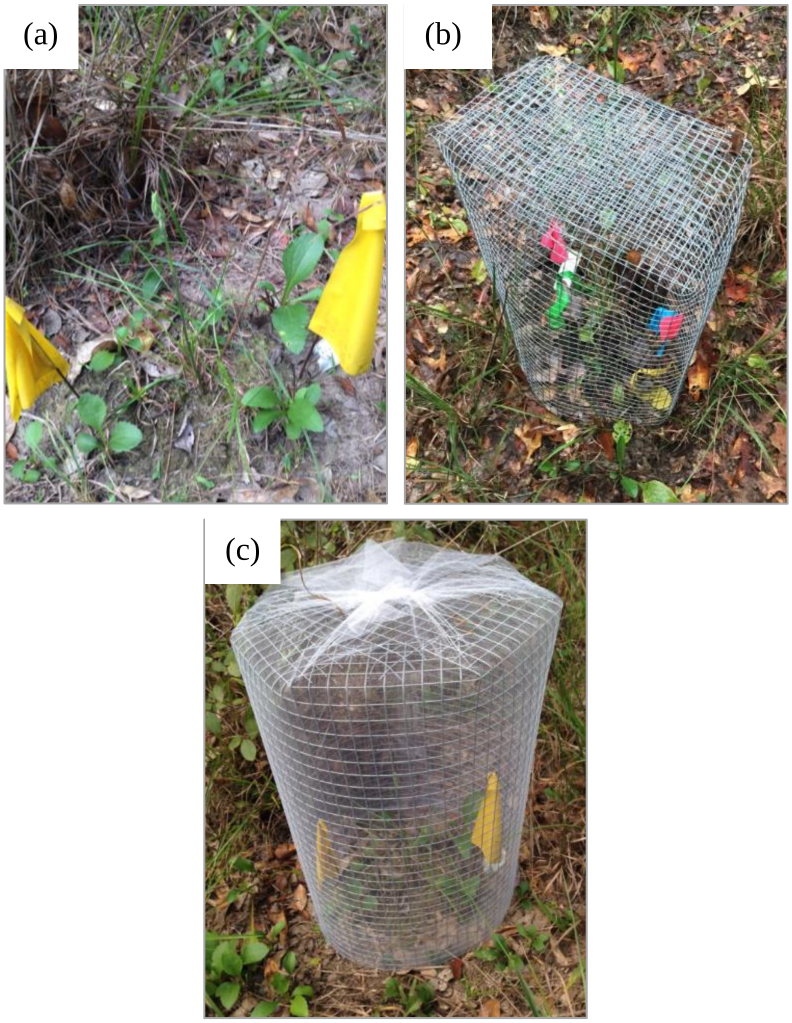

Figure 4.3. Uncaged (a), caged (b) and mesh (c) treatments with north and south reference flags.

determined that the 30 mesh treatment plants included 4 *S. parksii* and 26 *S. cernua*. In later inferences by Nally (2016), it was suggested that protective cages around plants were too small; rosette leaves touching the galvanized wire may have been more prone to desiccation and natural flora around the *Spiranthes* specimen was disrupted by constant cage removal and replacement. In response, in fall 2016 all wire enclosures for both caged and mesh treatments were replaced with cages widened to 30 cm diameters.

Herbivory Determination and Demographic Data

Beginning in fall 2014, demographic data was collected every 7 to 10 days through spring 2018, after which one observation date in mid-October recorded fall 2018 data. Rosette season growth measurements consisted of leaf length and width along the major and minor axes, maximum number of leaves at one time, and percent herbivory of individual leaves. Herbivory per leaf was visually estimated in 5 percent increments and averaged across the entire rosette for total herbivory (Waller & Jones 1989; Wonkka 2010; Johnson et al. 2016). To reflect total leaf area (LA) per plant, an elliptical method was utilized where L is length, d is leaf diameter, and i is the number of leaves (Wonkaa 2010; Ariza 2013).

$$\mathrm{LA} = 0.7854\ (L_1d_1 + L_2d_2 + \ldots + L_id_i)$$

Flowering season records included plant presence or absence, number of stalk bracts (i.e. stalk leaves), plant height, inflorescence length, basal stalk diameter, how many florets are open, closed, or desiccated, any basal rosette dimensions, and separate estimated herbivory percentages for the inflorescence, stalk, and stalk leaves. All flower structure herbivory parameters were averaged to obtain an herbivory percentage for the entire reproductive individual.

While it was unlikely to witness vertebrate herbivores consuming *Spiranthes* species, evidence of their presence was noted by herbivory patterns or identifiable soil disturbances such as rodent holes or rooting by feral hogs. Invertebrates found inside cages or consuming *Spiranthes* were recorded and identified when possible. Amounts of vertebrate and invertebrate herbivory were noted and examined across both rosette and flower seasons to determine the influence on plant growth and survival.

Weather Data

Temperature and rainfall data were collected by an on-site weather station at BVSWMA using a Davis Vantage Pro 2, located approximately 800 meters from DRA 11 (Nally 2016). Temperature maximums were averaged between weeks and months, and rainfall records were summed between observation time points and monthly to infer weather-related growth responses.

Data Analysis

All analyses were performed using JMP Pro 13 (SAS Institute Inc. 2016) with alpha set at 0.05, and relationships significant at $p \leq 0.10$ were also reported. Due to the nonparametric and inconsistent nature of most of the variables, transformations to fit normality assumptions were not possible. Significance in presence by treatment was determined with Likelihood Ratio (LR) χ^2 tests. Reproductive structure and flowering plant percentages, and desiccation and herbivory rates utilized nonparametric Kruskal-Wallis χ^2 tests determined whether or not statistical differences in variables existed and Steel-Dwass All Pairs, the nonparametric version of the conservative Tukey-Kramer test, determined where those differences occurred (Ott & Longnecker 2016; Nally 2016).

When spring and fall growth variables were assessed individually, parametric and nonparametric analyses were utilized to conclude significance. Of the fall variables, inflorescence length utilized ANOVA and Tukey-Kramer tests while the others were not normally distributed and required Kruskal-Wallis and Steel-Dwass assessments. However, when comparisons were made across groups such as for all reproductive structures and only plants that flowered, analyses required uniformity so nonparametric evaluations were employed. Wilcoxon Signed Rank tests were used in place of Steel-Dwass All Pairs comparisons when only two means were assessed. When variables were assessed by both treatment and year, normality was tested for each variable and either parametric or nonparametric tests were utilized to achieve optimal results and within-year comparisons.

Herbivory parameters of both spring and fall required nonparametric Kruskal-Wallis tests for significance and Steel-Dwass means comparisons between years. When treatments were added to the analyses, all variables continued to utilize nonparametric analyses. Comparisons of only two parameter levels utilized Wilcoxon Signed Rank tests and Fisher's Exact Test, where applicable. Evaluations of herbivory correlations with weather variables utilized herbivory parameter means from each season, for each year, and multivariate analyses determined Pearson's correlations with summed precipitation and mean temperatures during months respective to the herbivory season assessed.

Results

Presence by Treatment

Due to uneven distribution of specimens amongst the five treatments, treatment presence as a percent of the sample population showed consistently higher presence in Mesh plants over time, and 30 individuals made up the treatment (Fig. 4.4a). Despite having the next largest sample size, the Uncaged Treated group remained in the lower presence percentages. Caged treatments had the two smallest sample sizes, yet exhibited mid-range presence percentages that at times dipped lower. Comparison of treatments as a percent of treatment sample sizes rather than the sample population aligned presence percentages more evenly (Fig. 4.4b), and while Mesh plants still maintained the highest presence, Uncaged Treated plants were clearly the least present across the years. Beginning in 2015, caged presences were unfailingly higher than Uncaged Untreated in all springs, yet Uncaged Untreated fall presence was always greater than one caged group, most often Caged Treated. The least amount of fall precipitation was seen in fall 2015 (Fig. 4.5), and effects were apparent by large decreases in presence. Fall 2018 presence exhibited the steepest overall drop in presence from spring to fall, but as only one observation date contributed to counts, it is possible that more individuals had been present than were witnessed. Two variations of the presence data are offered, because ordinal differences in treatments occur. For instance, in spring 2016, presence as a function of the sample population from high to low is Mesh, Caged Treated, Uncaged Untreated, and Caged Untreated and Uncaged Treated are equivalent; when presence is depicted as a function of treatment size, Mesh and Caged Treated are in the same order but Caged Untreated rises above both of the uncaged groups. While neither interpretation is incorrect, hereafter percentages will refer to proportions of treatments rather than the sample population.

Due to the herbivore allowances created by the treatments, it was expected that the order of plant presence in any given season from greatest to lowest would be Mesh, Caged Treated, Caged Untreated, Uncaged Treated, then finally Uncaged Untreated. Spring presence by treatment in every year was significantly different (Fig. 4.6a). Weather and environmental conditions (Fig. 4.5) leading up to and through spring 2015 were clearly favorable for rosette growth or

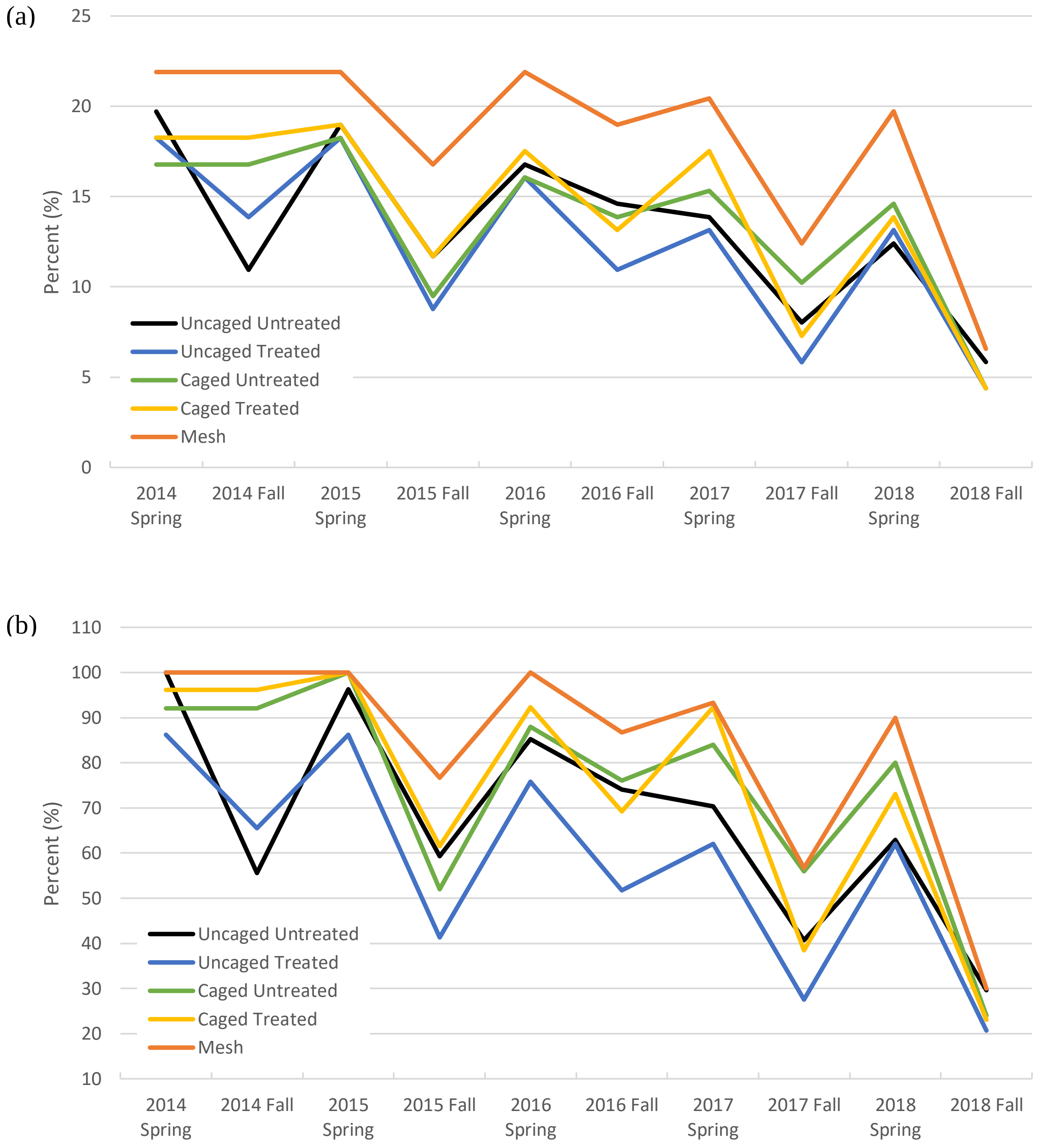

Figure 4.4. Treatment presence (a) as a function of the sample population, *n* = 137, and (b) as a percent of each treatment sample size. See Fig. 4.2 for treatment sizes.

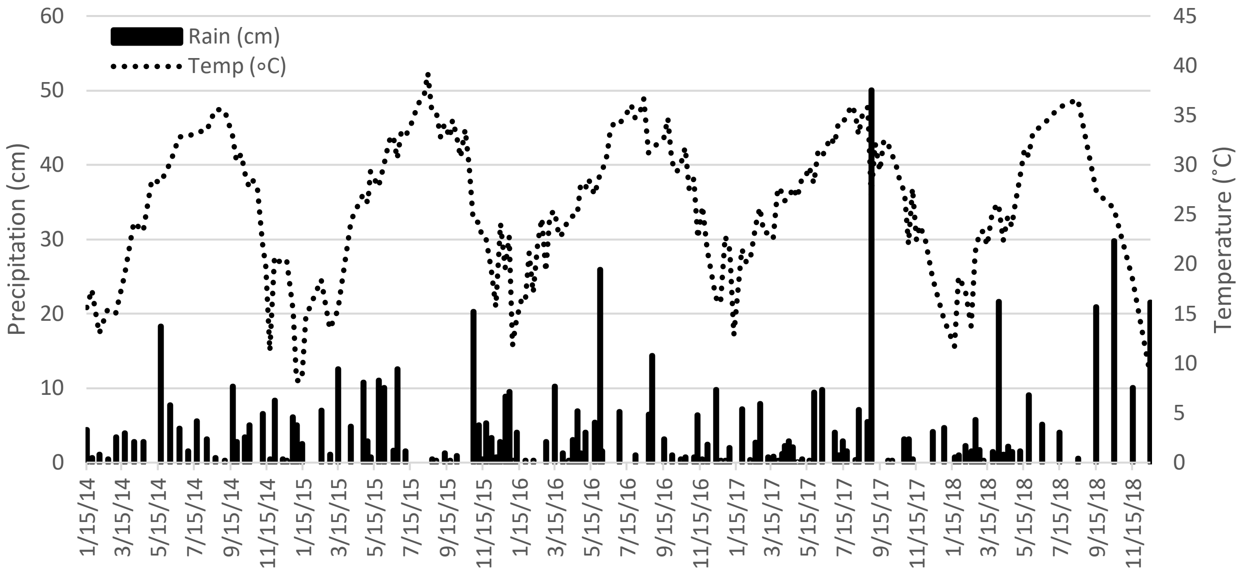

Figure 4.5. Summed precipitation per observation date and maximum temperatures averaged by month. Note the study period was from May 2014 to May 2018, but earlier and later data is also provided.

individuals were still young and robust, as more Mesh and Uncaged Untreated plants were present than expected but more Uncaged Treated were absent ($LR\ \chi^2$ = 9.588, p = 0.0480), the latter of which continued in spring 2015 ($LR\ \chi^2$ = 11.098, p = 0.0255). By spring 2016, all treatments except Mesh experienced reductions in rosette presence, and 2016 and 2017 were both characterized by more Mesh present than expected and less Uncaged Treated plants (2016: $LR\ \chi^2$ = 11.616, p = 0.0204; 2017: $LR\ \chi^2$ = 13.901, p = 0.0076). By spring 2018, Mesh plants were still present in greater numbers than anticipated while more plants within both uncaged treatments were absent ($LR\ \chi^2$ = 8.902, p = 0.0636). Over the study years, uncaged plants exhibited the steepest declines in rosette presence (untreated, 37.0%; treated, 24.1%), followed by caged (untreated, 12.0%; treated, 23.1%; both from 2015 to 2018), and Mesh had the smallest declines (10.0%).

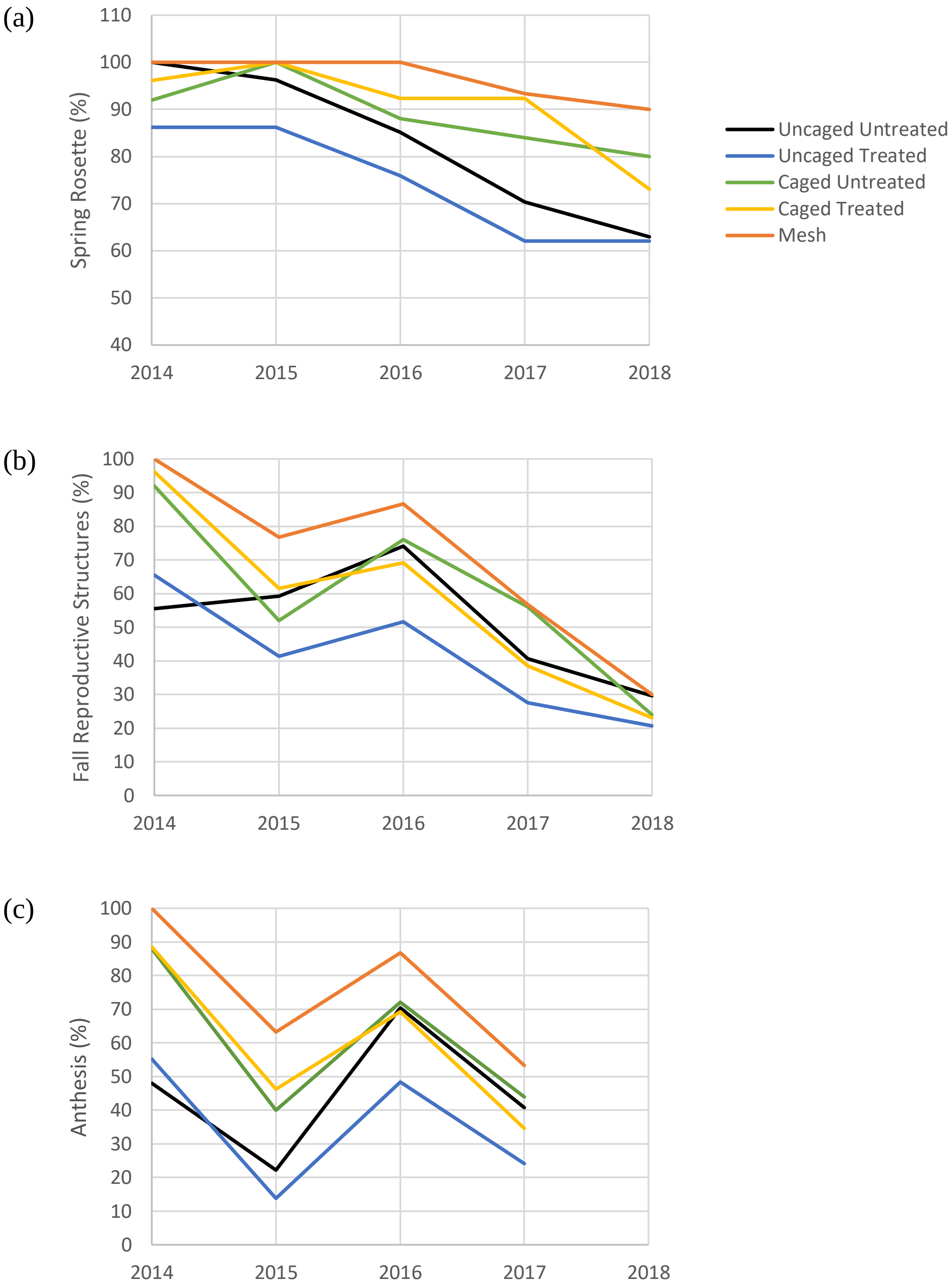

Figure 4.6. Annual percent presence of (a) spring rosettes, (b) fall reproductive structures, and (c) those that flowered, by treatment; *n* = 137.

Similar to spring, presence orders in the fall did not always occur regularly, and treatment presence percentages were significantly or marginally different in 2014 through 2016 (Fig. 4.6b). Mesh plants had the highest overall reproductive presence every year and Uncaged Treated most often had the lowest, which reflected spring presence, but Uncaged Untreated, Caged Untreated, and Caged Treated presence percentages fluctuated amongst each other and did not follow presence orders seen in the preceding nor subsequent spring. In fall 2014, more Mesh and Caged Treated plants were present than expected while less in the uncaged cohorts were present ($LR\ \chi^2$ = 33.313, $p < 0.0001$). In 2015 and 2016, more Mesh were present and more Uncaged Untreated were reproductively absent than expected (2015: $LR\ \chi^2$ = 8.348, *p* = 0..0796; 2016: $LR\ \chi^2$ = 9.381, $p = 0.0522$). While treatment proportions in 2017 and 2018 were not significantly different, both of the insecticide-treated cohorts had consistently lower reproductive presence values than untreated cohorts, which indicated that the insecticide treatment itself may have had long-term negative effects on growth and/or reproduction. Uncaged Untreated was the only group to exhibit an increase in presence higher than the 2014 starting value, but its presence in 2014 was also quite low. Overall, uncaged plants exhibited the smallest declines in reproductive presence (untreated, 44.5% from 2016 to 2018; treated, 44.8%) and caged and Mesh had the largest declines (untreated, 68.0%; treated, 73.1%; Mesh, 70.0%).

Fall 2014 anthesis values reflected the pattern of flower stalk presence, and 2014 through 2016 were significantly different by treatment (Fig. 4.6c). In 2014, more Mesh and less uncaged plants flowered than expected, respectively ($LR\ \chi^2$ = 37.045, $p < 0.0001$), while in 2015 and 2016 more Mesh and less Uncaged Untreated flowered (2015: $LR\ \chi^2$ = 20.038, $p = 0.0005$; 2016: $LR\ \chi^2$ = 10.586, $p = 0.0316$). In 2015, anthesis rates in uncaged cohorts were very low and Uncaged Untreated exhibited a 37.1% drop between reproductive presence and anthesis. Anthesis by treatment was not significantly different in fall 2017. Interestingly, Caged Untreated and both uncaged treatments displayed higher rates of anthesis in 2017 than 2015 while the other treatments were the opposite. During the fall 2018 observation date, not all flowers had yet opened so the percent of flowering structures was unavailable.

Over the study period, an average of 85.9% of reproductive structures each year were able to reach anthesis, and values ranged from 63.8% to 96.9%. However, given that the difference in reproductive and flowering presence was significantly different over time ($\chi^2{}_3 = 12.0636$, $p = 0.0072$), and that the difference in 2015 was marginally significantly greater than those of the other years (Steel-Dwass $|Z| > 2.40$, $p < 0.08$), recalculations without 2015 data led to an average of 93.3% of reproductive structures able to reach anthesis. Within treatments, the smallest differences in reproductive and flowering percentages each year tended to occur in the Mesh group but was variable amongst all treatments except Uncaged Treated. The largest differences occurred in both uncaged groups and Caged Untreated, with the latter the most frequent. As uncaged cohorts are the most exposed to herbivores, it is surprising that the Caged Untreated group lost greater percentages of flower stalks prior to anthesis in both 2016 and 2017. There was no significant difference, however, between treatments and the difference in reproductive and flowering presence ($\chi^2{}_4 = 2.3299$, $p = 0.6753$).

To determine one possible reason that fall 2015 had the greatest loss of potential flowering individuals, plants that had produced reproductive structures but did not flower were divided into those that experienced herbivory or desiccation prior to flower development (Table 4.1). Neither desiccation nor herbivory counts were significantly different by treatment (desiccation: $\chi^2{}_4 = 2.3378$, $p = 0.6739$; herbivory: $\chi^2{}_4 = 7.6242$, $p = 0.1064$), although Uncaged Untreated and Uncaged Treated had the highest cumulative numbers of individuals from 2014 to 2017 that experienced herbivory prior to flower development. When desiccation was assessed by year, differences arose ($\chi^2{}_3$ = 13.9840, p = 0.0029). The number of individuals in fall 2015 that desiccated prior to flower development was significantly greater than that of falls 2016 (Steel-Dwass Z = -2.7117, p = 0.0338), 2017 (Steel-Dwass Z = -2.5780, p = 0.0488), and marginally significantly greater than that of fall 2014 (Steel-Dwass Z = 2.4549, p = 0.0672). While the number of plants with debilitating herbivory in fall 2015 also exceeded that of the other years, differences were not significant ($\chi^2{}_3$ = 1.5168, p = 0.6784). Reproductive plants in the Caged Treated cohort only missed flower development when desiccation occurred, never herbivory. Caged Untreated plants exhibited 62.5% desiccation over the years that prevented flowering, Mesh had 40%, Uncaged Treated plants had 38.5%, and Uncaged Untreated had 30.8% desiccation. While premature desiccation occurred at least once per treatment over the four years, it appeared to be more common when treatment included a cage, with or without insecticide. Conversely, herbivory was most common in uncaged cohorts.

Table 4.1. Counts of plants by treatment with reproductive growth but no flower development due to either desiccation (Des) or herbivory (Hb).

	2014		2015		2016		2017	
	Des	Hb	Des	Hb	Des	Hb	Des	Hb
Uncaged Untreated	0	2	4	6	0	1	0	0
Uncaged Treated	1	2	4	4	0	1	0	1
Caged Untreated	1	0	3	0	0	1	1	2
Caged Treated	2	0	4	0	0	0	1	0
Mesh	0	0	2	2	0	0	0	1
Total	4	4	17	12	0	3	2	4

Growth by Treatment

Individuals were observed and recorded weekly to obtain maximum values of demographic growth parameters. Averaged maximum rosette leaf and flower stalk areas per plant at each date of observation are shown in Fig. 4.7, and details follow in the examinations of seasonal averages below. Spring 2014 measurements were not included due to incomplete data.

In all sample population fall parameter means over time, 2015 means were much smaller and significantly different from those of all other years ($F_{3,346} = 28.0033$ and $\chi^2_3 > 54.8$, $p < 0.0001$; Tukey-Kramer and Steel-Dwass $|Z| > 3.29$, $p < 0.01$) (Table 4.2). Mean plant areas and numbers of flowers were significantly lower in 2015 and greater in 2016 (Steel-Dwass $Z > 6.77$, $p < 0.0001$) than values in 2014 and 2017 (Steel-Dwass $|Z| > 3.29$, $p < 0.006$), although plant area means were only marginally different in 2014 and 2016 (Steel-Dwass $Z = 2.4482$, $p = 0.0683$) (Fig. 4.7). Whole plant heights and inflorescence lengths were similarly tall and long in 2014 and 2016, and significantly different from the short and mid-range heights and lengths seen in 2015 and 2017, respectively (Steel-Dwass $|Z| > 3.66$ and Tukey-Kramer, $p < 0.01$).

To determine potential impacts of herbivory, desiccation, or weather, data was divided into all plants present as reproductive structures each year (flowering and non-flowering) and only those that developed flowers, then growth parameters within each year were tested for differences between the two groups (Table 4.3). Years 2014 and 2017 exhibited some distinctions between means of the two reproductive groups. In 2014, inflorescence length (Wilcoxon $Z = -1.8046$, $p = 0.0711$) and number of flowers (Wilcoxon $Z = -1.9784$, $p = 0.0479$) were shorter and fewer for all plants with reproductive growth. Plant height and number of flowers were marginally greater in flowering plants in 2017 (Wilcoxon $Z = -1.7005$, $p = 0.0890$; $Z = -1.6517$, $p = 0.0986$, respectively). The most statistically significant differences were apparent in 2015; all of the flowering growth

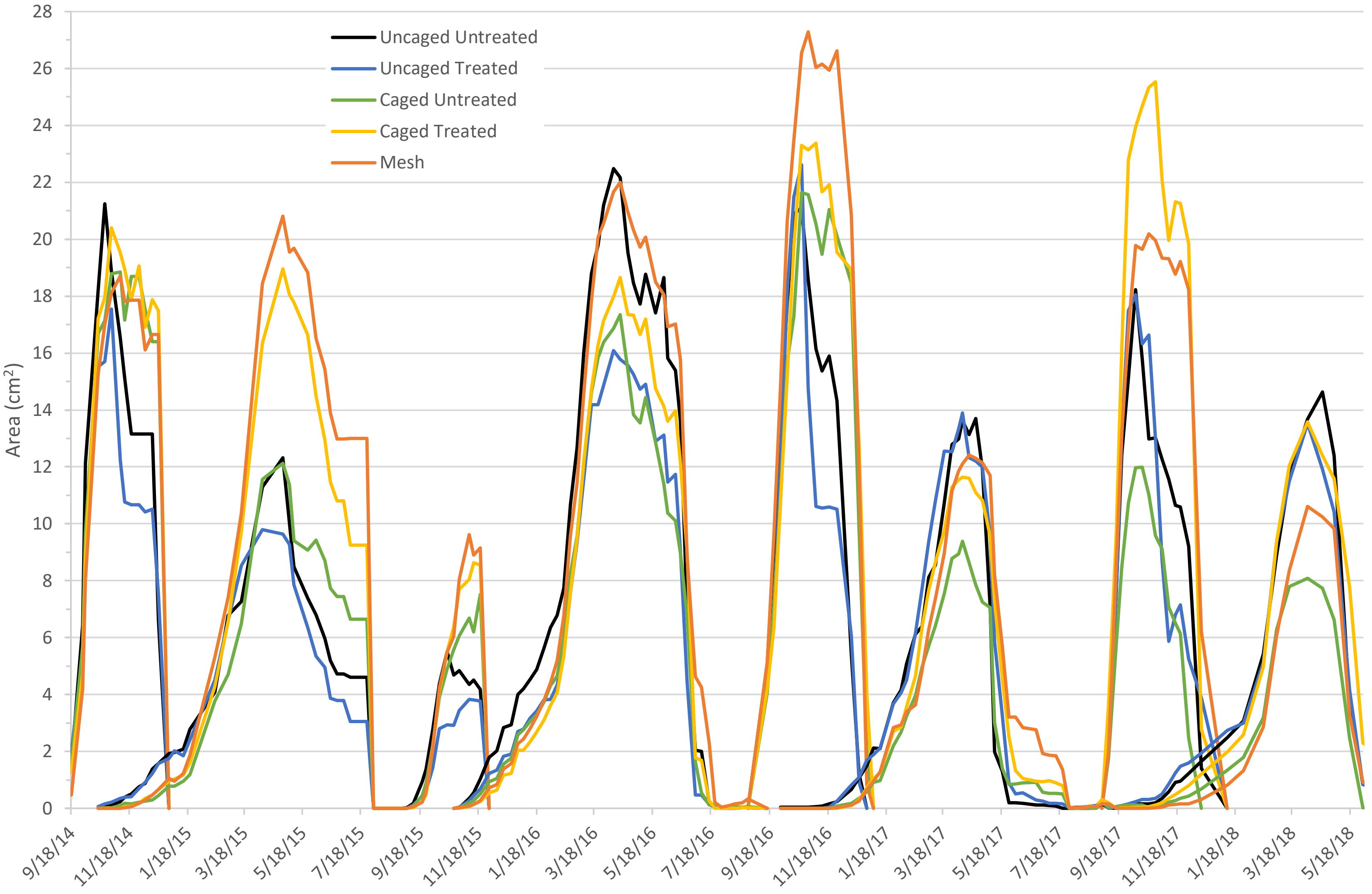

Figure 4.7. Rosette and reproductive structure plant area averages by treatment, fall 2014 to spring 2018. All present plants were included, regardless of premature herbivory or desiccation.

Table 4.2. Fall and spring growth parameter means each year for all present plants. Values are means ± SE. Capital letters represent Kruskal-Wallis significant differences between years for each variable. Different letters in a column indicate significant differences, and * denotes $p < 0.10$.

Fall[a]

	PA (cm^2)	PH (cm)	IL (cm)	#F
2014	22.18 ± 0.97	25.12 ± 0.65	6.48 ± 0.22	16.23 ± 0.71
	A*	A	A	A
2015	8.57 ± 2.24	11.44 ± 0.77	3.66 ± 0.26	9.93 ± 0.84
	B	B	B	B
2016	26.43 ± 1.03	26.67 ± 0.70	6.29 ± 0.24	19.27 ± 0.76
	C*	A	A	C
2017	20.56 ± 1.32	21.35 ± 0.89	4.94 ± 0.30	14.97 ± 0.97
	A	C	C	A

Spring[b]

	LA (cm^2)	#L	LL (cm)	LW (cm)
2015	17.02 ± 1.12	3.83 ± 0.08	12.71 ± 0.47	1.11 ± 0.03
	A	A*	A	AC
2016	21.55 ± 1.17	4.17 ± 0.08	12.68 ± 0.49	1.33 ± 0.03
	B	B	A	B
2017	13.49 ± 1.23	3.54 ± 0.09	8.57 ± 0.51	1.16 ± 0.03
	C	C*	B	A
2018	12.91 ± 1.28	3.42 ± 0.09	9.55 ± 0.53	1.01 ± 0.03
	C	C	B	C

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers; $n = 350$.

[b] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width; $n = 464$.

Table 4.3. Growth parameter means of all plants in each year that came up as reproductive structures, flowering or non-flowering ($n = 350$). Values represent means ± SE. Two-way ANOVA and Kruskal-Wallis were used to test for differences; Tukey-Kramer and Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$.[a]

		PA (cm^2)	PH (cm)	IL (cm)	#F
2014	All	22.18 ± 0.97 ***A***	25.12 ± 0.65 ***A***	6.48 ± 0.22 ***A****	16.23 ± 0.71 ***A***
	Only Flowering	23.25 ± 0.98 ***A***	26.10 ± 0.55 ***A***	6.84 ± 0.19 ***B****	17.48 ± 0.57 ***B***
2015	All	8.57 ± 2.24 ***A***	11.44 ± 0.77 ***A***	3.66 ± 0.26 ***A***	9.93 ± 0.84 ***A***
	Only Flowering	11.62 ± 1.39 ***B***	15.16 ± 0.79 ***B***	5.20 ± 0.27 ***B***	15.57 ± 0.81 ***B***
2016	All	26.43 ± 1.03 ***A***	26.67 ± 0.70 ***A***	6.29 ± 0.24 ***A***	19.27 ± 0.76 ***A***
	Only Flowering	26.77 ± 1.02 ***A***	26.99 ± 0.58 ***A***	6.42 ± 0.20 ***A***	19.87 ± 0.59 ***A***
2017	All	20.56 ± 1.32 ***A***	21.35 ± 0.89 ***A****	4.94 ± 0.30 ***A***	14.97 ± 0.97 ***A****
	Only Flowering	22.46 ± 1.35 ***A***	23.14 ± 0.77 ***B****	5.45 ± 0.26 ***A***	16.63 ± 0.79 ***B****

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

variables were significantly greater than those of all plants with reproductive growth (Wilcoxon $|Z| > 4.60$, $p < 0.0001$), which indicated severe environmental impacts on individuals that year (see also Table 4.1). In contrast, 2016 displayed no statistical dissimilarities between parameters of the two groups and also had some of the greatest values seen over the four fall seasons. It was expected that the number of flowers would always be significantly different across the two groups for each year, as non-flowering plants in the more inclusive group were expected to reduce the average; in 2016, however, only three individuals could not flower amongst the large fall sample size and so means were hardly affected.

Similarly, means amongst all reproductive structures and only flowering plants were assessed by treatment (Table 4.4). Marginally significant analyses by treatment were seen in both plant heights and plant areas in 2016 and 2017, respectively, but conservative means comparisons tests did not yield significant values. Therefore, means in falls 2014, 2016, and 2017 displayed no meaningful variations by treatment, regardless of the flowering category, and 2015 parameters did not differ by treatment within only flowering plants. In the category of all reproductive structures, however, fall 2015 exhibited significant differences within plant height ($F_{4,75} = 2.7517$, $p = 0.0341$), inflorescence length ($F_{4,75} = 3.6394$, $p = 0.0092$), and number of flowers ($\chi^2_4 = 14.0484$, $p = 0.0071$); plant area was marginally significant but means comparisons yielded only insignificant differences. Uncaged Treated plants had the smallest mean plant height in 2015 at 7.61 cm, which was significantly different from the tallest mean plant height in Mesh plants at 14.20 cm (Tukey-Kramer, $p = 0.0386$). Mean inflorescence length of Uncaged Untreated plants at 2.28 cm was lower that of Caged Treated (Tukey-Kramer, $p = 0.0830$) and Mesh plants (Tukey-Kramer, $p = 0.0232$) at 4.44 cm and 4.65 cm, respectively, and Uncaged Treated mean inflorescence length at 2.43 cm was marginally significantly shorter than that of Mesh (Tukey-

Table 4.4. Averaged maximum growth parameter means by treatment, year, and whether or not plants flowered. Values represent means ± SE. Two-way ANOVA and Kruskal-Wallis were used to test for differences; Tukey-Kramer and Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$.[a]

	All Reproductive Structures				Only Flowering Plants			
	2014 ($n = 112$)				2014 ($n = 104$)			
Treatment	PA (cm^2)	PH (cm)	IL (cm)	#F	PA (cm^2)	PH (cm)	IL (cm)	#F
Uncaged Untreated	24.27 ± 2.61	24.30 ± 1.66	6.23 ± 0.55	15.87 ± 1.73	26.43 ± 2.63	26.30 ± 1.42	6.83 ± 0.48	18.31 ± 1.43
	A	A	A	A	A	A	A	A
Uncaged Treated	20.74 ± 2.32	24.31 ± 1.48	5.64 ± 0.49	13.74 ± 1.53	22.42 ± 2.37	25.34 ± 1.28	6.28 ± 0.44	16.31 ± 1.29
	A	A	A	A	A	A	A	A
Caged Untreated	22.61 ± 2.11	26.17 ± 1.34	6.78 ± 0.44	17.17 ± 1.39	23.43 ± 2.02	27.02 ± 1.09	7.05 ± 0.37	17.95 ± 1.10
	A	A	A	A	A	A	A	A
Caged Treated	22.21 ± 2.02	25.14 ± 1.29	6.66 ± 0.42	16.40 ± 1.34	23.89 ± 1.98	26.81 ± 1.07	7.14 ± 0.36	17.83 ± 1.08
	A	A	A	A	A	A	A	A
Mesh	21.68 ± 1.85	25.20 ± 1.18	6.75 ± 0.39	17.13 ± 1.22	21.68 ± 1.73	25.20 ± 0.93	6.75 ± 0.32	17.13 ± 0.94
	A	A	A	A	A	A	A	A
	2015 ($n = 80$)				2015 ($n = 51$)			
	PA (cm^2)	PH (cm)	IL (cm)	#F	PA (cm^2)	PH (cm)	IL (cm)	#F
Uncaged Untreated	6.73 ± 1.41	9.28 ± 1.60	2.28 ± 0.59	5.06 ± 2.01	11.64 ± 1.96	15.05 ± 1.77	4.28 ± 0.72	13.50 ± 2.15
	A	AB	A*	A	A	A	A	A
Uncaged Treated	5.87 ± 1.63	7.61 ± 1.85	2.43 ± 0.68	5.50 ± 2.32	12.25 ± 2.40	15.10 ± 2.16	5.60 ± 0.88	16.50 ± 2.64
	A	A	AB*	AB	A	A	A	A
Caged Untreated	8.40 ± 1.57	11.23 ± 1.77	3.81 ± 0.65	11.31 ± 2.23	10.62 ± 1.52	14.25 ± 1.37	4.84 ± 0.56	14.70 ± 1.67
	A	AB	ABC	AB	A	A	A	A
Caged Treated	9.27 ± 1.41	12.64 ± 1.60	4.44 ± 0.59	12.25 ± 2.01	11.23 ± 1.38	15.34 ± 1.25	5.51 ± 0.51	16.33 ± 1.52
	A	AB	BC*	AB	A	A	A	A
Mesh	10.88 ± 1.18	14.20 ± 1.33	4.65 ± 0.49	13.22 ± 1.67	12.25 ± 1.10	15.57 ± 0.99	5.39 ± 0.41	16.00 ± 1.21
	A	B	C*	B	A	A	A	A

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

Table 4.4. Continued.

	All Reproductive Structures				Only Flowering Plants			
	2016 (*n* = 98)				2016 (*n* = 95)			
Treatment	PA (cm^2)	PH (cm)	IL (cm)	#F	PA (cm^2)	PH (cm)	IL (cm)	#F
Uncaged Untreated	24.87 ± 2.63	25.36 ± 1.48	6.36 ± 0.49	19.70 ± 1.63	24.36 ± 2.65	25.25 ± 1.46	6.45 ± 0.48	20.74 ± 1.50
	A	A	A	A	A	A	A	A
Uncaged Treated	24.99 ± 3.03	25.20 ± 1.71	5.37 ± 0.56	18.40 ± 1.89	26.33 ± 3.09	26.29 ± 1.71	5.76 ± 0.55	19.71 ± 1.75
	A	A	A	A	A	A	A	A
Caged Untreated	23.70 ± 2.70	25.29 ± 1.52	6.19 ± 0.50	17.89 ± 1.68	24.66 ± 2.73	26.02 ± 1.50	6.46 ± 0.49	18.89 ± 1.54
	A	A	A	A	A	A	A	A
Caged Treated	27.26 ± 2.77	25.97 ± 1.56	6.61 ± 0.52	19.89 ± 1.72	27.26 ± 2.73	25.97 ± 1.50	6.61 ± 0.49	19.89 ± 1.54
	A	A	A	A	A	A	A	A
Mesh	29.87 ± 2.30	30.02 ± 1.30	6.60 ± 0.43	20.00 ± 1.43	29.87 ± 2.27	30.02 ± 1.25	6.60 ± 0.41	20.00 ± 1.28
	A	A	A	A	A	A	A	A
	2017 (*n* = 60)				2017 (*n* = 54)			
	PA (cm^2)	PH (cm)	IL (cm)	#F	PA (cm^2)	PH (cm)	IL (cm)	#F
Uncaged Untreated	19.86 ± 3.58	21.49 ± 2.42	4.88 ± 0.78	15.27 ± 2.34	19.86 ± 3.31	21.49 ± 1.85	4.88 ± 0.67	15.27 ± 1.91
	A	A	A	A	A	A	A	A
Uncaged Treated	20.60 ± 4.20	20.74 ± 2.84	4.24 ± 0.92	13.75 ± 2.75	23.23 ± 4.15	23.37 ± 2.32	4.84 ± 0.84	15.71 ± 2.39
	A	A	A	A	A	A	A	A
Caged Untreated	13.75 ± 3.18	17.20 ± 2.15	3.71 ± 0.70	11.64 ± 2.08	16.48 ± 3.31	20.33 ± 1.85	4.60 ± 0.67	14.82 ± 1.91
	A	A	A	A	A	A	A	A
Caged Treated	27.77 ± 3.76	23.85 ± 2.54	6.27 ± 0.82	17.20 ± 2.46	30.59 ± 3.66	26.09 ± 2.05	6.97 ± 0.74	19.11 ± 2.11
	A	A	A	A	A	A	A	A
Mesh	22.37 ± 2.88	23.48 ± 1.95	5.54 ± 0.63	16.76 ± 1.88	23.46 ± 2.75	24.46 ± 1.54	5.84 ± 0.56	17.81 ± 1.58
	A	A	A	A	A	A	A	A

[a] PA = flower stalk plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

Kramer, $p = 0.0736$). In the flower parameter, Uncaged Untreated had the lowest mean number of flowers at 5.06 and was significantly different than the 13.22 mean number of flowers of Mesh plants (Steel-Dwass $Z = -2.9255$, $p = 0.0284$). As desiccation and herbivory frequencies were highest in fall 2015, it appears that uncaged cohorts may have experienced more extensive damage. The fact that mean values tended to increase from uncaged to caged to Mesh treatments also suggests that vertebrate herbivory in fall 2015 was more abundant than seen in other years. Analysis of only the flowering specimens indicated that when non-flowering plants were excluded from growth parameter means, there were no significant differences by treatment in individual years, even in fall 2015; this implies that treatments themselves may not have had an effect on growth, which limits potential confounding variables when interpreting results. Previous researchers of *S. parksii* had conducted analyses using all individuals available and not just those that flowered (Wonkka 2010; Ariza 2013; Nally 2016), therefore, other analyses in this study maintain consistence and utilize data from all reproductive structures in each fall.

Changes in spring demographic parameter means from 2015 to 2018 were significantly different over the years ($\chi^2_3 > 36.6$, $p < 0.0001$), but 2017 and 2018 leaf area means tended to resemble each other the most, with smaller values than those seen in both 2015 (Steel-Dwass $Z < -2.75$, $p < 0.03$) and 2016 (Steel-Dwass $Z < -4.67$, $p < 0.0001$) (Table 4.2; Fig. 4.7). Mean numbers of leaves followed the same pattern, and mean leaf lengths were similar except 2015 and 2016 were also statistically comparable, with greater values than 2017 and 2018 (Steel-Dwass $Z < -4.15$, $p < 0.001$). Mean leaf widths were more versatile; 2016 through 2018 were all significantly different and exhibited decreasing values (Steel-Dwass $Z < -3.00$, $p < 0.02$), and the 2015 mean was statistically similar to those of 2017 and 2018.

Mean rosette parameter values were also assessed by treatment. At least one parameter displayed significant differences by treatment each year, but spring 2015 had two parameters with dissimilarities (Table 4.5). In spring 2015, Uncaged Treated and Caged Untreated mean leaf areas were significantly or marginally significantly smaller than that of Mesh ($\chi^2{}_4 = 9.9489$, $p = 0.0413$; Steel-Dwass $|Z| > 2.52$, $p < 0.09$), and uncaged and Caged Untreated leaf lengths were meaningfully shorter than that of Mesh ($\chi^2{}_4 = 20.9111$, $p = 0.0003$; Steel-Dwass $|Z| > 3.08$, $p < 0.02$). Years 2016 and 2017 both exhibited leaf width differences by treatment, and in 2016, Uncaged Untreated widths were considerably wider than those of Caged Untreated and Mesh ($\chi^2{}_4 = 9.5224$, $p = 0.0493$; Steel-Dwass $|Z| > 2.53$, $p < 0.09$) while in 2017, Uncaged Untreated widths were significantly wider than Caged Treated leaf widths ($F_{4,105} = 2.3402$, $p = 0.0598$; Tukey-Kramer, $p = 0.0430$). In 2017, the mean number of leaves also showed significant distinctions ($\chi^2{}_4 = 10.6539$, $p = 0.0307$), but Steel-Dwass means comparisons did not yield any differences with $p < 0.10$. The mean number of leaves was also significant in spring 2018, where Uncaged Treated was appreciably greater than Mesh plants ($F_{4,96} = 2.6724$, $p = 0.0366$; Tukey-Kramer, $p = 0.0809$).

Herbivory

Herbivory data was collected for all emergent plants over the entire study period, and maximum herbivory averages were calculated for each treatment and observation date (Fig. 4.8). Overlapping lines of the same treatment indicate transitions between physical states, such as basal rosette development before complete flower stalk senescence. Beginning in fall 2015, stalk herbivory observations were separated into stalk and stalk leaf herbivory and each available stalk parameter was given equal weight in average herbivory calculations. Flower stalk herbivory typically spanned from early September to mid- or late November, and rosette herbivory

Table 4.5. Averaged maximum growth parameter means by treatment and year. Values represent means ± SE. Two-way ANOVA and Kruskal-Wallis were used to test for differences; Tukey-Kramer and Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$.[a]

	2015 (n = 132)				2016 (n = 121)			
Treatment	LA (cm^2)	#L	LL (cm)	LW (cm)	LA (cm^2)	#L	LL (cm)	LW (cm)
Uncaged Untreated	15.37 ± 2.25	3.88 ± 0.16	10.90 ± 1.03	1.08 ± 0.06	25.40 ± 3.15	4.52 ± 0.19	13.32 ± 1.13	1.54 ± 0.08
	AB	A	A	A	A	A	A	A*
Uncaged Treated	12.67 ± 2.30	3.96 ± 0.16	10.26 ± 1.05	1.20 ± 0.06	19.41 ± 3.22	4.05 ± 0.19	11.71 ± 1.16	1.29 ± 0.08
	A	A	A	A	A	A	A	AB
Caged Untreated	14.02 ± 2.30	3.60 ± 0.16	11.65 ± 1.05	1.04 ± 0.06	18.76 ± 3.22	4.09 ± 0.19	12.24 ± 1.16	1.27 ± 0.08
	A*	A	A	A	A	A	A	B*
Caged Treated	19.97 ± 2.25	3.81 ± 0.16	14.31 ± 1.03	1.07 ± 0.06	20.26 ± 3.08	3.88 ± 0.19	11.89 ± 1.11	1.32 ± 0.07
	AB	A	AB	A	A	A	A	AB
Mesh	22.00 ± 2.10	3.87 ± 0.15	15.84 ± 0.96	1.14 ± 0.06	23.25 ± 2.76	4.27 ± 0.17	13.84 ± 0.99	1.25 ± 0.07
	B*	A	B	A	A	A	A	B
	2017 (n = 110)				2018 (n = 101)			
	LA (cm^2)	#L	LL (cm)	LW (cm)	LA (cm^2)	#L	LL (cm)	LW (cm)
Uncaged Untreated	15.66 ± 2.74	4.05 ± 0.24	9.26 ± 1.16	1.36 ± 0.08	16.24 ± 3.01	3.59 ± 0.21	10.96 ± 1.31	1.11 ± 0.07
	A	A	A	A	A	AB	A	A
Uncaged Treated	15.32 ± 2.81	3.83 ± 0.25	9.22 ± 1.19	1.21 ± 0.09	14.31 ± 2.92	3.78 ± 0.20	10.48 ± 1.27	1.07 ± 0.07
	A	A	A	AB	A	A*	A	A
Caged Untreated	10.60 ± 2.61	3.43 ± 0.23	7.90 ± 1.10	1.11 ± 0.08	9.40 ± 2.77	3.15 ± 0.19	8.45 ± 1.21	0.99 ± 0.07
	A	A	A	AB	A	AB	A	A
Caged Treated	12.89 ± 2.44	3.17 ± 0.22	7.54 ± 1.03	1.04 ± 0.07	14.86 ± 2.84	3.63 ± 0.19	9.93 ± 1.24	0.99 ± 0.07
	A	A	A	B	A	AB	A	A
Mesh	13.53 ± 2.26	3.39 ± 0.20	9.07 ± 0.95	1.11 ± 0.07	11.11 ± 2.39	3.11 ± 0.16	8.61 ± 1.04	0.95 ± 0.06
	A	A	A	AB	A	B*	A	A

[a] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width.

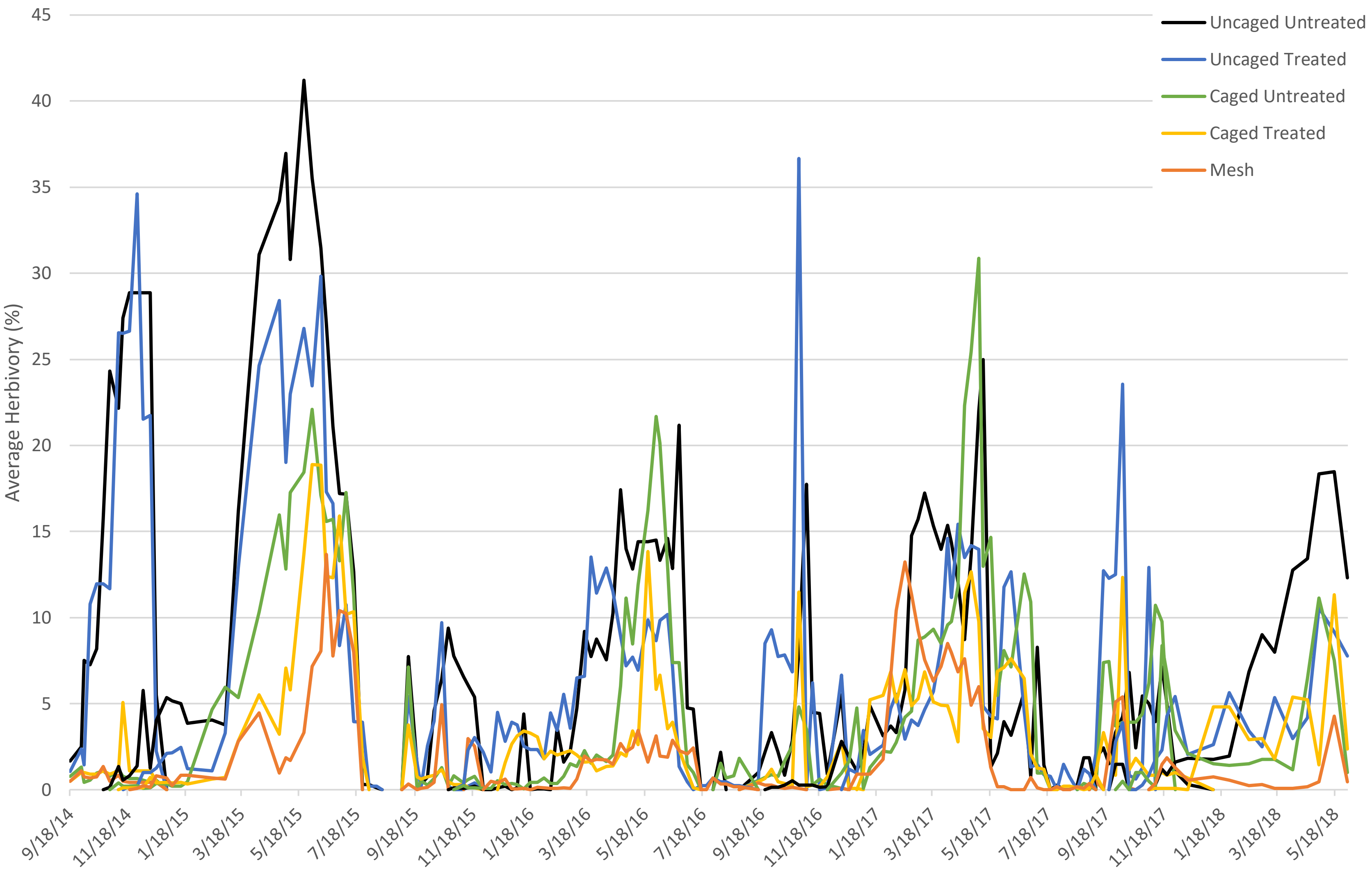

Figure 4.8. Average rosette and reproductive structure maximum herbivory by treatment, fall 2014 to spring 2018. All present plants were included, regardless of premature herbivory or desiccation.

encompassed both the winter and spring seasons, often from late November through June or early July, depending on rosette persistence. Few individuals sporadically broke summer dormancy during the hot summer months of July and August and even fewer persisted continuously, but herbivory upon these individuals was low due to the inconspicuous physical state which consisted of one to two conical bracts that reached heights and widths no greater than 1.0 and 0.5 cm, respectively.

When fall herbivory means were averaged into one parameter value per year and treatment, there were few differences over time (Table 4.6). Stalk herbivory by year procured significant results ($\chi^{2}{}_{3} = 47.1933$, $p < 0.0001$) and the 2014 mean value was significantly greater than that of all other years (Steel-Dwass $Z < -3.66$, $p < 0.002$) (Fig. 4.8). There were no significant differences amongst the other herbivory parameters and years ($p > 0.80$), but inflorescence herbivory means were always the greatest.

Table 4.6. Mean maximum herbivory (%) parameters across treatments, by year for reproductive structures (stalk leaf: $n = 238$; other parameters: $n = 350$) and rosette leaves ($n = 464$). Values represent means ± SE. Kruskal-Wallis was utilized to test for differences; Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$. Stalk leaf herbivory data was not collected in fall 2014.

Reproductive Structures				
	Stalk	Stalk Leaf	Inflorescence	Average
2014	16.21 ± 2.90		18.30 ± 3.47	16.86 ± 2.90
	A		A	A
2015	7.55 ± 3.43	10.14 ± 3.28	14.01 ± 4.10	10.35 ± 3.43
	B	A	A	A
2016	17.81 ± 3.10	22.07 ± 2.97	22.50 ± 3.70	20.36 ± 3.10
	B	A	A	A
2017	15.33 ± 3.96	18.83 ± 3.79	22.33 ± 4.73	18.39 ± 3.96
	B	A	A	A

Rosettes	
	Leaf
2015	40.90 ± 3.11
	A
2016	26.39 ± 3.24
	B*
2017	38.13 ± 3.40
	A*
2018	23.98 ± 3.55
	B

Herbivory parameters in every year exhibited significant or marginally significant differences by treatment. In fall 2014, Uncaged Untreated and Uncaged Treated plants sustained the highest rates of herbivory and had significantly greater means than those in the other three treatments ($\chi^2_4 > 28.9$, $p < 0.0001$; Steel-Dwass $Z > 3.15$, $p < 0.02$) (Table 4.7). Fall 2015 brought different results, but Uncaged Untreated herbivory means were always the greatest and significantly or marginally different than the lowest values, seen in caged and Mesh plants (stalk and inflorescence: $\chi^2_4 > 9.94$, $p < 0.05$; Steel-Dwass $Z > 2.59$, $p < 0.08$; stalk leaves and average: $\chi^2_4 > 15.9$, $p < 0.001$; Steel-Dwass $Z > 2.77$, $p < 0.05$). In 2016, Uncaged Treated plants had the greatest herbivory means in every parameter ($\chi^2_4 > 22.7$, $p < 0.0001$). Their means were significantly different from both caged and Mesh means for stalk (Steel-Dwass $Z > 2.57$, $p < 0.08$), stalk leaf (Steel-Dwass $Z > 3.10$, $p < 0.02$), and average herbivory (Steel-Dwass $Z > 2.76$, $p < 0.05$), while all uncaged and caged inflorescence herbivory means were notably greater than Mesh plants (Steel-Dwass $|Z| > 2.68$, $p < 0.06$). Uncaged Untreated plants had the next highest herbivory means in 2016 and were always significantly different from Mesh plant values (Steel-Dwass $Z > 3.10$, $p < 0.02$). Likewise, Uncaged Treated plants also had the greatest herbivory means in 2017 ($\chi^2_4 > 9.38$, $p < 0.06$), although mean values were the same across Uncaged Treated and Caged Untreated inflorescence herbivory. Uncaged Treated stalk and uncaged stalk leaf herbivory means were significantly greater than those of Mesh (stalk: Steel-Dwass $Z = 2.9562$, $p = 0.0259$; stalk leaf: Steel-Dwass $Z > 2.53$, $p < 0.09$). Caged Untreated inflorescence and uncaged and Caged Untreated average herbivory means were also greater than Mesh means (inflorescence: Steel-Dwass $Z = -2.9051$, $p = 0.0302$; average: Steel-Dwass $|Z| > 2.66$, $p < 0.06$).

As leaves are the only organ seen in the rosette state, spring herbivory measurements made up only one parameter, the averaged maximum leaf herbivory. In addition, visual determination of

Table 4.7. Averaged maximum reproductive structure herbivory (%) parameter means by treatment and year. Values represent means ± SE. Kruskal-Wallis was used to test for differences; Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$. Stalk leaf herbivory data was not collected in fall 2014.

	2014 (n = 112)				2015 (n = 80)			
Treatment	Stalk		Inflorescence	Average	Stalk	Stalk Leaf	Inflorescence	Average
Uncaged Untreated	42.20 ± 6.59		57.00 ± 7.27	49.27 ± 6.68	20.19 ± 5.38	21.88 ± 3.60	30.31 ± 7.50	20.00 ± 4.06
	A		A	A	A*	A	A*	A
Uncaged Treated	41.84 ± 5.86		51.32 ± 6.46	45.92 ± 5.94	8.67 ± 6.21	14.17 ± 4.15	20.42 ± 8.66	14.83 ± 4.68
	A		A	A	AB	AB	AB	AB
Caged Untreated	3.91 ± 5.32		1.96 ± 5.87	2.61 ± 5.40	0.00 ± 5.97	8.16 ± 3.99	2.38 ± 8.32	5.92 ± 4.50
	B		B	B	B*	AB	B*	B
Caged Treated	6.72 ± 5.11		5.60 ± 5.63	5.86 ± 5.18	0.94 ± 5.38	7.19 ± 3.60	2.50 ± 7.50	4.48 ± 4.06
	B		B	B	AB	AB	B*	B
Mesh	4.33 ± 4.66		1.17 ± 5.14	2.33 ± 4.73	7.04 ± 4.49	3.04 ± 3.00	13.91 ± 6.25	7.90 ± 3.38
	B		B	B	AB	B	B*	B
	2016 (n = 98)				2017 (n = 60)			
	Stalk	Stalk Leaf	Inflorescence	Average	Stalk	Stalk Leaf	Inflorescence	Average
Uncaged Untreated	26.25 ± 6.91	39.20 ± 6.82	34.50 ± 7.87	32.23 ± 6.87	10.00 ± 9.38	16.36 ± 9.39	18.18 ± 11.21	14.85 ± 9.52
	AB	AB	A	AB	AB	A*	AB	A
Uncaged Treated	57.00 ± 7.97	56.60 ± 7.88	60.33 ± 9.09	57.62 ± 7.93	35.63 ± 10.99	37.50 ± 11.01	40.00 ± 13.14	36.88 ± 11.16
	B*	A	A	A	A	A*	AB	A*
Caged Untreated	7.37 ± 7.08	11.84 ± 7.00	13.42 ± 8.08	10.44 ± 7.05	24.64 ± 8.31	26.43 ± 8.32	40.00 ± 9.94	29.05 ± 8.44
	AC	BC	A	B	AB	AB	A	A
Caged Treated	12.22 ± 7.28	14.17 ± 7.19	19.44 ± 8.30	15.09 ± 7.24	10.00 ± 9.83	16.00 ± 9.85	16.00 ± 11.76	13.83 ± 9.98
	AC*	BC	A*	B	AB	AB	AB	AB
Mesh	0.19 ± 6.06	1.92 ± 5.99	0.19 ± 6.91	0.64 ± 6.03	4.71 ± 7.54	7.06 ± 7.55	5.88 ± 9.02	5.88 ± 7.66
	C	C	B*	C	B	B*	B	B*

herbivory caused by invertebrates versus vertebrates is not always discernable, so suspected herbivores were inferred from treatments, environmental clues such as hog activity and mouse holes, or witnessed events. Spring herbivory from 2015 to 2018 followed the slight peak variations seen in Fig. 4.8, in that average herbivories in 2015 and 2017 were pointedly greater than those in 2016 and 2018 ($\chi^2{}_3 = 25.8988$, $p < 0.0001$; Steel-Dwass $|Z| > 2.49$, $p < 0.07$) (Table 4.6).

When herbivory was divided by treatment, significant differences were apparent in every year ($\chi^2{}_4 > 9.69$, $p < 0.05$) and the Uncaged Untreated cohort consistently sustained the greatest levels of herbivory while Mesh plants always assumed the lowest values (Table 4.8). In spring 2015, both uncaged treatments were marginally different from Caged Treated herbivory (Steel-Dwass $Z > 2.47$, $p < 0.10$) and significantly greater than Mesh (Steel-Dwass $Z > 3.74$, $p < 0.002$). Similarly, in 2016 Uncaged Untreated herbivory was considerably greater than both Caged Treated and Mesh (Steel-Dwass $Z = 2.5206$, $p = 0.0861$ and Steel-Dwass $Z = 4.0151$, $p = 0.0006$, respectively) while Uncaged Treated and Caged Untreated were only notably greater than Mesh

Table 4.8. Averaged maximum rosette leaf herbivory (%) means by treatment and year. Values represent means ± SE. Kruskal-Wallis was used to test for differences; Steel-Dwass tested means comparisons. Different letters in a column indicate significant differences, and * denotes $p < 0.10$.

Treatment	2015 (n = 132)	2016 (n = 121)	2017 (n = 110)	2018 (n = 101)
Uncaged Untreated	60.15 ± 7.04	46.38 ± 6.60	53.25 ± 8.26	40.99 ± 7.53
	A*	A*	A	A
Uncaged Treated	55.30 ± 7.18	31.77 ± 6.75	36.70 ± 8.49	35.56 ± 7.32
	A*	AB	AB	A
Caged Untreated	36.77 ± 7.18	30.89 ± 6.75	46.92 ± 7.86	24.48 ± 6.95
	AB	AB*	AB	A
Caged Treated	33.22 ± 7.04	20.50 ± 6.46	38.77 ± 7.35	22.54 ± 7.13
	B*	BC*	AB	A
Mesh	22.30 ± 6.55	8.54 ± 5.78	21.64 ± 6.80	6.20 ± 5.98
	B	C*	B	B

(Steel-Dwass Z = 2.9245, p = 0.0285 and Steel-Dwass Z = -2.5941, p = 0.0714, respectively). In 2017 the Uncaged Untreated mean herbivory was significantly greater than Mesh (Steel-Dwass Z = 2.8995, p = 0.0307) whereas in 2018 all uncaged and caged treatments were meaningfully greater than Mesh (Steel-Dwass $Z > 3.42$, $p < 0.006$). Overall, herbivory averages tended to decrease with treatments from uncaged to caged to Mesh except in spring 2017 when Caged Untreated held a higher average herbivory than Uncaged Treated.

Lastly, it was expected that more overall or acute herbivory in one season might affect growth in the next. Spring herbivory was separated into plants that had received ≤ 25%, 50%, and 75% herbivory and those that had more; subsequent fall growth across all reproductive structures and flower counts of only plants that reached anthesis were then assessed by these categories (Table 4.9). In all results, ≤ 25% spring herbivory was the smallest level tested that produced significant results. Mean plant area and number of flowers in 2015 were greater for those that sustained ≤ 25% herbivory (PA: Wilcoxon $Z > 3.18$, $p < 0.002$; #F: Wilcoxon $Z > 2.54$, $p < 0.02$). Plant area and flower count means in 2015 decreased as the level of herbivory increased. There were no differences in fall plant areas nor flowers in 2016 and 2017. These assessments were also done for fall plant areas of only those that flowered, but results did not differ. Loss of an

Table 4.9. Mean reproductive plant areas and flower counts by how much herbivory was received in the preceding spring. Values represent means ± SE. Wilcoxon Signed Rank was utilized to test for differences. Different letters in a column indicate significant differences, and * denotes $p < 0.10$. See Table 4.4 for sample sizes.[a]

	2015 PA	2016 PA	2017 PA
≤ 25% Hb	12.74 ± 0.80 **A**	27.52 ± 1.39 **A**	23.57 ± 2.27 **A**
> 25% Hb	9.87 ± 1.00 **B**	24.75 ± 2.26 **A**	21.44 ± 2.18 **A**
	2015 #F	**2016 #F**	**2017 #F**
≤ 25% Hb	17.42 ± 0.83 **A**	19.99 ± 0.78 **A**	16.96 ± 1.24 **A**
> 25% Hb	12.70 ± 1.04 **B**	19.58 ± 1.27 **A**	16.32 ± 1.20 **A**

[a] PA = reproductive plant area (cm^2); #F = number of flowers per individual.

Table 4.10. Mean rosette leaf areas by what type of herbivory was received in the preceding fall. Values represent means ± SE. Wilcoxon Signed Rank was utilized to test for differences. Different letters in a column indicate significant differences, and * denotes $p < 0.10$. See Table 4.5 for sample sizes.[a]

	2015 LA	2016 LA	2017 LA	2018 LA
Flowered	18.13 ± 1.15 **A***	27.64 ± 1.98 **A**	15.45 ± 1.15 **A**	17.90 ± 1.57 **A**
Non-flowering	12.86 ± 2.22 **B***	17.11 ± 1.69 **B**	3.50 ± 2.60 **B**	7.62 ± 1.62 **B**

[a] Hb = herbivory; LA = rosette leaf area (cm^2).

inflorescence is a major setback to any individual that attempts to flower, but flower production also requires an immense amount of energy, so mean rosette leaf areas were compared to flowering capability in the previous fall (Table 4.10). It must be noted that lack of flower production may also have been due to desiccation (Table 4.1). In spring 2015 through 2018, mean rosette leaf areas from flowering individuals were consistently greater than those of plants that did not flower (Wilcoxon $Z > 3.97$, $p < 0.0001$), and only 2015 mean leaf areas were marginally different (Wilcoxon $Z = 1.6977$, $p = 0.0896$).

In addition, plants were more likely to be reproductively present in the fall (rather than dormant or vegetative) if the maximum average herbivory in the preceding spring was less than or equal to 25% (Table 4.11). Fall 2015 showed higher significance of reproductive presence as levels of herbivory increased, but 2016 values decreased in significance with increasing herbivory. Absence probability in fall 2017 interestingly was marginally significant when plants experienced 26% to 74% spring rosette herbivory rather than 25% rosette herbivory or less.

Herbivory and Weather

In the previous chapter, assessments found that precipitation in October and mean August temperatures were negatively correlated with fall reproductive growth, while summed rainfall

Table 4.11. Fisher's Exact Test *p*-values, indicating the probability of fall reproductive presence or absence in the respective year by the amount of herbivory sustained in the same year's preceding spring season; $n = 137$. Significant *p*-values ($\alpha = 0.10$, in bold) indicate increased likelihood of the respective fall status. Only 2017 "Absent" values had significance levels of $p < 0.10$.

	Fall Status	≤ 25%	< 50%	< 75%
2015	Present	**0.0670**	**0.0325**	**0.0380**
2016	Present	**<0.0001**	0.2928	0.4685
2017	Present	**0.0565**	0.9689	0.9645
	Absent	0.9735	**0.0692**	**0.0838**

from January to May and February precipitation were positively and negatively correlated with spring rosette growth, respectively. Despite the lack of significance here, precipitation in August through September is believed to influence fall plant fitness and reproductive success (Ariza 2013; Nally 2016). The 30-year average rainfall in these months is 14.9 cm; in fall 2015, the study site only received 3.18 cm while in 2017, Hurricane Harvey brought 63.3 cm. Rainfall in August and September of 2014 and 2016 were closer to the average, at 17.2 cm and 25.0 cm, respectively. Growth within treatments should not be affected by precipitation given the small scale of the experiment location and the equal access to rainfall; therefore, weather was assessed in respect to overall herbivory sustained by plants.

Overall, climatic variables were negatively related to averaged maximum herbivory parameters (Table 4.12). Despite several strong correlations related to stalk leaf herbivory, this parameter was not included in 2014 observations and so had a smaller sample size that rendered more scrupulous analyses, therefore none of the correlation coefficients had *p*-values less than 0.10 (Table 4.12). On the other hand, stalk herbivory was significantly and marginally correlated with both October and December precipitation, respectively, inflorescence herbivory was associated with rainfall parameters from September through December, and average herbivory correlated strongly with October precipitation. Most fall herbivory is generally received from September through November when inflorescence spikes are developing and flowering, but if flower stalks are still green in December, they are still vulnerable to herbivores. However, December herbivory upon reproductive structures is quite limited, so correlations with December precipitation presented here must be assessed with scrutiny. Mean August temperatures correlated negatively with stalk, inflorescence, and average herbivories while mean August through September temperatures were marginally related to inflorescence and average herbivory. It was expected that hotter temperatures would be positively correlated with increased herbivory, but perhaps rainfall has more influence.

Spring herbivory was more sparsely related to climatic variables. Precipitation from February through April was negatively yet marginally correlated with average rosette leaf herbivory and there were no statistically significant associations between rosette herbivory and mean temperatures (Table 4.12). This suggests that factors other than precipitation and temperature may affect herbivore activity, or that more detailed analyses must be performed.

Discussion

Orchids are prone to both specialist and generalist herbivores, some of which may be destructive in large numbers or when plants are already stressed by environmental variables (Light & MacConaill 2011). Similarly, the effect of herbivory and grazing upon orchids probably depends on its type and timing (Kindlmann & Balounová 2001). Seasonal herbivore presence is subject to a complex combination of variables and is stochastic from year to year, and while weather and climate change may greatly impact plant-herbivore relationships, the effects are largely unexplored (Light & MacConaill 2011). Like herbivore presence, various data sources on terrestrial orchids

Table 4.12. Pearson’s correlations between fall and spring average maximum herbivory parameters, summed precipitation, and mean temperatures. Only pairings with at least one significant relation are shown. Bold numbers are statistically significant at α = 0.05, and * denotes $p < 0.10$.

Fall		Stalk	Stalk Leaf	Inflorescence	Average
∑ Rainfall	Sep-Oct	-0.68	-0.98	**-0.94***	-0.83
	Oct	**-0.95**	-0.99	**-0.98**	**-0.99**
	Nov-Dec	-0.77	-0.81	**-0.93***	-0.85
	Dec	**-0.95***	-0.94	-0.83	-0.90
Mean Temperature	Aug	**-0.92***	-0.96	**-0.99**	**-0.97**
	Aug-Sep	-0.89	-0.89	**-0.93***	**-0.92***

Spring		Average
∑ Rainfall	Feb-Apr	**-0.84***

suggest that flowering pattern and incidence is extremely irregular and unpredictable over time. Kindlmann and Balounová (2001) suggested that several influential factors affect both the site and species, and include weather, above- and belowground herbivory by vertebrates and invertebrates, diseases, reproduction costs, and habitat management and degradation.

This study assessed the effects of several consumer deterrents on *S. parksii* and *S. cernua* seasonal growth, herbivory, and seedling recruitment. The measures of protection included insecticide spray, cages, and fine mesh barriers which were meant to exclude invertebrates, vertebrates, and all herbivores, respectively, either alone or in combination. Climatic variables of precipitation and temperature were compared to seasonal herbivory rates to determine any correlations, as weather changes may facilitate invertebrate outbreaks or push herbivores to graze more indiscriminately (Light & MacConaill 2011).

Presence

Treatments affected how many individuals came up seasonally, and plants in the Mesh treatment were always the most abundant (Fig. 4.6). It is possible that the treatment created a favorable microclimate inside the tulle fabric by providing shade during extreme temperatures and light intensity, and reducing soil moisture loss (Nally 2016; Heinze & Joshi 2018); however, since temperatures were not measured inside and outside of the fabric, they may also have increased within the tulle. In addition, Mesh plants began treatment almost six months later than the other treatments, so higher numbers in 2014 through 2016 may be attributed to the additional time they were not touched and disturbed. Conversely, the Uncaged Treated cohort exhibited the lowest presence at the beginning of the study in spring 2014 (Fig. 4.6a), which might suggest early mortality. However, all absent Uncaged Treated plants in spring 2014 returned aboveground for a minimum of two cumulative seasons during the study, and all were present in fall 2014 and spring 2015. It appears that due to random treatment assignment, more individuals in the Uncaged Treated cohort just happened to exhibit more irregular aboveground activity.

The smallest overall declines in reproductive presence were seen in the uncaged cohorts (Fig. 4.6b), so while these plants were more exposed to herbivores, it is possible that less frequent reproductive development allowed them to persist more steadily. The insecticide spray may have had a long-term negative effect on reproduction, as fall presence in both treated cohorts was the lowest of all treatment groups, beginning in 2016. Similarly, while caged cohorts had greater rates of anthesis in 2014 and 2015, by 2017 all untreated groups experienced more flowering individuals than those that were sprayed (Fig. 4.6c). Overall, the percentages of reproductive structures that were able to flower varied greatly with the highest rates in 2016 (96.9%) and 2014 (92.9%). Based on this data, it is possible that the percent of flowering plants may increase again in the near future.

However, the number of annual flowering plants is limited by the number of reproductive structures in the population, and there appears to be a constant decline in reproductive growth.

Weather

Weather effects on *S. parksii* and *S. cernua* presence and growth were discussed in detail in Chapter III, but in general it appeared that spring presence and growth were positively affected by rainfall in the preceding fall and current spring, and lower November but greater September and December temperatures. Fall presence was positively correlated with rainfall one fall prior while fall growth was negatively associated with spring and October rainfalls; both presence and growth were negatively related to summer temperatures. Despite the lack of significance in this study, precipitation in August through September is also believed to influence fall presence, plant fitness, and reproductive success (Hammons 2008; Ariza 2013; Nally 2016). In general, terrestrial orchids in regions with dry summers depend upon cool, moist late fall and early spring conditions for adequate carbon and nutrient storage each year (Rasmussen 1995).

Precipitation and temperature can also affect plant-herbivore interactions. While spring rainfall was positively correlated with leaf lengths in rosettes, it was also negatively associated with rosette herbivory; springs with less rainfall might lead to increased herbivory rates, which could translate to shorter leaf lengths. During the spring observations, armyworms (Order Lepidoptera: Family Noctuidae) and grasshoppers (Order Orthoptera: Family Acrididae) were abundant and voracious herbivores of *Spiranthes* species. Similarly, greater fall herbivory was related to less precipitation, and the most prominent herbivores again included armyworms (Family Arctiinae). Fire ant mounds were also occasionally found around both rosettes and flower stalks after rainfall events, and while these invertebrates did not consume *Spiranthes*, they have been observed attacking an armyworm (Nally 2016), and herbivory measurements on plants never increased while ants still surrounded it. Past research has also documented the role of ants as protection against insect herbivores (Crawley 1989). Additionally, carnivorous sundew plants (*Drosera annua*) emerged each spring and study plants located near them usually received less invertebrate herbivory.

The long-term average rainfall in August and September is 14.9 cm and in fall 2014, rainfall was just above this 30-year average and vertebrates caused the greatest amounts of average herbivory (Table 4.7). At this time, plants donned tall flower stalks (average 25.1 cm), were possibly under little water stress, and white-tail deer probably targeted *Spiranthes* (Nally 2016). In fall 2015, vertebrate herbivory decreased and invertebrate herbivory increased in Caged Untreated and Mesh treatments. The study site only received 3.18 cm of rainfall in August and September 2015 and more reproductive structures failed to flower from desiccation rather than herbivory. The Mesh treatment received its greatest average herbivory, as invertebrates such as immature armyworms can easily pass through the mesh fabric. Flower stalks were about 14 cm shorter than in the previous fall and less visible to large herbivores. Fall 2016 brought 25.0 cm of rain and tall average plant heights of about 26.7 cm, which possibly contributed to the vertebrate herbivory increase. Interestingly, invertebrate consumers of caged plants also increased from 2015 to 2016, possibly due to amiable environmental conditions. Vertebrate herbivory decreased overall from 2016 to 2017 while invertebrate herbivory continued to climb, most notably in the Caged Untreated group. Plants averaged 21.3 cm in height and August and September 2017 yielded 63.3 cm of precipitation from Hurricane Harvey, although 55.5 cm of it fell within a seven-day period. Overall, invertebrates became a formidable threat to *S. parksii* and *S. cernua* reproductive structures and caused increasing damage from 2014 to 2017 while vertebrates predated on plants when fall precipitation levels were closer to the long-term average.

Growth and Herbivory

Mean flower stalk heights across the sample population in 2014 and 2016 were similar to those found by Ariza (2013), signifying that ~25.0 cm reproductive structures are standard for *S. parksii* and *S. cernua* in this location when environmental conditions are close to the long-term averages (Table 4.2). However, growth parameters in fall 2015 were significantly different between groups of all plants and only those that flowered, which further supported the fact that herbivory and desiccation played large roles in the limitation of all aspects of reproductive growth that year rather than, for instance, only a reduction in flower counts (Table 4.3). Similarly, uncaged reproductive structures in 2015 exhibited much smaller plant heights, inflorescence sizes, and flower counts than those of the Mesh treatment, which had the largest growth parameter values of both flowering and non-flowering structures (Table 4.4). Interestingly, however, in 2015 Mesh plants experienced their greatest level of herbivory as armyworm observations recorded high activity and abundance (Nally 2016). Aside from fall 2015, reproductive stalk growth and flower production within years was not significantly different between treatments. However, uncaged treatments with full vertebrate access had the highest cumulative numbers of individuals from 2014 to 2017 that experienced inflorescence herbivory, and also had most of the greatest mean flower stalk herbivories. Interestingly, Caged Treated plants never lost entire inflorescences to herbivory, which indicated that both the cage and insecticide were effective deterrents.

Spring growth parameters did not display many significant differences amongst treatments, but in 2015 Mesh plants tended to have the greatest leaf areas, lengths, and widths (Table 4.5).

Surprisingly in every other year, Uncaged Untreated plants that were exposed to both invertebrate and vertebrate herbivory had the largest leaf areas. This was possibly due to the mitigation of herbivory effects by induction of compensatory growth, even overcompensation, as was observed in the field (Crawley 1987). Treated plants had the second largest areas in 2017 and 2018, while Mesh leaf areas slowly declined and were only greater than Caged Untreated areas. Part of the decline in leaf areas within treatments may be attributed to the general trends seen in Chapter III; after spring 2016, *S. cernua* growth became progressively smaller than *S. parksii*, and Mesh and Caged Untreated cohorts had the smallest amounts of *S. parksii* per treatment (Fig. 4.2). Plants in the Uncaged Treated cohort that only permitted vertebrate consumers may have experienced herbivory too early in the season that resulted in debilitated growth capability and plant fitness (García & Ehrlén 2002). Additionally, insecticide treatment may have had inhibitory effects on growth, as Uncaged Treated plants consistently received less herbivory than the Uncaged Untreated plants each year yet the latter always had greater mean leaf areas.

In these *Spiranthes* species, overall spring herbivory damage did not affect fall plant area growth except in 2015, when the fall season also suffered unusually low precipitation (Table 4.9). In this year, any amount of mean total spring herbivory > 25% led to reduced reproductive plant area and number of flowers in the next season whereas in 2016 and 2017, plant area and flower means were no different regardless of whether spring herbivory was ≤ 25% or ≤75%. Even in spring 2015 plants were highly stressed, as evidenced by the high amounts that did not break dormancy after the summer, and it is possible that any amount of rosette herbivory greatly weakened their ability to prepare for fall reproduction. As noted by Kindlmann and Balounová (2001), the decision amongst terrestrial orchids on whether to flower, be vegetative, or remain dormant in the fall, is made during the preceding rosette season or late summer. Factors that led to the poor 2015 performance perhaps went beyond just those of weather, but when microhabitat environmental conditions are deemed favorable by these species and they have plenty of reserved carbon and nutrients (Wells & Willems 1991), it appears that spring herbivory does not have an effect on fall growth.

Conversely, flowering state in the fall had significant effects on rosette leaf area the following spring, where plants that flowered produced larger rosettes than those that did not. These results contradict the long-standing belief that reproductive costs result in unaffected or smaller rosette leaf output in the next season (Antlfinger & Wendel 1997; Hammons 2008), and Ariza's (2013) findings that rosette size was significantly lower following reproduction. Here, perhaps, the tubers suffered critical exhaustion from reproduction and so the aboveground organs had to be larger in order to compensate for and replenish lost resources (Kindlmann & Balounová 2001). Further research would benefit by assessing this data by rosette size classes before and after flowering, to determine if smaller rosettes lead to inflorescences of fewer flowers. Chapter III assessments touched on this briefly (see also Appendix B), and found that slight positive correlations existed between the number of flowers and subsequent rosette leaf area ($0.48 \leq \rho \leq 0.58$, $p < 0.0003$) as well as between leaf area and subsequent flower counts per individual ($0.51 \leq \rho \leq 0.70$, $p < 0.0001$).

Overall, Uncaged Untreated, Mesh, and Caged Treated plants achieved the greatest growth areas in different falls from 2014 to 2017 (Fig. 4.7, Table 4.4). Interestingly, none of these treatments had the lowest rates of average herbivory in their respective years of peak growth except Mesh in 2016 (Table 4.7). Rather, the timing of herbivory on plants in the other treatments appeared to have the greatest effect on growth success or limitation (Fig. 4.8). Fall 2014 appeared to be an exception in which Caged Untreated and Mesh plants did not have peak growth despite very low herbivory, but in fall 2015, all treatments except Mesh received notable herbivory around mid-September when flower stalks were developing, which perhaps put plants at a disadvantage for full reproductive development before mid-November when peak flowering occurred. Similarly, uncaged plants in fall 2016 experienced an higher levels of herbivory in early October than other treatments, and while caged plants received less, the herbivory may have been targeted at the inflorescence or in the middle of the stalk where nutrient flow could have been interrupted. In fall 2017, Caged Treated plants had the greatest growth yet still experienced some herbivory prior to anthesis, but perhaps most of the herbivory at this time occurred on the stalk leaves where plant fitness and reproductive success was not affected as greatly.

Spring growth and herbivory interacted comparably. Spring 2015 exhibited near-perfect inverse relations between treatment leaf areas and leaf herbivory, except Uncaged Untreated plants achieved peak growth even after herbivory levels rose above 30%, whereas Uncaged Treated and Caged Untreated cohorts did not exhibit continued increases in leaf area once their rates of herbivory rose to 25% and 16%, respectively (Figs. 4.7, 4.8). The high levels of consumption across all treatments in spring 2015 probably also assisted the low reproductive outcome that fall, along with climatic influences. Shefferson et al. (2005) found that defoliated *Cypripedium* and *Cephalanthera* orchids entered prolonged dormancy more frequently than controls. Uncaged Untreated plants held the greatest rosette leaf areas in the remaining springs yet also had the greatest average herbivory each year (Tables 4.5, 4.8). In springs 2016 through 2018, this treatment was able to reach its time of peak growth before the effects of herbivory became noticeable, regardless of whether noteworthy amounts of herbivory had occurred prior, and always received even more herbivory after peak growth.

Although not proven, treatments have been suspected of growth inhibition or vegetation disruption, and Uncaged Untreated plants potentially benefitted from the absence of insecticide and constant cage removal during measurements (Nally 2016). It is also possible that Uncaged Untreated plants experienced most herbivory from the leaf tips down rather than near the petioles or in the middle of leaves, so compensatory growth was able to recover some of the lost surface area each time (Crawley 1987). Rosettes are present for longer periods of time each year than reproductive structures and despite the additional exposure time to herbivores, it has been suggested that they contribute up to 92% of carbon stored in the tubers (Antlfinger & Wendel 1997). Therefore, rosettes may have adapted a greater resilience to herbivory than their reproductive stage. This resilience may allow them both persist long enough to adequately replenish exhausted nutrients and carbon from the previous fall's demands, and prepare for summer sustenance and strains of the next fall.

It is standard belief that herbivory reduces leaf area, interrupts leaf function, and thus affects productivity within the disturbed organ. However, it is possible that herbivory can induce altered photosynthesis within even undamaged leaf tissue; factors such as severed vasculature, altered sink demands and biomass redistribution, defense-induced autotoxicity or photosynthesis down-regulation, and modified plant-soil feedbacks can all lead to suppressed fitness in the individual, and in some cases these indirect effects are more detrimental than the herbivory itself (Nabity et al. 2009; Heinze & Joshi 2018). Due to the high levels of herbivory sustained by *S. parksii* and *S. cernua* in spring 2015, is it possible that rosettes lost much of their photosynthetic ability before acquiring suitable amounts of nutrient storage for the seasons ahead, so injurious herbivory in combination with other biotic and abiotic factors probably led to the dismal performance in fall 2015. However, not all herbivory is detrimental to plant productivity. Heinze and Joshi (2018) found that herbivore presence induced root biomass in some grass species, and Crawley (1987) noted that some plants are capable of compensatory growth that can actually increase fitness above that of ungrazed plants. More research on herbivore-influenced compensatory growth in *S. parksii* and *S. cernua* species might unveil characteristics of the rosette stage that allow individuals to persist for so long without reproducing each year.

CHAPTER V

SUPPLEMENTAL WATER

Literature Review

Plants that have their perennating structures, or meristems, belowground (geophytes) commonly exhibit adult, whole-plant dormancy (Shefferson et al. 2005). Whole-plant dormancy is a condition in which a perennial herbaceous plant does not produce aboveground growth during one or more growing seasons with subsequent reemergence and has been studied in numerous wild orchid populations (Lesica & Steele 1994; Shefferson et al. 2005).

Many rare or geographically restricted long-lived orchids exhibit vegetative dormancy that can last one or more years (Hutchings 1987; Shefferson et al. 2005; Shefferson & Tali 2007; Ariza 2013). Unlike seed dormancy, which is characterized by absence of growth, adult whole-plant dormancy periods exhibit root growth and metabolic activity without aboveground growth (Lesica & Steele 1994; Shefferson et al. 2003; Shefferson et al. 2005). This behavior indicates mixotrophy, the capability of some orchids to acquire carbon and nutrients from both mycorrhizal fungi (mycoheterotrophy) and photosynthesis (autotrophy) (Rasmussen & Rasmussen 2009; Ariza 2013; Shefferson et al. 2018).

Through observational studies of wild orchids and other geophytes, several trends in dormancy and aboveground growth were discovered. Shefferson and Tali (2007) found that *Neotinea ustulata* plants that flower in one year tend to flower again in subsequent years, and those that are dormant in the reproductive season tend to continue dormancy in following years. Shefferson et al. (2005) suggests that the probability of dormancy in *Cypripedium calceolus* and *Cephalanhera longifolia* tends to decrease with greater plant growth, while Primack and Stacy (1998) similarly found that nonflowering *Cypripedium acaule* plants were smaller than those that flowered. Conversely, Primack and Stacy (1998) also found that consecutive years of fruiting could only be sustained for a maximum of two to four years before entering dormancy. Therefore, results are suspected to be species-specific and may not apply across all geophytic terrestrial orchids. Long-term studies have associated dormancy with reduced survival, especially in species with shorter lifespans of 2 to 6 years (Shefferson & Tali 2007), but in longer-lived species (i.e. 20-year lifespans) it is possible that vegetative dormancy serves to extend the life of a population (Juárez et al. 2014). For example, the low cost of a dormancy state can compensate for high costs of growth and flowering by extending lifespan and creating more opportunities for reproduction; similarly, in species with low annual recruitment, trade-offs between survival and reproduction favor longer lifespans with higher dormancy prevalence (Shefferson et al. 2018).

Spiranthes parksii is estimated to have an overall life expectancy of 5 to 12 years, a timeframe that includes a 2- to 3-year period from seedling establishment to maturity that is common across several terrestrial orchid species (Wells & Willems 1991; Hutchings 2010; Ariza 2013). Additionally, the proportion of seeds surviving from dispersal to maturity is remarkably low. An *in situ* study found that less than one percent of seedling protocorms survived their first year, so seasonal dormancy may have evolved as a trade-off to extend lifespans (Ariza 2013).

Geophytes can also enter vegetative dormancy when triggered by environmental conditions such as extreme heat, frost, low precipitation, and herbivory; this transition can occur at any developmental state, provided that belowground structures remain viable (Shefferson et al. 2001; Juárez et al. 2014; Wang et al. 2015). The proposed *S. parksii* life expectancy is between those of previously observed species with shorter or longer terms, so the effects of trade-off or stress-induced vegetative dormancy on survival are inconclusive as either positive or negative.

Similar to trends in vegetative dormancy, the costs of growth and reproduction in one year generally result in opposite development in following years. Primack and Stacy (1998) found that *Cypripedium acaule* terrestrial orchids that had more flowers develop into fruit suffered consequences in subsequent years in the form of reduced flowering probability and smaller leaf areas. Shefferson et al. (2017) analyzed three clonal orchids and found that highest survival rates for individual species had different optimal growth patterns involving dormancy, vegetative states, and small and large plant sizes. Between the three species, however, it was apparent that growth was more expensive in terms of survivability. Therefore, juvenile, vegetative plants were consistently small, possibly due to physiological and energetic costs, while larger plants were more vulnerable to mortality, perhaps due to greater age (Shefferson et al. 2017). Due to the high versatility between species growth patterns and environment-driven reactions, endangered geophyte species exhibiting vegetative dormancy must be assessed individually to determine the most viable demographic patterns and conservation goals.

Water is important to all plants and is involved in numerous processes including germination, respiration, nutrient and oxygen transport through plants and soil, photosynthesis, and mycorrhizal associations (Bidlack & Jansky 2011). Research on the terrestrial orchid *Ophrys sphegodes* concluded that inflorescence length and number of leaves were positively correlated to rainfall during inflorescence extension, although dormancy prevalence was unaffected by precipitation, temperature, and sunlight hours (Hutchings 2010). Morrison et al. (2015) found in the perennial geophyte orchid, *Platanthera praeclara*, a precipitation or soil moisture threshold in one season may be required to promote root growth that will lead to emergence in a subsequent flowering season as a type of lag effect; moisture thresholds may also exist during an emergence event, to support aboveground growth. If a geophyte has additional water during a season in which it is usually limited, it might be expected that excess resource acquisition and storage could enhance reproductive prospects during its flowering season, no matter the overall plant size (Shefferson & Tali 2007). In some orchids, older rather than larger plants exhibit increased flowering, possibly a function of greater nutrient and resource access over a lifespan or larger root systems that can compensate for greater reproductive costs (Hutchings 1987; Shefferson et al. 2017). However, Pileri (1998) found that tuber numbers decreased in *S. cernua* from rosette to reproductive seasons, which indicates significant demands from root nutrients. Similarly, research by Ariza (2013) suggested that *S. parksii* and *S. cernua* tubers last only one to two years as they are utilized and exhausted during reproductive growth, and each fall new root protuberances from the meristematic rhizome begin development as replacements (Ariza 2013). The root system consists of up to eight thick, unbranched roots with highly developed cortices, which does not equip them for water and mineral uptake as per fibrous or adventitious roots, and are more dependent upon mycotrophy (Ariza 2013). If supplemental water is provided when ordinarily an orchid is dormant and dependent solely upon mycoheterotrophy, reproductive fitness may increase.

Soil is an important determinant of orchid distribution, and most orchids have specific soil and cover requirements (Liggio & Liggio 1999; Wang et al. 2015; Wang et al. 2019). Ariza (2013) found that *S. parksii* prefer areas of higher soil moisture content and organic matter than their congener, *S. cernua*. This corresponds to prior findings that *S. parksii* reside along drainage banks and eroded stream margins, allowing access to subsurface water flow (Wilson 2002; USFWS 2009). Organic matter is possibly similarly important due to its higher water-holding capacity than sand, the dominant soil component in *S. parksii* habitat (Ariza 2013; Wang et al. 2015). Associated vegetation also has a central role in *S. parksii* habitat preferences, possibly due to mycorrhizal fungal distributions, canopy driplines, and light filtering (Wang et al. 2015). Ariza (2013) created a model to determine *S. parksii* habitat predictors and found that patchy woody cover, leaf litter depth, and soil moisture levels were the strongest correlates.

It is hypothesized that scheduled summertime watering events will decrease the prevalence and duration of summer rosette dormancy and increase survival and inflorescence growth in the fall. Objectives include:

(1) Locate and monitor flowering individuals with a recorded history of inflorescence and rosette emergence and apply treatments (control, watered) to assess growth variations.

(2) Assess biophysical factors of soil moisture, slope, and depth to claypan to determine how habitat relates to *S. parksii* growth variations.

Methods

Treatments

Dry summer months may inhibit aboveground growth of *S. parksii* and *S. cernua* as the plants reside as rhizomes beneath the soil surface to survive elevated temperatures and low soil moisture (Ariza 2013; Wang et al. 2015). No prior studies have examined how summertime drought might affect *Spiranthes* survival and rates of inflorescence growth following summer dormancy with long periods of water stress. To gain insight into these effects, growth history of caged and uncaged plants from a herbivory study (see Chapter IV) were analyzed. By examining growth and demographic data from 2014 to spring 2017, plants were selected based on their growth history and aboveground persistence each season, while considering that many terrestrial orchids are inconsistent in yearly rosette and inflorescence production (Wonkka et al. 2012), but *S. cernua* individuals tend to be more consistent if they produced 16 to 30 florets the season prior, while *S. parksii* are less predictable due to a lack of long-term studies and low sample sizes (Hammons et al. 2010).

Sixty *S. parksii* and *S. cernua* individuals were used to assess variable outcomes by categorizing them into histories of large growth size and small growth size (Fig. 5.1). Large growth size was defined as those with one or more rosette leaves of 10 cm or greater and/or whole plant heights of 30 cm or greater at least once within the past two years, and small growth size plants were designated as those with rosette leaf length or plant heights less than 10 or 30 cm, respectively. Large and small sized plants were randomized separately to ensure equivalent representation within treatments, as small plants were more common. Treatments were applied during the dry summer months of July and August, when plants are typically experiencing vegetative dormancy. Following growth initiation in the fall, growth measurements such as leaf area, length, and width, plant height, inflorescence length, and number of flowers on individuals in the cohort were taken at regular intervals.

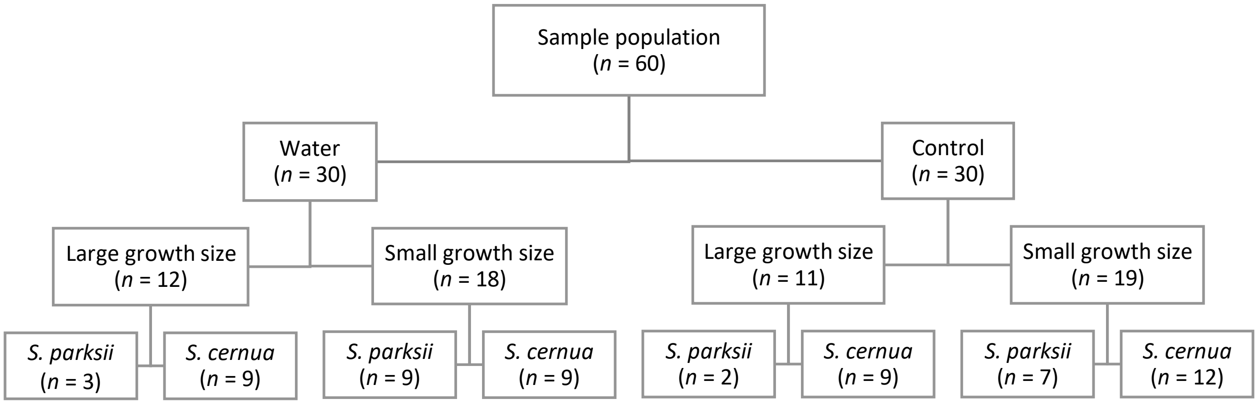

Figure 5.1. Supplemental water treatments and sample sizes (n) per treatment.

Water Application

PVC pipe rings were utilized to contain deionized water during treatment. To be compliant with findings that lateral root growth maximized at a radius of 8 cm (Hammons et al. 2010), 20.32 cm (8 in) diameter PVC pipe was cut into 15 cm lengths with the bottom edges beveled to ease entry into the soil. Pipe rings were centered over water-treated plants then gently twisted into the soil about 1 to 2 cm, or enough to avoid water seepage from the bottom. Using the equation for cylindrical volume ($V = \pi r^2 h$), 3.81 cm (1.5 in) of water in a 20.32-cm pipe is equivalent to 1236 mL. During each water application, deionized water was poured onto a sponge in the pipe ring to allow minimal soil disturbance. Beginning July 7th, 2017, plants receiving water treatment had 3.81 cm of deionized water delivered twice a week at even intervals until October 19th, three weeks after the first inflorescences reached anthesis.

Soil Moisture

Soil infiltration rates were collected for each plant in the water treatment cohort, and each round of timing was completed in a single day. The process was replicated a total of three times at each plant and occurred on scheduled treatment days that coincided with volumetric water contents relatively equivalent to those from the day of the initial infiltration rate measurements. Infiltration times were measured with a stopwatch beginning from complete water deposition into the pipe ring to the time of complete water infiltration and percolation into the soil.

Early sample experiments with a FieldScout soil moisture analyzer (FieldScout TDR200, Spectrum Technologies, Inc.) indicated that watering 3.81 cm (1.5 in) within a pipe ring allowed the soil to remain at a volumetric water percentage near to that of field capacity for up to 3 to 4 hours after watering. The FieldScout was fit with 12-cm-long rods in order to encompass the full depth of the root system. In mature plants, Ariza (2013) measured tuber depths of 4 to 8 cm from the stem base while Hammons et al. (2010) found that maximum tuber depth from the stem base was 9 cm.

Once a week during water treatments and demographic data collection, three soil moisture readings were taken immediately prior to water delivery (pretreatment) and three readings 3 to 4 hours after water delivery (hereafter referred to as "post-treatment"), per plant. Pretreatment moisture values were taken within 3 cm outside the pipe ring and post-treatment values were taken within 3 cm inside the ring. On midweek water treatment days, pretreatment volumetric water content readings were randomly collected to ensure pretreatment soil water percentages were below field capacity. When Hurricane Harvey occurred in late August, the study area received rainfall sufficient to raise the pretreatment soil moisture content above field capacity for two consecutive weeks. Preliminary experiments showed that if the soil was already at or above field capacity, applying the water treatment had no effect on soil moisture levels 3 or 4 hours later. Therefore, pretreatment moisture readings continued during this time but water applications were postponed until pretreatment values returned below field capacity. Soil water content continued to be collected for all 60 plants after treatment cessation (hereafter addressed as "post-experiment") until May 2018.

Microhabitat Characterization

To identify other potential influences in *S. parksii* and *S. cernua* survivability and year-to year emergence or dormancy, slope and depth to claypan were assessed. Slope of a 30-cm downward gradient around each plant was determined by adjusting meter sticks secured at 90-degree angles to each other to yield the slope rise and run. The meter sticks were equipped with a cross check level to ensure horizontal and vertical precision at each measurement. Additionally, slope was measured on a 1-m downward gradient from the plant location to assess longer slope patterns. Rise and run measurements were converted into percent slope. Depth to claypan was determined by driving a 2.2-cm diameter soil corer into the soil near each plant. Soil cores were analyzed in 5 cm increments until clay was detected and confirmed by hand texturing.

Data Analysis

Dormancy data was obtained by presence-absence notations during field observations. As summer rosette appearances can be sporadic and ephemeral, summer dormancy was assessed as the lack of consistent aboveground growth for greater than 30 days from July through August. Data was assessed back to spring 2016 to determine whether supplemental water treatments delivered notable effects during and after treatment application. One observation date each of presence-absence data was available for summer and fall 2018, and the seasons were included in analyses where applicable. Emergence records and demographic measurements were also obtained from the dataset. Variables were tested with Fisher's Exact Test and Likelihood Ratio (LR) χ_2 tests for significance (Ott & Longnecker 2016).

Two sample *t*-tests and the nonparametric Wilcoxon Test were used to determine if treatments (control, water) and growth history (small, large) produced statistically significant growth in the fall 2017 flowering and spring 2018 rosette seasons following supplemental water treatments. Two-way ANOVA and nonparametric Kruskal-Wallis tests were utilized to assess significant differences between group means (Control Small, Control Large, Water Small, Water Large), and Tukey-Kramer HSD and the nonparametric counterpart Steel-Dwass All Pairs compared means between the groups. Individuals were excluded in demographic analyses on a seasonal basis when they remained belowground or experienced severe environmental effects before reaching the time of peak growth, therefore sample sizes differed from initial experiment design. Severe environmental effects are defined here as total inflorescence herbivory before measurements could be taken and/or premature senescence before anthesis, rosette senescence, and greater than or equal to 70% entire rosette herbivory early in the season that drastically limited growth capability.

Treatment was not assessed in analyses of edaphic and microsite properties because it did not alter the physical characteristics of microsites. The Wilcoxon Test and two sample *t*-tests assessed whether or not there were significant edaphic differences across microsites that each species and growth form resided upon. To determine if microsites varied across interactions between species and growth, variables that met parametric assumptions were analyzed by two-factor ANOVA and Tukey-Kramer HSD while the nonparametric tests Kruskal-Wallis and Steel-Dwass All Pairs were utilized for the rest. As *S. parksii* and *S. cernua* predominantly favor sandy loam surface strata (Ariza 2013; Wang et al. 2015; Wang et al. 2019), four plants were excluded from soil moisture parameters due to their location in atypically clayey surface soil. All other individuals in the study were included in analyses regardless of aboveground presence or absence during the experimental period.

Multivariate restricted maximum likelihood (REML) method of correlation estimations was utilized to compare soil moisture and microhabitat parameters with each other and with seasonal demographics. Significant correlations were utilized to assist interpretation of microhabitat means to species and growth. All correlation estimations between edaphic properties and most correlations between demographic growth variables and microhabitat parameters met the criteria for Spearman ρ correlation estimations.

When post-experiment moisture converged with inflorescence length, number of flowers, number of rosette leaves, leaf length, and leaf width, estimations were made with Pearson's pairwise correlations. Given the limited availability of data for some parameters, correlations for interacting species and growth history combinations were tested but then discarded due to small sample sizes (frequently $n < 10$) and suspect *p*-values as indicated in the tests. Four clayey soil individuals remained excluded, and two additional individuals were excluded from soil moisture parameters due to high values in multivariate analyses. Individuals were excluded in demographic analyses on a seasonal basis when they remained belowground or experienced severe environmental effects.

For each analysis and variable pair the Shapiro-Wilk W Test was utilized to check that residuals met normality assumptions while homogeneity of variance was tested by the Brown-Forsythe Test. Analyses were performed using JMP Pro 13 (SAS Institute Inc. 2016) with alpha set at 0.05, and relationships significant at $p \leq 0.10$ were also reported.

Results

Supplemental Water Effects on Dormancy and Emergence

Supplemental water treatments were applied to 30 of 60 plants from July 2017 through mid-October with the expectation that summer dormancy prevalence would be reduced in watered individuals. For the following analyses, fall dormancy refers only to the absence of inflorescence development; vegetative rosettes may have been present but will be addressed later. In the year prior to treatments, both the control and water cohorts exhibited 90% summer dormancy, followed by fall reproductive season dormancy rates of 20% or less (Fig. 5.2a). During the treatment period in 2017, the control group rose to 100% summer dormancy while the supplemental water

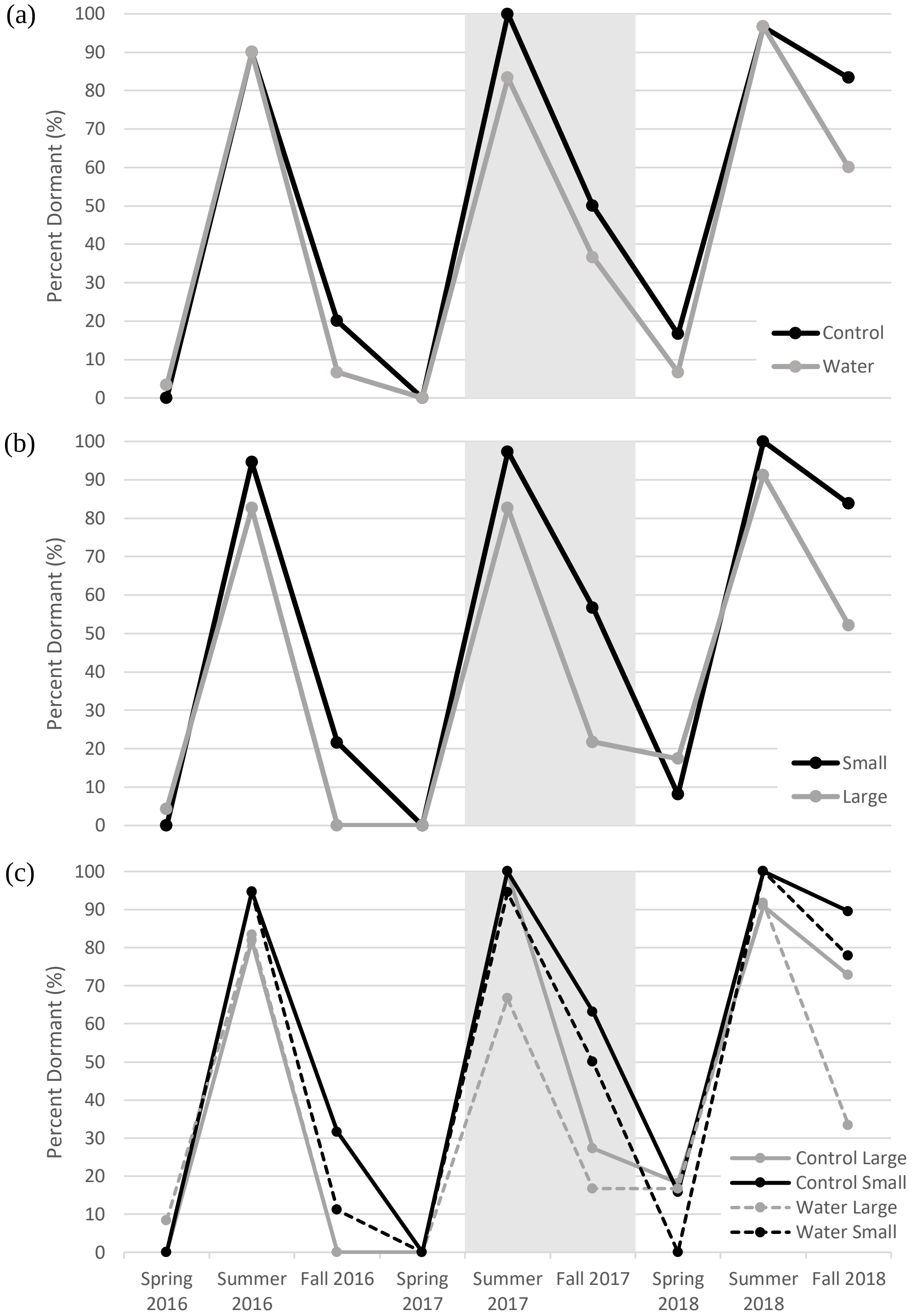

Figure 5.2. Dormancy rates over time by (a) treatment, Control *n* = 30, Water *n* = 30; (b) growth history, Small *n* = 37, Large *n* = 23; and (c) treatment *x* growth history, Control Large *n* = 11, Control Small *n* = 19, Water Large *n* = 12, Water Small *n* = 18; from Spring 2017 to Summer 2017, Control Large and Control Small overlap. Shaded area indicates treatment period, from 07/07/17 to 10/19/17.

treatment experienced 83.3% dormancy. The difference between summer 2016 and summer 2017 dormancies across the entire sample was significant (Fisher's Exact Test, $p = 0.0735$), as was the difference in the water cohort from summer to summer (Fisher's Exact Test, $p = 0.0640$). One year after treatment initiation, however, 2018 summer dormancy percentages resembled those of both 2016 (Fisher's Exact Test, $p > 0.9000$) and 2017 summer dormancies (Fisher's Exact Test, $p > 0.1500$), and there was no difference in water cohort dormancies between summers 2016 and 2018, and 2017 and 2018.

Because the control cohort had no individuals present during summer 2017, we were unable to test differences between all years, but in summer 2017 the probability of dormancy was significantly different across treatments (Fisher's Exact Test, $p = 0.0522$). Of the five watered plants aboveground in summer, four had been present for several weeks before treatment initiation and one emerged after two weeks of treatment; the latter and one of the former of which had resisted dormancy the prior summer.

While treatment effects decreased 2017 summertime dormancy as expected, dormancy prevalence was substantially greater overall in fall 2017 (Fisher's Exact Test, $p = 0.0006$) and spring 2018; average monthly temperatures for September and October 2017 were slightly below those of 2016, but excessive rainfall from the hurricane in August 2017 (63 cm) or plant aging may have contributed to the discrepancies. Similarly, fall 2018 dormancy rates were significantly greater than those of fall 2017 (Fisher's Exact Test, $p = 0.0031$). Of the plants that had been dormant in fall 2016, all produced rosettes in spring 2017, none persisted through summer, and all were dormant again in fall 2017, no matter the treatment. These plants followed a similar trend in 2018 except one that had been in the water treatment appeared aboveground in the fall. Despite the steady increase in dormancy between the three fall seasons, water-treated plants continued to have reduced dormancy than control (except in summer 2018) yet there were not many statistical differences; 50% of control and 36.7% of watered plants were dormant in fall 2017 (Fisher's Exact Test, $p = 0.4348$), and 16.7% of control and 6.7% of watered plants were dormant in spring 2018 (Fisher's Exact Test, $p = 0.4238$). Conversely, the difference in treatments in fall 2018 was marginally significant; 83.3% of control and 60% of watered plants were dormant (Fisher's Exact Test, $p = 0.0840$).

Large growth history plants exhibited significantly less dormancy than small in all summer and fall seasons except summers 2016 and 2018, which were not statistically significant (Fisher's Exact Test: summer 2016, $p = 0.1911$; fall 2016, $p = 0.0188$; summer 2017, $p = 0.0662$; fall 2017, $p = 0.0150$; summer 2018, $p = 0.1429$; fall 2018, $p = 0.0168$) (Fig. 5.2b). Summer dormancy percentages in large plants remained constant at 82.6% from 2016 to 2017, but only one (25%) of the summer-emergent plants during these timepoints was consistent between the years. In summer 2018 large plant dormancy rose to 91.3%; again, one emergent plant was consistent between 2017 and 2018, but no plants resisted summer dormancy all three years. Small plant summertime dormancy increased steadily from 94.6% to 100% over time, and only one of the emergent individuals was aboveground in two summers. In the fall reproductive seasons more small plants were dormant than large plants (Fisher's Exact Test: fall 2016, $p = 0.0188$; fall 2017, $p = 0.0150$; fall 2018, $p = 0.0168$), and all small individuals dormant in fall 2016 were also underground in falls 2017 and 2018, except for one which flowered in 2018. Conversely, in spring 2018 more of the large plants (17.4%) were dormant than small (8.1%), but the difference was not significant (Fisher's Exact Test, $p = 0.4116$). It is possible that smaller plants opted for survival over reproduction by postponing inflorescence emergence for a year with more favorable climatic conditions.

By assessing treatment and growth history together, the effects of supplemental water on summer dormancy became more pronounced (Fig. 5.2c). The range of dormancy across cohorts in summer 2016 only spanned about 13 percentage points ($LR\chi_{23} = 2.206$, $p = 0.5308$) whereas during treatment application in 2017 it ranged from 66.7% dormancy in Water Large, 94.4% in Water Small, to 100% dormancy in both Control Large and Control Small groups ($LR\chi_{23} = 11.420$, $p = 0.0097$), and in summer 2018 the range was only 9.1 percentage points ($LR\chi_{23} = 3.951$, $p = 0.2668$). By fall 2017 the four groups had significantly variable rates of reproductive dormancy ($LR\chi_{23} = 8.442$, $p = 0.0377$); Control Small exhibited the highest percent of dormant individuals, followed closely by Water Small. Fall 2018 exhibited a similar trend but with increased dormancy prevalence ($LR\chi_{23} = 11.505$, $p = 0.0093$).

Interestingly, in spring 2018 only the Water Small group had returned to 0% dormancy, while the other groups leveled at 15.8% to 18.2% dormancy, which suggests that small growth history plants with additional resources are better equipped for environmental stress but may still forgo reproduction in favor of long-term survival. Furthermore, all individuals dormant in spring 2018 continued to be dormant in both summer and fall 2018, which insinuates mortality within the population. The interaction between treatment and growth factors for 2018 spring rosette dormancy was insignificant ($LR\chi_{23} = 5.409$, $p = 0.1442$).

Results indicate that large growth history plants have increased reproductive fitness over small plants in the form of greater inflorescence emergence, however, the higher rates of absence in spring and fall 2018 suggest extreme climatic response or even mortality. Historically, most plants do not bypass spring rosette emergence but may do so when under environmental stress and often return aboveground in the next spring. There were no significant differences in dormancy by species for any of the assessed seasons (Fisher's Exact Test, $p > 0.4000$).

As expressed previously, the analyses considered dormancy as the complete lack of reproductive growth. In this section, vegetative rosettes were removed from the dormancy category and evaluated as their own parameter in order to elucidate nonflowering plant behavior in the fall. Rosette counts were taken as those with complete lack of reproductive development and were considered from the time of reproductive growth emergence to the time of peak flowering, after which winter rosette growth becomes widespread across all individuals, flowering or not. Fall seasons were assessed for the percent of individuals present as reproductive structures, vegetative rosettes, and those belowground. More nonflowering plants that received supplemental water came up as rosettes than nonflowering control plants, but all years were marginally or not significantly different between treatments (2016: $LR\chi^2_2 = 5.657$, $p = 0.0591$; 2017: $LR\chi^2_2 = 3.898$, $p = 0.1424$; 2018: $LR\chi^2_2 = 5.859$, $p = 0.0534$) (Fig. 5.3a).

Small and large growth history plant forms were significantly different in each fall assessed (Fig. 5.3b). In fall 2016, the percentage of dormant plants was the greatest difference between the growth history cohorts ($LR\chi^2_2 = 8.487$, $p = 0.0144$), in fall 2017 it was the percentage of rosettes ($LR\chi^2_2 = 7.885$, $p = 0.0194$), and in fall 2018 the percentage of reproductive structures differed the most ($LR\chi^2_2 = 7.313$, $p = 0.0258$). Overall, small plants exhibited greater dormancy but also rosette presence each fall than large plants.

Presence from percentages divided by treatment and growth history cohorts provided further insight (Fig. 5.3c). There were significant differences across cohorts in each year, and the greatest digressions from expected cell counts were noted. In falls 2016 and 2017, more Control Small plants were dormant than expected (2016: $LR\chi^2_6 = 14.120$, $p = 0.0283$; 2017: $LR\chi^2_6 = 11.905$, $p = 0.0641$) and indeed had the greatest dormancy percentages of all the groups. In fall 2018, Water Large plants had more reproductive structures than expected and Water Small had more rosettes

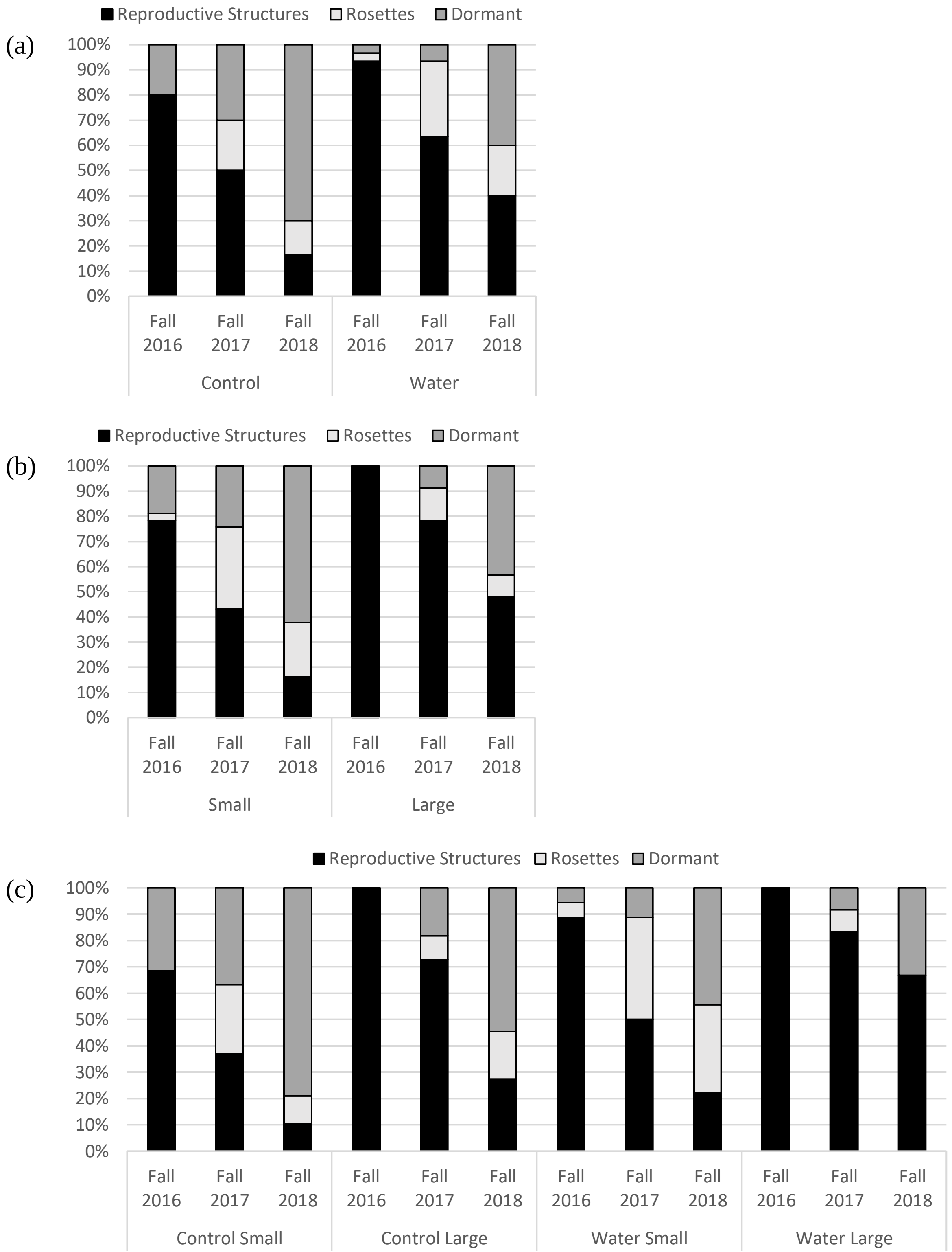

Figure 5.3. Percent reproductive growth, Fall rosettes, and dormancy in Fall 2016 to 2018 by (a) treatment, Control *n* = 30, Water *n* = 30; (b) growth history, Small *n* = 37, Large *n* = 23; and (c) treatment *x* growth history, Control Small *n* = 19, Control Large *n* = 11, Water Small *n* = 18, Water Large *n* = 12.

($LR\chi_{26}$ = 17.713, $p = 0.0070$). It may be inferred that even in fall 2018 the water-treated small and large plants behaved more like they had in fall 2017 after receiving treatments than the control cohorts.

Supplemental water may also affect the timing of emergence in the fall. In August, the month preceding the typical onset of flowering season, sprouting is variable and spike and rosette development cannot be differentiated, so both growth forms were assessed. After excluding the five water-treated plants that evaded summertime dormancy in 2017 (four of large growth, one of small), no plants had yet emerged in treatment week 5 at the beginning of August (Fig. 5.4). By treatment weeks 6 and 7, 5.9% and 11.8% of plants in the study had emerged, all of which were in the Water Small cohort. By week 8, large growth history plants from both control and water treatments had emerged. At week 9, just before sprouting became discernible as either spikes or rosettes, 75% of Water Large, 63.6% of Control Large, and 52.9% of Water Small individuals had emerged aboveground, while 31.6% of the Control Small plants had just begun sprouting.

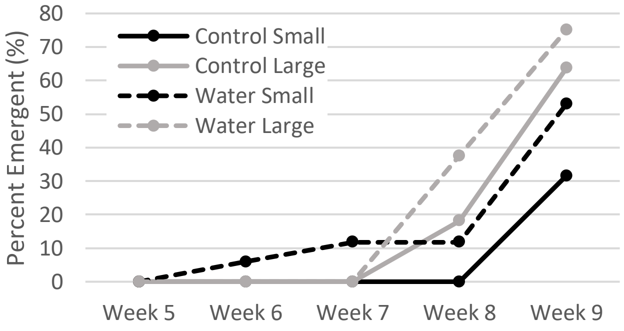

Figure 5.4. Rates of emergence from dormancy from August 3 – 31, 2017 (weeks 5 to 9) by treatment and growth history; five individuals that evaded summer dormancy were excluded (Control Small *n* = 19, Control Large *n* = 11, Water Small *n* = 17, Water Large *n* =8).

Supplemental water appears to have allowed small growth history plants to begin emergence from dormancy approximately three weeks earlier than small plants that did not receive the water treatment. Large plants of both treatments began emerging after week 7, but a greater percentage of the Water Large individuals had broken dormancy by week 9 than those of Control Large, similarly with Water Small versus Control Small cohorts.

The small growth history plant that had eluded dormancy in summers 2016 and 2017 exhibited increased fall plant area (+3.7 cm$_2$), whole-plant height (+6.9 cm), inflorescence length (+5.1 cm), and number of flowers (+18) after receiving the water treatments for 3.5 months in 2017 (Table 5.1). Growth of Water Small plants that had emerged early in August 2017 (weeks 6 and 7, Fig. 5.4) showed mixed results when compared to the prior fall, where after treatment one had increased height (+2.2 cm) but fewer flowers (-10) and the other had lower values all around. Of the large plants that didn't have summer dormancy in 2017 (all of which were water-treated), subsequent fall flowering measurements varied greatly from fall 2016 data. One plant had increased values for all variables from fall 2016 to 2017, while the other three all had reduced plant

Table 5.1. Differences in growth parameters from fall 2016 to fall 2017 amongst individuals that evaded summer dormancy in 2017, summers 2016 and 2017 (denoted by *), and those that exhibited early emergence in weeks 6 and 7 of treatment in summer 2017 (see Fig. 5.4). Each row represents an individual plant.[a]

Small Growth History	PA (cm$_2$)	PH (cm)	IL (cm)	#F
No summer dormancy	+3.7*	+6.9	+5.1	+18
Weeks 6 & 7 emergence	+2.2	+2.4	-1.0	-10
	-2.9	-3.1	-1.8	-7

Large Growth History	PA (cm$_2$)	PH (cm)	IL (cm)	#F
No summer dormancy	+3.9	+2.4	+1.4	+4
	-2.3	-1.7	+0.1	+0
	-4.8	-3.8	+0.8	+3
	-9.2*	-6.4	-1.6	-12

[a] PA = plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

Table 5.2. Fall 2017 flowering and spring 2018 rosette season demographic parameter values after supplemental water treatments. Values represent mean ± SE. In each group and variable, means with the same letter are not significantly different from each other. * denotes α < 0.10.

Fall 2017[a]	PA (cm^2)	PH (cm)	IL (cm)	#F
Control (*n*=12)	19.05 ± 3.25 ***A***	20.65 ± 1.77 ***A***	4.06 ± 0.62 ***A***	15.75 ± 1.82 ***A***
Water (*n*=19)	26.52 ± 2.59 ***B***	25.19 ± 1.40 ***B***	6.67 ± 0.49 ***B***	17.74 ± 1.44 ***A***
Small (*n*=14)	20.06 ± 3.05 ***A***	20.57 ± 1.59 ***A***	5.39 ± 0.67 ***A***	16.64 ± 1.70 ***A***
Large (*n*=17)	26.56 ± 2.77 ***A***	25.79 ± 1.45 ***B***	5.88 ± 0.61 ***A***	17.24 ± 1.54 ***A***
Control Small (*n*=5)	14.61 ± 4.96 ***A****	16.98 ± 2.51 ***A***	3.72 ± 0.98 ***A****	16.00 ± 2.90 ***A***
Control Large (*n*=7)	22.21 ± 4.19 ***B***	23.27 ± 2.13 ***AB***	4.30 ± 0.83 ***A****	15.57 ± 2.45 ***A***
Water Small (*n*=9)	23.08 ± 3.69 ***B****	22.57 ± 1.87 ***AB***	6.32 ± 0.73 ***AB***	17.00 ± 2.16 ***A***
Water Large (*n*=10)	29.61 ± 3.50 ***AB***	27.56 ± 1.78 ***B***	6.98 ± 0.69 ***B****	18.40 ± 2.05 ***A***

Spring 2018[b]	LA (cm^2)	#L	LL (cm)	LW (cm)
Control (*n*=25)	13.43 ± 2.79 ***A***	3.36 ± 0.19 ***A***	9.33 ± 1.10 ***A***	1.05 ± 0.06 ***A***
Water (*n*=28)	15.93 ± 2.63 ***A***	3.64 ± 0.18 ***A***	11.14 ± 1.04 ***A***	1.08 ± 0.06 ***A***
Small (*n*=34)	10.89 ± 2.23 ***A***	3.32 ± 0.16 ***A***	8.42 ± 0.85 ***A***	1.01 ± 0.05 ***A***
Large (*n*=19)	21.65 ± 2.98 ***B***	3.84 ± 0.21 ***B***	13.62 ± 1.14 ***B***	1.17 ± 0.07 ***B***
Control Small (*n*=16)	8.72 ± 3.28 ***A****	3.13 ± 0.23 ***A***	6.94 ± 1.23 ***A***	0.96 ± 0.07 ***A***
Control Large (*n*=9)	21.80 ± 4.38 ***B****	3.78 ± 0.30 ***A***	13.57 ± 1.64 ***B***	1.22 ± 0.10 ***A***
Water Small (*n*=18)	12.82 ± 3.09 ***AB***	3.50 ± 0.21 ***A***	9.73 ± 1.16 ***AB***	1.05 ± 0.07 ***A***
Water Large (*n*=10)	21.52 ± 4.15 ***AB***	3.90 ± 0.29 ***A***	13.67 ± 1.55 ***B***	1.12 ± 0.09 ***A***

[a] PA = plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

[b] LA = rosette leaf area, #L = number of leaves, LL = leaf length, LW = leaf width.

area, were shorter by about 2 to 7 cm, and exhibited mixed results for number of flowers: +3, +0, -12, the latter of which pertained to the individual that had evaded dormancy in both summers. Large plants that emerged in week 8 were also smaller in fall 2017 (-4 to -7.5 cm) and had fewer flowers (-4 to -11) than their 2016 records.

Supplemental Water Effects on Demographic Data

Treatment and growth history not only affected dormancy, but also demographic growth variables of individuals. Due to drastic variabilities across the years of data that may have been caused by climatic, edaphic, and/or genetic factors, it was more feasible to compare treatment effects on demographic parameters within each assessed season rather than with prior years (see Chapter III). In the 2017 fall flowering season that followed the supplemental water experiment, three of the four measured demographic parameters displayed statistically significant differences by treatment (Table 5.2). Mean plant area (Wilcoxon $Z = -1.9874$, $p = 0.0469$) (Fig. 5.5), plant height (Wilcoxon $Z = -2.1093$, $p = 0.0349$), and inflorescence length ($t = 3.3112$, $p = 0.0025$) of the watered cohort were significantly larger than those of the control cohort. There was no significant difference between the number of flowers produced by control and watered individuals ($t = 0.8564$, $p = 0.3988$). Plants with large growth history had significantly greater plant heights than small (Wilcoxon $Z = -2.2630$, $p = 0.0236$), but no significant differences were found in inflorescence lengths ($t = -0.5366$, $p = 0.5956$) and number of flowers ($t = -0.2580$, $p = 0.7983$). These results were reflected when the demographics were broken down into combined treatment and growth factors. Whole plant area of Control Small individuals was significantly lower than that of Control Large and Water Small ($\chi_{23} = 7.9486$, $p = 0.0471$; Steel-Dwass $Z = -2.5984$, $p = 0.0462$ and Steel-Dwass $Z = 2.4000$, $p = 0.0771$, respectively). Water Large had the greatest plant area mean but was not significantly different from Control Small due to the rank-based nature of the nonparametric statistical tests. Control Small plant heights were significantly less than those of Water Large individuals ($F_{3,27}$ = 4.0646, p = 0.0166; Tukey-Kramer, p = 0.0098). Additionally, Control Small and Control Large inflorescence lengths were both significantly lower than those of Water Large ($F_{3,27}$ = 3.6929, p = 0.0239; Tukey-Kramer, p = 0.0521 and p = 0.0855, respectively). Number of flowers was insignificant between groups ($F_{3,27}$ = 0.3072, p = 0.8199). Supplemental water rather than growth history appeared to have the greatest effect on fall 2017 reproductive structure growth. Incidentally, many of the control cohort flowering stalks began to senesce much earlier than those that had received water treatments, creating the steep decline in plant area seen in Fig. 5.5 around early October 2017.

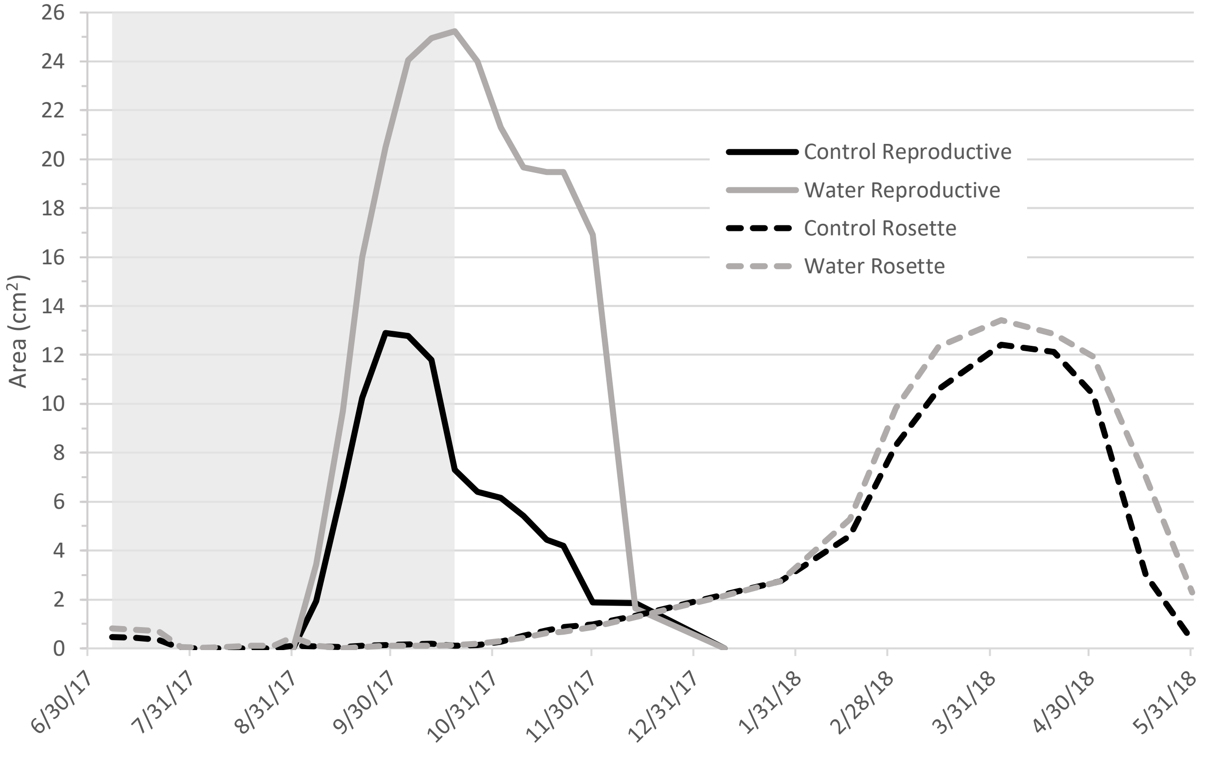

Figure 5.5. Average fall 2017 reproductive plant areas and spring 2018 rosette leaf areas by treatment. Shaded area indicates treatment period, from 07/07/17 to 10/19/17. Fall: Control n = 15, Water n = 19. Spring: Control n = 25, Water n = 28.

Spring 2018 rosette parameters displayed no significant differences across treatment groups (Fig. 5.5), but all variables were significantly different between growth histories (Table 5.2). Plants that had histories of large growth had significantly larger mean values as rosettes in all measured variables than those with histories of small growth ($p < 0.05$). Treatment and growth groups combined exhibited fewer differences. The Control Small group had a mean leaf area significantly lower than that of Control Large ($\chi_{2}3 = 9.7575$, $p = 0.0207$; Steel-Dwass $Z = -2.2929$, $p = 0.0996$), and Control Small mean leaf length was significantly shorter than those of Control Large and Water Large ($F_{3,49} = 5.4581$, $p = 0.0026$; Tukey-Kramer, $p = 0.0073$ and $p = 0.0114$, respectively). These findings suggest that growth history rather than treatment influenced spring rosette growth. In both fall 2017 and spring 2018, Water Large plants exhibited a wide range of values in the majority of parameters so fewer differences were significant than expected.

Growth is variable year to year, but reductions or gains in flowering stalk height are often offset by increased or reduced rosette leaf lengths in the next. However, only 61.9% of the large cohort continued to fit the large growth history size classifications in the fall and spring seasons after supplemental water treatments. Large plants in the 2017 flowering season had relatively short plant heights compared to fall 2016, with the majority (83.3%, $n = 18$) under 30 cm tall whereas 73.9% ($n = 23$) were 30 cm or taller in the year prior. Large plant rosette leaf lengths generally increased in spring 2018 compared to spring 2017, where 68.4% ($n = 19$) of plants had longer maximum leaf lengths. Smaller plants tended to remain small overall; 100% ($n = 29$) of plants were under 30 cm tall in fall 2016 and 93.8% ($n = 16$) stayed below this level in fall 2017. And similar to the large cohort, 68.8% of small plants had shorter flowering stalks in fall 2017 than fall 2016 and 73.5% ($n =34$) exhibited longer rosette leaves in spring 2018 than in the prior spring, but generally (76.5%) remained under 10 cm in length.

Table 5.3. Referenced treatment terminology during the supplemental water study and the associated dates and applicable sample sizes (*n*).

	Dates	*n*
Pretreatment	7/7/17 - 10/19/17	56
Post-treatment (3-4 hrs)	7/7/17 - 10/19/18	28
Post-experiment	10/20/17 - 5/1/18	56
Summer	7/7/17 - 8/31/17	56
Fall	9/1/17 - 11/30/17	56
Spring	12/1/17 - 5/1/18	56

Soil Moisture and Microhabitat Characteristics

During the supplemental water study, soil moisture readings were taken once a week on all plants (pretreatment), on the treated plants three to four hours after treatment (post-treatment), and again on all plants every one to two weeks after treatment cessation (post-experiment). Due to the overlap of the treatment period with summer dormancy and fall reproductive stages in the *Spiranthes* growth cycle, pretreatment and post-experiment volumetric water content averages were also divided into summer, fall, and spring seasons (Table 5.3).

Comparison of *S. parksii* and *S. cernua* soil moistures from treatment initiation in July 2017 to observation cessation in May 2018 indicated that volumetric water content around *S. parksii* remained almost consistently lower than *S. cernua* (Fig. 5.6a). Analysis of *S. parksii* values confirmed this, and were significantly lower in pretreatment (Wilcoxon $Z = 3.2968$, $p = 0.0010$), post-experiment ($t = 3.3032$, $p = 0.0017$), and all three indicated seasons (summer: Wilcoxon $Z = -3.4700$, $p = 0.0005$; fall: Wilcoxon $Z = -2.7949$, $p = 0.0052$; spring: $t = 3.4689$, $p = 0.0010$) (Table 5.4). Hurricane Harvey resulted in the large peak in soil moisture in late August seen in Fig. 5.6. Growth history comparisons were less variable, and often the soil moisture

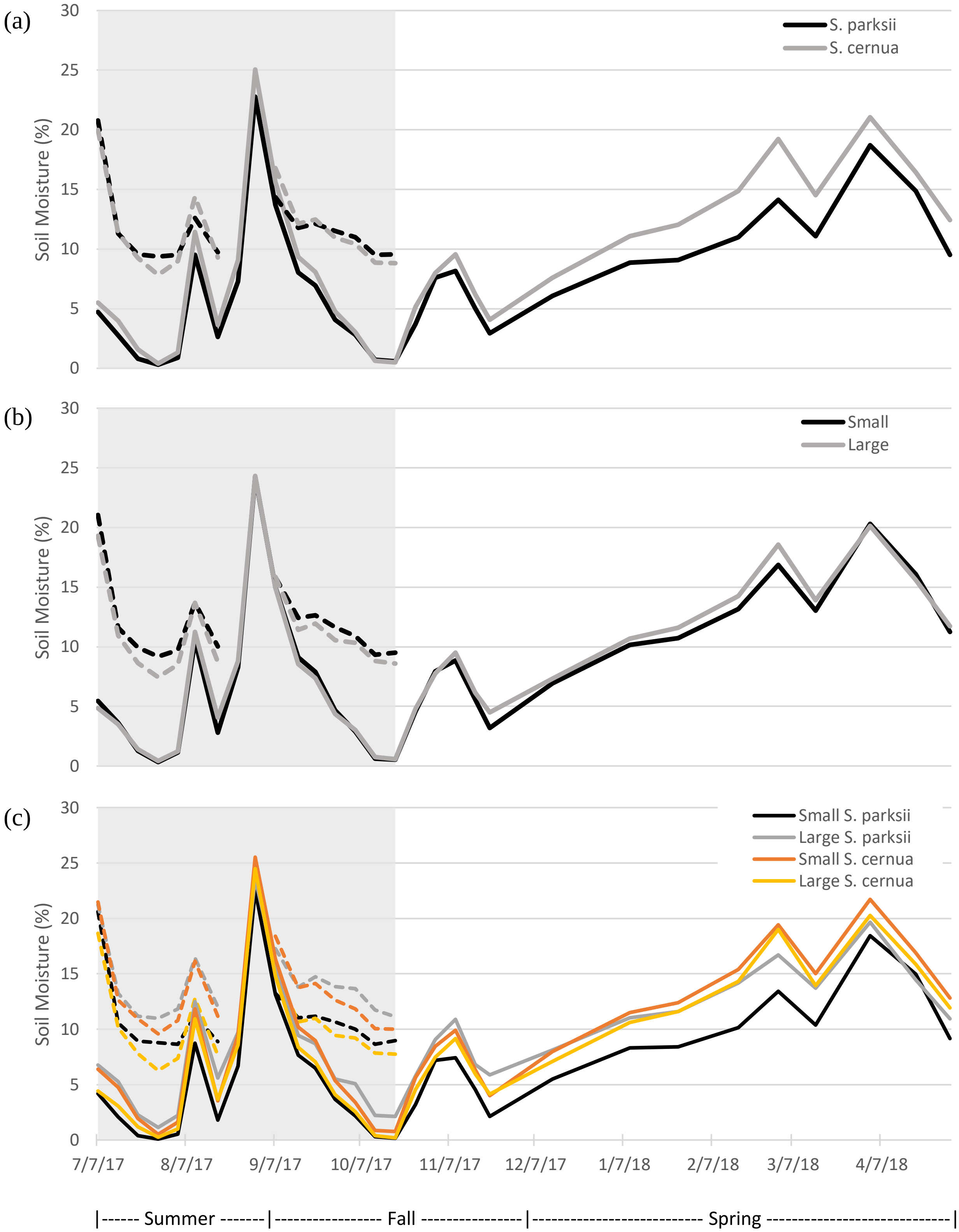

Figure 5.6. Average soil moisture values by (a) species, (b) growth history, and (c) species and growth history. Shaded area indicates treatment period. Solid and dashed lines within treatment period indicate pretreatment and post-treatment values, respectively. Solid lines after treatment period are post-experiment averages. Referenced seasons are indicated. Sample sizes in Tables 5.3 and 5.4.

Table 5.4. Soil moisture means by species and growth history, with soil moisture samples divided into treatment phases and seasons. Values represent mean ± SE (*n*). Rows not connected by the same letter are significantly different (∝ = 0.05).

Treatment Phases	Pretreatment (%)	Post-treatment (%)	Post-experiment (%)
S. parksii	5.53 ± 0.38 ***A*** (*19*)	10.91 ± 0.83 ***A*** (*11*)	9.34 ± 0.55 ***A*** (*19*)
S. cernua	6.49 ± 0.27 ***B*** (*37*)	10.88 ± 0.67 ***A*** (*17*)	11.59 ± 0.40 ***B*** (*37*)
Large	6.21 ± 0.38 ***A*** (*21*)	10.42 ± 0.78 ***A*** (*12*)	11.17 ± 0.57 ***A*** (*21*)
Small	6.14 ± 0.29 ***A*** (*35*)	11.25 ± 0.68 ***B*** (*16*)	10.62 ± 0.44 ***A*** (*35*)
Large *S. p*	7.36 ± 0.77 ***AB*** (*4*)	13.22 ± 1.38 ***AB*** (*3*)	11.35 ± 1.17 ***AB*** (*4*)
Small *S. p*	5.04 ± 0.40 ***A*** (*15*)	10.05 ± 0.85 ***A*** (*8*)	8.81 ± 0.61 ***A*** (*15*)
Large *S. c*	5.94 ± 0.37 ***AB*** (*17*)	9.48 ± 0.80 ***A*** (*9*)	11.13 ± 0.57 ***B*** (*17*)
Small *S. c*	6.97 ± 0.35 ***B*** (*20*)	12.46 ± 0.85 ***B*** (*8*)	11.97 ± 0.52 ***B*** (*20*)

Seasons	Summer (%)	Fall (%)	Spring (%)
S. parksii (*n* = 19)	5.74 ± 0.37 ***A***	5.36 ± 0.38 ***A***	11.48 ± 0.68 ***A***
S. cernua (*n* = 37)	6.88 ± 0.26 ***B***	6.24 ± 0.27 ***B***	14.36 ± 0.48 ***B***
Large (*n* = 21)	6.65 ± 0.37 ***A***	6.02 ± 0.37 ***A***	13.75 ± 0.71 ***A***
Small (*n* = 35)	6.41 ± 0.29 ***A***	5.90 ± 0.29 ***A***	13.16 ± 0.55 ***A***
Large *S. p* (*n* = 4)	7.68 ± 0.74 ***AB***	7.26 ± 0.76 ***AB***	13.38 ± 1.46 ***AB***
Small *S. p* (*n* = 15)	5.23 ± 0.38 ***A***	4.85 ± 0.39 ***A***	10.97 ± 0.75 ***A***
Large *S. c* (*n* = 17)	6.41 ± 0.36 ***B***	5.72 ± 0.37 ***AB***	13.84 ± 0.71 ***B***
Small *S. c* (*n* = 20)	7.29 ± 0.33 ***B***	6.69 ± 0.34 ***B***	14.80 ± 0.65 ***B***

averages of small and large growth plants overlapped (Fig. 5.6b); only post-treatment values within the treatment period displayed significant differences, of which the soil around small plants retained more moisture three to four hours after treatment than the soil around large plants (Wilcoxon $Z = 2.1587$, $p = 0.0309$) (Table 5.4). Analysis of species factored by growth history provided more expressive results, and all treatment phases and seasons exhibited significant interactions between species and growth, suggesting that soil moisture may influence large or small growth, depending on the species (Fig. 5.6c).

Soil of small *S. parksii* consistently retained the lowest moisture averages in pretreatment, post-experiment, and seasonal variables. Small *S. parksii* soil moisture was significantly lower than that of both small and large *S. cernua* in the summer ($\chi^2_3 = 18.7591$, $p = 0.0003$; Steel-Dwass $Z = -4.0170$, $p = 0.0003$ and Steel-Dwass $Z = -2.8144$, $p = 0.0252$, respectively), small *S. cernua* in the fall ($\chi^2_3 = 14.8574$, $p = 0.0019$; Steel-Dwass $Z = -3.9010$, $p = 0.0006$), and small and large *S. cernua* in the spring ($F_{3,52} = 5.1517$, $p = 0.0034$; Tukey-Kramer, $p = 0.0018$ and Tukey-Kramer, $p = 0.0367$, respectively) (Table 5.4). In post-treatment measurements, small *S. cernua* soil moisture was significantly greater several hours after water deposition than both small *S. parksii* and large *S. cernua* ($\chi^2_3 = 12.6496$, $p = 0.0055$; Steel-Dwass $Z = -2.7831$, $p = 0.0276$ and Steel-Dwass $Z = 3.2235$, $p = 0.0069$, respectively). Large *S. parksii* displayed no significant differences from the other three groups in all seasons and treatment phases, but retained soil moisture averages closer to those of *S. cernua* groups than its own species' counterpart; however, the sample of large *S. parksii* was considerably lower and more varied than that of the other groups, so it may not be meaningful to make inferences on large *S. parksii* from this dataset alone.

Microhabitat parameters showed fewer degrees of variation in regards to species and plant growth (Table 5.5). There were no significant differences in infiltration rate amongst the four species-growth groups ($F_{3,26} = 0.3123$, $p = 0.8163$), and neither of the main effects were significant ($p > 0.40$). Scores of claypan depth measurements indicated that *S. parksii* were located in areas where the claypan was significantly deeper than areas inhabited by *S. cernua* (Wilcoxon $Z = 2.6989$, $p = 0.0070$). Similarly, small *S. parksii* claypan depth was significantly deeper than that of small *S. cernua* ($\chi^2_3 = 10.1046$, $p = 0.0177$; Steel-Dwass $Z = 2.8581$, $p = 0.0222$). Slope percent at 30 cm run length yielded significant differences by growth history (Wilcoxon $Z = 2.1610$, $p = 0.0307$), where large plants grew on significantly steeper slopes than small plants. Slope percent

Table 5.5. Microhabitat parameter means by species and growth history. Values represent mean ± SE (*n*). Rows not connected by the same letter are significantly different (∝ = 0.05).

	Infiltration (sec)	Claypan (cm)	Slope, 30 cm (%)	Slope, 1 m (%)
S. parksii	104.54 ± 16.08 ***A*** (*12*)	40.40 ± 4.16 ***A*** (*21*)	8.98 ± 1.68 ***A*** (*21*)	6.55 ± 1.66 ***A*** (*21*)
S. cernua	111.97 ± 13.13 ***A*** (*18*)	26.17 ± 3.05 ***B*** (*39*)	10.55 ± 1.23 ***A*** (*39*)	9.45 ± 1.22 ***B*** (*39*)
Large	119.25 ± 15.92 ***A*** (*12*)	30.48 ± 4.22 ***A*** (*23*)	11.99 ± 1.58 ***A*** (*23*)	10.60 ± 1.58 ***A*** (*23*)
Small	102.17 ± 13.00 ***A*** (*18*)	31.56 ± 3.33 ***A*** (*37*)	8.77 ± 1.24 ***B*** (*37*)	7.09 ± 1.24 ***B*** (*37*)
Large *S. parksii*	128.67 ± 32.86 ***A*** (*3*)	29.85 ± 8.37 ***AB*** (*5*)	15.67 ± 3.34 ***A*** (*5*)	8.02 ± 3.40 ***AB*** (*5*)
Small *S. parksii*	96.5 ± 18.97 ***A*** (*9*)	43.70 ± 4.68 ***A*** (*16*)	6.90 ± 1.87 ***A*** (*16*)	6.09 ± 1.90 ***A*** (*16*)
Large *S. cernua*	116.11 ± 18.97 ***A*** (*9*)	30.66 ± 4.41 ***AB*** (*18*)	10.96 ± 1.76 ***A*** (*18*)	11.32 ± 1.79 ***B*** (*18*)
Small *S. cernua*	107.83 ± 18.97 ***A*** (*9*)	22.32 ± 4.09 ***B*** (*21*)	10.19 ± 1.63 ***A*** (*21*)	7.85 ± 1.66 ***AB*** (*21*)

Table 5.6. Multivariate correlation between soil moisture and microhabitat parameters, compared by demographic characterization. Values represent Spearman ρ estimations and number of individuals contributing to the statistic (*n*). Significant correlations are noted by + $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

	Pretreatment	Post-treatment	Post-experiment	Infiltration	Claypan	Slope, 30 cm
Post-treatment	**0.55** (*28*) **					
Post-experiment	**0.85** (*56*) ***	**0.37** (*28*) +				
Infiltration	**0.33** (*28*) +	0.18 (*28*)	0.18 (*28*)			
Claypan	**-0.52** (*56*) ***	**-0.45** (*28*) *	**-0.51** (*56*) ***	**-0.48** (*30*) **		
Slope, 30 cm	-0.09 (*56*)	-0.24 (*28*)	-0.62 (*56*)	0.03 (*30*)	-0.20 (*60*)	
Slope, 1 m	0.02 (*56*)	-0.30 (*28*)	0.13 (*56*)	0.20 (*30*)	**-0.25** (*60*) +	**0.57** (*60*) ***

of one-meter run length continued to support this variation by growth history (Wilcoxon $Z = 1.9846$, $p = 0.0472$), and also indicated that *S. cernua* resided on significantly steeper slopes than *S. parksii* (Wilcoxon $Z = 2.7128$, $p = 0.0067$). Additionally, large *S. cernua* slope scores were significantly greater than those of small *S. parksii* ($\chi^2_3 = 11.1001$, $p = 0.0112$; Steel-Dwass $Z = -3.2626$, $p = 0.0061$).

Soil moisture and microhabitat parameter values were assessed further to determine whether edaphic correlations occurred as expected, or if the study plants exhibited sporadic tendencies that might instead be due to unassessed factors (Table 5.6). Many of the multivariate Spearman ρ correlation estimations between soil moisture parameters were significant, strong, and positive across factors as was expected due to predictable and consistent soil behavior across the study site in response to time, precipitation, and temperature. Likewise, the correlation between infiltration rates and pretreatment soil moisture percentages were not surprising as infiltration measurements were obtained during the treatment phases. It is suspected that seasonal changes in edaphic properties led to the lack of correlation between post-experiment soil moistures and infiltration rates. Areas with lower pretreatment soil moisture therefore tended to have faster infiltration rates and deeper claypans, which indicated a higher percentage of sand in the top 12 cm of soil. Slope at 30 cm run length displayed no significant correlations; one-meter slope length was significantly positively correlated to 30-cm slope, but also negatively correlated to claypan depth. Therefore, steeper slopes of one-meter length had shallower claypans, but shorter slope distances within the meter may have variable claypan depths due to surface disturbance. *Spiranthes parksii* are known to thrive in moderately disturbed soil (Wilson 2002) and due to the sandy nature of the soil upon which they are found, wind, precipitation, runoff, and wildlife are constantly disrupting the surface.

Multivariate analyses were performed on moisture, microhabitat, and fall 2017 demographic growth variables to determine whether edaphic characteristics had a role in determining growth (Table 5.7). Despite the removal of one additional outlier in both *S. parksii* and *S. cernua* soil moisture parameters, minimal significant correlations were found. Post-treatment moisture percentages were positively and significantly related to *S. parksii* reproductive structure plant area, inflorescence length, and number of flowers, indicating that individuals in soil that retained more moisture from July to October were able to increase their growth. *S. cernua* exhibited a negative correlation between post-treatment moisture and plant height, so higher moisture levels after water treatment may have actually decreased the plants' potential to grow taller. Similarly, cooler and drier conditions in the post-experiment phase possibly led to the significantly positive relation between soil moisture and plant area in *S. cernua* and control plants. Slope at 30 cm and inflorescence length of *S. parksii* indicated a significant negative relationship, where increasingly steep slopes led to shorter inflorescences.

Analyses of spring 2018 demographic parameters with multivariate techniques showed that rosette leaf area and leaf width were negatively related to pretreatment soil moisture within the water cohort (Table 5.8). Generally, soil moisture percentages returned to baseline levels between water applications, but drought and hurricane climatic factors during the pretreatment phase caused pretreatment values to fluctuate drastically and may have led to the negative relation with spring growth. Post-treatment soil moisture relations to spring growth was similar to that of growth in fall 2017; rosette leaf area, length, and width were significantly positively correlated in *S. parksii* and negatively correlated in *S. cernua*. Again, soil with greater water-holding capacity appeared to assist *S. parksii* growth while hindering *S. cernua* rosettes. Increasing claypan depth produced significantly negative correlations from *S. parksii* rosette growth; results suggest that the shallower

Table 5.7. Multivariate correlations between 2017 fall flowering demographics and soil moisture and microhabitat parameters. Spearman ρ and Pearson's correlations were utilized. Significant values are indicated by + $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.

		PA	PH	IH	#F	*n*
Pretreatment	*S. parksii*	0.38	0.31	0.50	0.24	*8*
	S. cernua	0.18	-0.16	0.25	0.13	*18*
	Large	0.05	-0.24	0.18	-0.1	*15*
	Small	0.00	-0.13	-0.13	-0.02	*13*
	Water	-0.26	-0.35	-0.13	-0.23	*18*
	Control	0.30	0.07	-0.02	0.35	*10*
	All	0.01	-0.14	0.06	-0.07	*28*
Post-treatment	*S. parksii*	**0.71**+	0.50	**0.89****	**0.92****	*7*
	S. cernua	-0.50	**-0.82****	-0.30	-0.30	*9*
	Large	-0.08	-0.10	0.27	0.26	*10*
	Small	-0.33	-0.36	-0.14	0.00	*8*
	Water	-0.19	-0.33	0.13	0.20	*18*
	Control	N/A	N/A	N/A	N/A	*0*
	All	-0.19	-0.33	0.13	0.20	*18*
Post-experiment	*S. parksii*	0.05	0.00	0.00	0.12	*8*
	S. cernua	**0.53***	0.27	0.37	-0.06	*18*
	Large	0.01	-0.23	-0.06	-0.28	*15*
	Small	-0.04	-0.10	-0.11	-0.09	*13*
	Water	-0.26	-0.21	-0.28	-0.32	*18*
	Control	**0.56**+	0.31	0.21	-0.27	*10*
	All	0.1	0.03	-0.01	-0.20	*28*
Infiltration	*S. parksii*	-0.17	-0.33	0.11	-0.25	*8*
	S. cernua	-0.01	-0.03	-0.28	-0.24	*11*
	Large	-0.12	0.03	-0.03	-0.15	*10*
	Small	0.03	-0.37	-0.43	-0.36	*9*
	All	-0.13	-0.09	-0.19	-0.25	*19*
Claypan	*S. parksii*	-0.04	0.08	-0.16	-0.32	*10*
	S. cernua	0.00	0.09	0.05	-0.24	*21*
	Large	0.35	0.34	0.27	-0.13	*17*
	Small	-0.39	-0.23	-0.27	-0.41	*14*
	All	0.00	0.09	0.01	-0.25	*31*
Slope, 30 cm	*S. parksii*	-0.32	-0.35	**-0.68***	-0.20	*10*
	S. cernua	-0.08	-0.07	-0.01	0.06	*21*
	Large	-0.14	-0.22	-0.29	0.09	*17*
	Small	-0.24	-0.20	-0.3	-0.42	*14*
	All	-0.12	-0.15	-0.27	-0.07	*31*
Slope, 1 m	*S. parksii*	-0.04	-0.15	-0.04	0.13	*10*
	S. cernua	-0.01	0.08	0.03	-0.03	*21*
	Large	-0.18	-0.12	-0.26	0.07	*17*
	Small	0.10	0.12	0.14	-0.12	*14*
	All	0.01	0.04	-0.08	0.00	*31*

Table 5.8. Multivariate correlations between 2018 spring rosette demographics and soil moisture and microhabitat parameters. Spearman ρ and Pearson's correlations were utilized. Significant values are indicated by + $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.

		LA	#L	LL	LW	*n*
Pretreatment	*S. parksii*	-0.12	-0.07	-0.02	-0.08	*17*
	S. cernua	0.02	0.13	-0.01	-0.11	*30*
	Large	0.03	0.10	0.01	-0.01	*17*
	Small	-0.21	-0.16	-0.20	-0.26	*32*
	Water	**-0.38**+	-0.13	-0.30	**-0.43***	*26*
	Control	-0.02	-0.06	-0.04	-0.01	*23*
	All	-0.17	-0.09	-0.15	-0.19	*49*
Post-treatment	*S. parksii*	**0.68***	-0.34	**0.76***	**0.64***	*10*
	S. cernua	**-0.46**+	-0.21	**-0.46**+	**-0.46**+	*14*
	Large	0.03	0.09	0.04	0.10	*10*
	Small	-0.08	-0.05	0.07	-0.26	*16*
	Water	-0.16	-0.04	-0.08	-0.12	*26*
	Control	N/A	N/A	N/A	N/A	*0*
	All	-0.16	-0.04	-0.08	-0.12	*26*
Post-experiment	*S. parksii*	0.05	0.03	0.01	0.1	*17*
	S. cernua	0.20	0.25	0.17	0.05	*30*
	Large	0.18	0.22	0.04	0.04	*17*
	Small	-0.20	-0.15	-0.22	-0.20	*32*
	Water	-0.28	-0.04	-0.20	-0.33	*26*
	Control	0.18	-0.03	0.11	0.20	*23*
	All	-0.03	0.02	-0.03	-0.08	*49*
Infiltration	*S. parksii*	-0.13	-0.17	0.04	-0.21	*12*
	S. cernua	0.06	-0.19	-0.04	-0.20	*16*
	Large	-0.24	-0.44	0.02	-0.36	*10*
	Small	0.12	0.03	-0.05	-0.09	*18*
	All	-0.01	-0.11	0.01	-0.17	*28*
Claypan	*S. parksii*	**-0.63****	**-0.40**+	**-0.67****	-0.38	*20*
	S. cernua	0.20	0.04	0.27	0.18	*33*
	Large	0.02	-0.21	-0.09	0.04	*19*
	Small	-0.16	0.00	-0.07	-0.02	*34*
	All	-0.07	-0.05	-0.05	-0.01	*53*
Slope, 30 cm	*S. parksii*	-0.06	-0.01	-0.03	-0.21	*20*
	S. cernua	0.18	0.25	0.18	0.23	*33*
	Large	-0.04	0.16	-0.05	0.01	*19*
	Small	0.09	0.06	0.05	0.03	*34*
	All	0.09	0.12	0.09	0.06	*53*
Slope, 1 m	*S. parksii*	0.12	0.14	0.05	0.16	*20*
	S. cernua	0.19	0.17	0.19	0.24	*33*
	Large	0.04	0.05	0.15	0.01	*19*
	Small	0.11	0.04	0.05	0.20	*34*
	All	0.15	0.10	0.13	0.20	*53*

the claypan, the larger, longer, and wider the rosettes became. Interestingly, no significant correlations between edaphic parameters and demographic measurements were found for small and large growth histories in fall 2017 and spring 2018.

Discussion

Studies in population ecology are essential for endangered species persistence, particularly for geophytes like *Spiranthes parksii* that spend intermittent periods of time belowground to either evade harsh climatic conditions or restore nutrient supplies after expensive reproductive growth, all the while exhibiting continued but slow root growth. The more data collected and inferences made regarding the species' behavior, the more conservation efforts can be specifically tailored to protect populations by augmenting their chances of survival or determining suitable transplant sites. In this study, population dynamics of a subsample of 60 *S. parksii* and *S. cernua* individuals were investigated in an attempt to understand how their responses to supplemental water treatment, past growth behavior, and natural habitat influenced the growth cycle phases of summer dormancy, flowering, and vegetative growth.

After delivery of supplemental water to 30 plants once a week from early July to mid-October 2017, summer dormancy prevalence was significantly reduced in large watered individuals while large untreated plants reached 100% dormancy (Fig. 5.2). Likewise, small watered plants remained at the same percentage of dormancy in summers 2016 and 2017, while the small untreated group increased to 100%. It is possible that without treatment both watered small and large groups would have been at or close to 100% summer dormancy like their control group counterparts. Jäkäläniemi et al. (2011) found similar behavior in the terrestrial orchid *Epipactis atrorubens*, where dormancy was more common at a dry rather than mesic site. The effects of supplemental water on summer dormancy appeared to diminish one year after treatment, as cohort dormancies in summer 2018 returned to the same pattern seen in summer 2016 of large rather than watered individuals with the lowest dormancy rate; although, latent effects may have led to the capability of large watered individuals to be at least twice as prevalent aboveground in fall 2018 when average reproductive dormancy was at its highest rate yet recorded. Analyses concerning summer and fall 2018 should be considered lightly, however, as only one data collection date per season contributed to the results; therefore, presence counts are perhaps lower than would have been found with more data observations. Additionally, water-treated individuals exhibited reduced fall dormancy both before and after supplemental water treatments took place, possibly indicating unforeseen bias despite randomized assignment of individuals to treatments.

By altering the previous definition of dormancy to exclude vegetative rosettes in the fall seasons, more details on nonflowering plant behavior were elucidated. Comparisons of falls 2016 through 2018 by treatment showed that from year to year, rates of dormancy, rosettes, and reproductive growth in the control cohort differed significantly as overall reproductive growth declined (Fig. 5.3). The same comparisons in the water cohort yielded no significant differences, which indicated that the three growth phases changed as would be expected over time. Therefore, control cohort dormancy prevalence increased at a rate much faster than was expected from the data; this implied that water treatments helped to sustain higher percentages of both reproductive growth and fall rosettes. Overall, almost half of small plant aboveground presence in falls 2017 and 2018 was as rosettes whereas in the same years, far smaller percentages of large plants were present as rosettes. Small plants perhaps favor rosette development over absolute fall dormancy because it offers the chance to obtain photosynthates.

The Control Small group began fall 2016 with only about 68% reproductive growth whereas Water Small was closer to 90%. Regardless, when plants were both small and watered, fall rosette development exceeded that of Control Small plants, especially in falls 2017 and 2018. Control Large and Water Large groups both had 100% reproductive growth in 2016 and so offer a more accurate display of treatment effects; the increase in dormancy over time was much greater in Control Large plants, but in 2018 the group also displayed a great increase in rosettes that was not seen in the watered counterparts. Water treatment did not inhibit the downward trends in reproductive growth from 2016 to 2018 but instead lessened the slope of the decrease in Water Large plants. Additionally, fall rosettes in Water Small plants far outnumbered those of the Control Small Group, especially in 2018. It is possible that the effects of supplemental water application in summer and early fall 2017 not only affected inflorescence and rosette counts in 2017 but also had latent effects on plants in 2018.

By encouraging aboveground growth during summer months when vegetative dormancy normally occurs, it is possible that fitness increased; all 2017 summer emergent plants maintained upper-range measurements in flowering plant height and rosette leaf lengths in the subsequent fall and spring seasons. However, reproductive fitness was not affected as predicted since the number of flowers in summer-emergent and early-emergent plants varied widely from negative to positive differences from the year prior, and the number of flowers was no different from the rest of the population (Table 5.1). Delivery of water during hot summer months perhaps allowed earlier development of new tubers that typically initiate in the fall, thus providing plants with more resources and a higher chance of flowering (Ariza 2013). Supplemental water also allowed small plants to emerge up to three weeks earlier than the majority of plants which typically only began sprouting in the week prior to reproductive structure or fall rosette determination (Fig. 5.4). The additional access to photosynthetic nutrients from early emergence may give the small watered plants an advantage in future seasons or overall survival, but more research is needed.

Dormancy across all groups rose in fall 2017 compared to 2016, and as prior research has shown, the proportion of dormant plants in a given year is often correlated to climatic conditions, herbivory, or reproductive costs prior to or during the growing season (Miller et al. 2004; Hutchings 2010; Jäkäläniemi et al. 2011; Shefferson et al. 2018), therefore the unexpected overall increase in fall 2017 dormancy may have been influenced by a number of natural phenomena occurring anytime from the prior fall through the onset of the reproductive season. Furthermore, Jäkäläniemi et al. (2011) suggested that reproductive emergence that differs stochastically among individuals may be a method to facilitate outbreeding among different conspecifics, so fluctuating dormancy prevalence might be affected by both weather and genetics. Small plants were more likely to go dormant in the summer and fall than large, which suggests that larger, more robust individuals can adapt more efficiently to environmental stochasticity and that small plants might not risk reproduction until they have accrued enough resources to compensate the physiological costs and herbivore vulnerability of flowering (Shefferson et al. 2003; Shefferson et al. 2005; Jäkäläniemi et al. 2011). Small plants that regularly fluctuate between sprouting and dormancy most often remain small in stature upon emergence and in some species, this allows higher survival; consistently large and emergent plants have also been found to have high survival (Jäkäläniemi et al. 2011; Shefferson et al. 2017), but the atypical and drastically increased levels of dormancy in spring 2018 and fall 2018 (Fig. 5.2) might be a sign of reduced plant fitness and/or impending mortality. Based on the few seasons of data assessed, it can be inferred that supplemental water in the summer had a positive influence on emergence in the summer and fall for both small and large plants, with possible residual effects on fall presence one year later.

Results are less clear for the spring season as dormancy rates were unprecedentedly high, so future data on individuals dormant in spring 2018 will be of interest for reemergence and survival records.

From August 23 to 29, 2017, Hurricane Harvey deposited 55.5 cm of rain upon the study site and led to a monthly sum of 63 cm of precipitation during the supplemental water treatment period. Despite the prodigious amount of rainfall received by not only water treated plants but also the controls, growth in treatment cohorts responded quite differently (Fig. 5.2). Flowering plant area, whole height, and inflorescence length in fall 2017 were all significantly greater in the water cohort than the control, but there were no differences in amount of flowers produced. Conversely, only average plant height was significantly different between small and large plants in fall 2017, signifying that the size of the individual does not lead to greater flower production.

Due to the additional growth in the treated cohort, it was expected that future rosette growth might be limited. However, treatment effects did not continue into the spring rosette season during which only large and small growth history effects were significantly different. In perennial geophytes reproduction is considered a high-cost state (Primack & Stacey 1998; Shefferson et al. 2003; Jacquemyn & Hutchings 2010), but this was not evident in *S. parksii* nor *S. cernua* from assessments of the subsequent spring; therefore, it is possible that the full effects of larger growth in treated plants in fall 2017 may not become evident until fall 2018. In addition, over half of the large plants continued to exhibit growth measurements from fall 2017 to spring 2018 that kept them classified as large, and the majority of small plants stayed within small growth classifications. As growth classes weighed more heavily upon past flowering plant heights rather than rosette leaf lengths within the two years prior to the treatment period, it is possible that more large category plants will regain their typical spike heights of greater than or equal to 30 cm by the next fall if environmental conditions are favorable.

Contrary to previous studies, edaphic assessments determined that soil around *S. parksii* exhibited lower volumetric water content than that of *S. cernua* (Table 5.4). Ariza. (2013) found that soil moisture in *S. parksii* microsites was consistently higher than *S. cernua* sites in three of four sampling dates spanning March 2010 to February 2011. The data collection techniques differed, however, where Ariza (2013) took water availability measurements within 1 m_2 microsites per species, with at least one plant residing within the quadrat; the proximity of the soil moisture instrument to the species of interest is unknown. In this study, *S. cernua* may more consistently receive subsurface water due to its tendency to reside on steeper slopes with shallower claypans. Future studies could therefore follow the flow of water from upslope to downslope and thus account for the variability of subsurface water and claypan depths that can vary drastically within a 1 m_2 space, then compare results between *S. parksii* and *S. cernua*.

Spiranthes parksii were significantly associated with areas of deeper claypan and less slope over one meter than *S. cernua* (Table 5.5). This finding does not fully support the generally accepted *S. parksii* habitats such as drainage banks and eroded stream margins with shallow subsurface water flow (Wilson 2002; USFWS 2009), and may instead represent a local site-specific adaptation. Large plants occupied sites with lower water holding capacity, as seen through post-treatment measurements taken three to four hours after water delivery, and resided on steeper slopes compared to small growth plants. This suggests that large plants may achieve their growth capability from access to subsurface water flow, but claypan depth was insignificant between growth history sizes yet correlated negatively with post-treatment soil moisture and infiltration rate. The sample size of large *S. parksii* presented an issue because more available *S. parksii* were small than large, therefore analyses of large *S. parksii* are unreliable and assessments of *S. parksii* as a species favored measurements of small individuals. Without the consideration of large *S.*

parksii, however, small *S. parksii* and large *S. cernua* occupied sites with very similar yet often significantly different soil moistures, the former with lower values, but the site slopes of large *S. cernua* remained significantly steeper. It is possible that these groups occupy soils with higher sand content while small *S. cernua* sites have more organic matter and shallower claypans. Neither *S. parksii* nor *S. cernua* were more likely to go dormant from summer 2017 through spring 2018, so the opposing site preferences indicate that other factors such as pH, organic matter content, and mycorrhiza species must be assessed. Ariza (2013) tested soil pH and percent organic matter but due to varying results between studies, these factors and soil water content should be addressed in each study of the congeners until there is more concrete assessment of these variables.

Studies that assess how *S. parksii* and its congener *S. cernua* respond to experimental or natural climatic and edaphic factors are imperative for a better understanding of *S. parksii* and how to conserve the species. Future research will benefit from a larger sample pool, as vegetative dormancy can greatly reduce the amount of observable subjects season to season and year to year (Shefferson et al. 2001), but the root system, vegetation change, seeds, and mycorrhizal associations must also be addressed. The root architecture of these species has been assessed before (Pileri 1998; Hammons et al. 2010; Ariza 2013) and while Hammons et al. (2010) found a correlation between total rosette leaf length and total tuber length from a sample of both small and large plants in spring, the root systems of summer-emergent rosettes has not been analyzed and it is suspected that fall tuber development is expedited in these individuals. Environmental stochasticity is a prominent factor in determining plant growth, but increasing woody encroachment may also reduce sunlight enough to limit photosynthetic capacity, leading to shorter and smaller growth on average (Jacquemyn & Hutchings 2010).

It is worth noting that several plants in open habitats achieved greater growth in fall 2017 than 2016, however, this did not apply to all plants in similar habitats. In *Spiranthes spiralis*, it is typical for rosettes to grow in conjunction with inflorescence emergence and flowering and provide nutrients to developing seeds; in *S. parksii* and *S. cernua*, simultaneous rosettes may or may not be present (if so, typically around time of fruit set), but greater flower stalk surface area and thus photosynthates may provide nutrients for ensured seed development that rosettes offer in *S. spiralis* (Jacquemyn & Hutchings 2010). In this study, the number of viable seeds within each fruit was not assessed but it is possible that larger capsules found on many large growth history plants contain more viable seeds than capsules of small plants, as has been found in capsules of some geophytes (Miller et al. 2004). Ariza (2013) estimated the number of seeds in *S. parksii* and *S. cernua* fruits and did not find significant differences, however, capsule size was not noted.

Lastly, as orchids depend fully or partially on mycorrhizal fungi for carbon and nutrients (McCormick et al. 2018), more research on preferred fungal species of *S. parksii* is imperative. As colonization of terrestrial orchid roots in one study ranged from 42% to 92%, it is probable that there is a correlation between aboveground size and fitness, root architecture, and mycorrhizal species, mycorrhizal dependence, and colonization extent (Lambers et al. 2006; Sathiyadash et al. 2012). Similarly, researchers have shown that plant growth, competitive ability, and abundance depended on which type of mycorrhizal fungi was associated (Klironomos 2003; McCormick & Jacquemyn 2014; McCormick et al. 2018). And of peak concern for this study, McCormick et al. (2018) found that orchid populations were more abundant, less prone to dormancy, and more likely to reemerge or sprout when orchid mycorrhizal fungi were abundant.

CHAPTER VI

GENERAL CONCLUSIONS

Orchidaceae contains the most threatened species of any plant family, and while only about one-third of these species are terrestrial, almost half of the already-extinct orchids are terrestrial perennials (Swarts & Dixon 2009). This draws attention to the protection of the remaining terrestrial orchids that pose a similar extinction risk. However, many of these species are rare due to both ecological and genetic dynamics. Ecological variables may include interspecific competition, environmental variability, dispersal, mutualisms, and herbivory (Schemske et al. 1994) and can be seen by terrestrial orchids' evolutionary tendency of hiding amongst associated species rather than monopolizing the habitat, like many grasses (Pierce & Belotti 2011); their endemic nature caused by highly specialized habitat preferences (Liggio & Liggio 1999; Swarts & Dixon 2009); and specialization with mycorrhizal fungi and pollinators (McCormick and Jacquemyn 2014). Therefore, while a thriving terrestrial orchid presence is thought to indicate a healthy and balanced ecosystem, habitat disruptions tend to impact these sensitive species first (Pierce & Belotti 2011). Genetic dynamics that influence vital rates can also lead to decreased population growth due to inbreeding, or increased population growth through allele richness and evolutionary potential (Schemske et al. 1994).

Conservation efforts can include both *in situ* and *ex situ* practices, and a combination of these techniques is suggested for terrestrial orchid populations (Swarts & Dixon 2009; Ariza 2013). *In situ* conservation entails habitat preservation techniques such as legal land protection, however, the land must be maintained by grazing, mowing, and/or coppicing during certain times of the year when the orchids won't be harmed (Pierce & Belotti 2011; Fay 2018). While terrestrial orchids favor disturbance, incorrect land management can be just as detrimental as no protection at all. Assisted migration, or plant transfer between habitats, is becoming a valuable technique for endangered species conservation in the face of climate change and its effects on species distributions, but is not without its own challenges nor should it be viewed as an alternative to habitat preservation (Swarts & Dixon 2009; Fay 2018). Problems associated with assisted migration include that it changes the natural vegetation, it is difficult to predict where a species will perform favorably, and seeds or plants must be maintained *ex situ* until transfer is complete (Pierce & Belotti 2011). Additionally, while seeds are often preferred for reintroduction of terrestrial orchids because they conserve genetic diversity, cultivation from seed for the purpose of transport can be extremely difficult in terrestrial orchids, and Raventós et al. (2015) found that translocated adults of two epiphytic species produced greater population momentum than relocated seedlings.

Ex situ practices include long-term storage of seeds, regenerative tissue, mycorrhiza, and living collections (Swarts & Dixon 2009; Fay 2018). However, genetically representative seeds and individuals must be selected to avoid inbreeding depressions and maximize evolutionary potential (Swarts & Dixon 2009). In addition, some species' seeds can survive storage at sub-zero temperatures while others do not, and cryopreservation in liquid nitrogen, while promising, is costly (Pierce & Belotti 2011). Furthermore, more research is needed to overcome difficulties of *in vitro* seedling survival after transfer to *in situ* conditions before techniques can be effectively applied to rare and endangered taxa (Swarts & Dixon 2009).

In this study, the demographics of *S. parksii* and *S. cernua* provide further data and understanding of the species' behavior that can contribute to *in situ* conservation techniques. Moreover, the small and weakly aggregated *S. parksii* subpopulations in the sample population suggest that within BVSWMA, the species will benefit greatly from *ex situ* conservation efforts despite their endemic nature and location in a habitat that, at least historically, is preferential yet threatened by canopy closure (Pierce & Belotti 2011; Ariza 2013). Annual counts of DRA 11 *S. parksii* and *S. cernua* outside of the sample population were not addressed in this study, but these values will be necessary to monitor adult and seedling census fluctuations over time in response to environmental conditions. For instance, long-term comparisons of demographic counts and life stages to temperature and precipitation may indicate that assisted migration to more suitable habitats is necessary for *S. parksii* preservation.

This study also assessed the effects of herbivory and supplemental water on plant performance. In general, invertebrate herbivory was more prevalent in the spring than fall as seen by minimal differences in sustained herbivory between treatments. While less herbivory in the spring led to a higher probability of fall reproductive structure development, in 2016 and 2017 the rates of spring herbivory had no effect on fall plant area nor flower production, possibly by compensating defoliation with increased stem and fruit photosynthesis (Gonneau et al. 2014), but fall inflorescence absence or herbivory always led to significantly reduced spring rosette leaf areas.

Within the supplemental water experiments, small plants consistently had greater rates of dormancy in summer and fall seasons, regardless of whether or not they received supplemental water, yet recovered and returned in high numbers each spring. However, those that were both small and watered during the treatment period achieved the greatest springtime return from dormancy, which indicated favoritism of survival over reproduction while large plants exhibited greater reproductive fitness. When treatments were applied, water delivery more so than growth history affected plant area. Contrary to previous research, *S. parksii* in this study maintained lower soil moisture values than those of *S. cernua*, and the claypans and ground slopes upon which *S.*

parksii grew were deeper and flatter than *S. cernua*, respectively. Results also suggested that growth history and species interact to influence soil moisture, or perhaps the soil properties and species dictate whether a plant will be small or large.

Research presented here increased our knowledge of *S. parksii* and *S. cernua* behavior yet expressed how different studies can produce opposing or indecisive results. This solidifies the need to continue field observations within the sample population so past, present, and future measurements can be compared, but to combat the declining numbers and to obtain more accurate assessments of the population elasticity over time, old and new individuals outside of the sample population should also be addressed and measured. A long-term observation schedule is encouraged so that individuals can be revisited several times during each spring, summer, and fall season, and elucidate aspects of the key processes of dispersal, recruitment, and early development. It is also important that *S. cernua* continue to be included in *S. parksii* research, as both species are facultative agamospermic and show minimal prevalence of sexual reproduction (Schmidt & Antlfinger 1992; Ariza 2013). This leads to low genetic variability, decreased preparedness to adapt to a changing environment, and inbreeding depression, all of which can be mitigated by integrated *in situ* and *ex situ* conservation methods (Schemske et al. 1994; Swarts & Dixon 2009; Ariza 2013).

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APPENDIX A

Spring and fall presence (green) and absence (red) by individual from 2014 to 2018 (*n* = 137). Plants were considered present in each respective fall if they exhibited reproductive growth during one or more observation dates. The water study (WS) treatments were implemented in summer 2017 on the indicated individuals (*n* = 60).

Species: P = *S. parksii*, C = *S. cernua*.

Treatment: UN = uncaged, UI = uncaged + insecticide, CN = caged, CI = caged + insecticide, M = mesh.

			2014		2015		2016		2017			2018	
Plant ID	Species	Treatment	Spring	Fall	Spring	Fall	Spring	Fall	Spring	WS	Fall	Spring	Fall
D13C41	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
D13C45	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟩
D13P4	P	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
D13U2	P	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
D14U38	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
D14U5	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
M13C3	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R13C30	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟩
R13C38	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟩
R13C48	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R13C54	C	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13P27	P	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13P45	P	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R13P48	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13P49	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13U1	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟩
R14U2	P	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R14U23	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R14U27	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R14U28	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩

			2014		2015		2016		2017			2018	
Plant ID	Species	Treatment	Spring	Fall	Spring	Fall	Spring	Fall	Spring	WS	Fall	Spring	Fall
R14U3	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R14U32	P	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
D13C34	C	UI	🟥	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
R13C61	C	UN	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13P37	P	UI	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟩
D13C46	C	UN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Water	🟩	🟩	🟩
D13P5	P	UI	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟩	🟩	🟩
D14U1	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟩
R13P46	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟩
R13P47	P	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟩
D13C1	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
D13C4	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
D13C47	C	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
D13C48	C	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
D13P6	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
D14U23	P	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R13C35	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
R13C40	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
R13C43	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
R13C46	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R13C51	C	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R13C55	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
R13P44	P	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
R13P50	P	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟩	🟩	🟥
R14U11	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U12	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U13	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U16	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U17	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U24	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R14U28A	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟥
R13C47	C	UN	🟩	🟥	🟩	🟥	🟩	🟩	🟩	Water	🟩	🟩	🟩
R13P32	P	UN	🟩	🟥	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟩
R13C32	C	CN	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟩	🟥
D13P1	P	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Water	🟥	🟩	🟩
D13C37	C	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟩	🟩	🟥
D13P8	P	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟩	🟩	🟥

			2014		2015		2016		2017			2018	
Plant ID	Species	Treatment	Spring	Fall	Spring	Fall	Spring	Fall	Spring	WS	Fall	Spring	Fall
R13C56	C	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟩	🟩	🟥
R13P31	P	UN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Water	🟩	🟩	🟥
R13P39	C	CI	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟩	🟩	🟥
D13C32	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟩	🟥
D13C33	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
D14U3	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟥
R13C44	C	CN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R13C57	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R13P28	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R13P29	P	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟩	🟥
R13P30	P	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R13P33	P	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R14U25	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟥
R14U26	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟩	🟥
R13C60N	C	CI	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Water	🟩	🟥	🟥
D13P10	P	CN	🟥	🟩	🟩	🟥	🟩	🟩	🟩	Water	🟩	🟩	🟥
D13C25	C	UN	🟩	🟥	🟩	🟥	🟩	🟩	🟩	Control	🟩	🟩	🟥
D13C26	C	UN	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Water	🟥	🟩	🟥
R13C41	C	UN	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟩	🟥
13U47	C	M	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟩	🟥
D13C38	C	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟥	🟩	🟥
D14U39	P	UI	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟥	🟩	🟥
R13C52	C	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟥	🟩	🟥
R14U10	C	M	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟩	🟥
R14U19	C	M	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟩	🟥
R13C28A	C	CN	🟩	🟩	🟩	🟥	🟩	🟩	🟩	Control	🟩	🟥	🟥
R13C42	C	CN	🟩	🟩	🟩	🟩	🟩	🟥	🟩	Control	🟥	🟩	🟥
R14U14	C	M	🟩	🟩	🟩	🟩	🟩	🟥	🟩		🟥	🟩	🟥
R13C49	C	UI	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟥	🟥
R13C59	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟥	🟥
R13C62	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟥	🟥
R14U20	C	M	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟥	🟥	🟥
D13C5	C	UN	🟩	🟥	🟩	🟥	🟩	🟥	🟩	Water	🟥	🟩	🟩
D14U19	C	UI	🟩	🟥	🟩	🟥	🟩	🟩	🟩		🟥	🟩	🟥
D14U20	C	UI	🟩	🟥	🟩	🟩	🟩	🟩	🟩		🟥	🟥	🟥
D14U36	P	UI	🟩	🟥	🟩	🟩	🟩	🟩	🟩	Control	🟥	🟥	🟥
D13C52	C	CI	🟩	🟩	🟩	🟥	🟩	🟥	🟩	Water	🟥	🟩	🟥

			2014		2015		2016		2017			2018	
Plant ID	Species	Treatment	Spring	Fall	Spring	Fall	Spring	Fall	Spring	WS	Fall	Spring	Fall
D13P3	P	UI	🟩	🟩	🟩	🟥	🟩	🟥	🟩		🟥	🟩	🟥
D13U3A	C	M	🟩	🟩	🟩	🟥	🟩	🟥	🟩		🟥	🟩	🟥
D14U18	C	CI	🟩	🟩	🟩	🟥	🟩	🟥	🟩		🟥	🟩	🟥
R13C39	C	CI	🟩	🟩	🟩	🟥	🟩	🟥	🟩	Control	🟥	🟩	🟥
R13P36	P	UI	🟩	🟩	🟩	🟥	🟩	🟥	🟩	Control	🟥	🟩	🟥
R13P38	P	CI	🟩	🟩	🟩	🟥	🟩	🟥	🟩	Control	🟥	🟩	🟥
R14U18	C	M	🟩	🟩	🟩	🟥	🟩	🟥	🟩		🟥	🟩	🟥
D14U12A	C	M	🟩	🟩	🟩	🟥	🟩	🟩	🟥		🟥	🟩	🟥
D14U33	C	CI	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟥	🟥
R13P35	P	UN	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟥	🟥
R14U10A	C	M	🟩	🟩	🟩	🟥	🟩	🟩	🟩		🟥	🟥	🟥
R13C58	C	UN	🟩	🟩	🟩	🟩	🟩	🟩	🟥		🟥	🟥	🟥
D13U9	C	UI	🟥	🟩	🟩	🟥	🟥	🟩	🟩		🟥	🟩	🟥
R13P42	P	UI	🟥	🟩	🟩	🟥	🟩	🟥	🟥		🟩	🟩	🟥
D13C49	C	CN	🟥	🟩	🟩	🟥	🟩	🟥	🟩	Control	🟥	🟩	🟥
R13P31A	P	UN	🟩	🟥	🟩	🟩	🟩	🟩	🟥		🟥	🟥	🟥
D13C2	C	UI	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟩	🟩
D13P2	P	UI	🟩	🟩	🟩	🟥	🟩	🟥	🟥		🟥	🟩	🟥
D13C3	C	CI	🟩	🟩	🟩	🟥	🟩	🟥	🟩	Control	🟥	🟥	🟥
D13C35	C	UN	🟩	🟩	🟩	🟩	🟩	🟥	🟥		🟥	🟥	🟥
D13C39A	C	CN	🟩	🟩	🟩	🟩	🟩	🟥	🟥		🟥	🟥	🟥
D13C54	C	CI	🟩	🟩	🟩	🟩	🟩	🟥	🟥		🟥	🟥	🟥
R14U31	C	M	🟩	🟩	🟩	🟩	🟩	🟥	🟥		🟥	🟥	🟥
D13C36	C	CI	🟥	🟩	🟩	🟥	🟥	🟩	🟩	Water	🟥	🟥	🟥
D13P11	P	CI	🟩	🟥	🟩	🟥	🟩	🟥	🟩		🟥	🟥	🟥
D14U24	P	UI	🟩	🟥	🟩	🟩	🟩	🟥	🟥		🟥	🟥	🟥
D13C30	C	UI	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟩	🟥
D13C57	C	UI	🟩	🟩	🟩	🟥	🟩	🟥	🟥		🟥	🟥	🟥
D13C31	C	UN	🟩	🟥	🟩	🟥	🟥	🟥	🟥		🟥	🟩	🟥
R13C29	C	UI	🟩	🟥	🟩	🟥	🟩	🟥	🟥		🟥	🟥	🟥
R13P43	P	UN	🟩	🟥	🟩	🟥	🟩	🟥	🟥		🟥	🟥	🟥
D14U34	P	CN	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
D14U35	P	CN	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
R13C26	C	UN	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
R13C27	C	CI	🟩	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
R13P34	P	UN	🟩	🟥	🟥	🟥	🟥	🟥	🟥		🟥	🟥	🟥
R13C34A	C	UI	🟥	🟩	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥

			2014		2015		2016		2017			2018	
Plant ID	Species	Treatment	Spring	Fall	Spring	Fall	Spring	Fall	Spring	WS	Fall	Spring	Fall
D14U37	P	UI	🟩	🟥	🟥	🟥	🟩	🟥	🟥		🟥	🟥	🟥
D13C6	C	UN	🟩	🟥	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
R13C64	C	CN	🟩	🟥	🟩	🟥	🟥	🟥	🟥		🟥	🟥	🟥
D13C58	C	UI	🟩	🟥	🟥	🟥	🟥	🟥	🟥		🟥	🟥	🟥
D14U40	C	UI	🟩	🟥	🟥	🟥	🟥	🟥	🟥		🟥	🟥	🟥
D14U41	P	UI	🟩	🟥	🟥	🟥	🟥	🟥	🟥		🟥	🟥	🟥

APPENDIX B

Spearman ρ multivariate correlations between averaged maximum spring and fall parameters from 2014 to 2018. Plants without documented flowers (either immature buds or fully-developed open or closed-form flowers) were excluded from analyses in individual falls. Levels of significance are indicated by ++ $p < 0.10$, + $p < 0.05$, * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

Fall: PA = plant area, PH = plant height, IL = inflorescence length, #F = number of flowers.

Spring: LA = leaf area, #L = number of leaves at one time, LL = leaf length, LW = leaf width.

		Spring 2015				Fall 2015			
		LA	#L	LL	LW	PA	PH	IL	#F
Fall 2014	PA	0.57***	0.45***	0.47***	0.51***	0.33 +	0.26 ++	0.34 +	0.36 +
	PH	0.41***	0.19 ++	0.41***	0.27*	0.27 ++	0.22	0.15	0.27
	IH	0.46***	0.25*	0.43***	0.37***	0.18	0.13	0.06	0.10
	#F	0.48***	0.41***	0.36**	0.46***	0.15	0.09	0.19	0.28 ++
		(*n* = 104)				(*n* = 43)			

		Fall 2015				Spring 2016			
		PA	PH	IL	#F	LA	#L	LL	LW
Spring 2015	LA	0.65***	0.45*	0.69***	0.70***	0.71***	0.61***	0.61***	0.58***
	#L	0.29 +	0.01	0.35 +	0.34 +	0.48***	0.54***	0.29*	0.44***
	LL	0.47***	0.42*	0.69***	0.67***	0.56***	0.42***	0.58***	0.36***
	LW	0.63***	0.39*	0.66***	0.66***	0.62***	0.58***	0.48***	0.55***
		(*n* = 51)				(*n* = 120)			

		Spring 2016				Fall 2016			
		LA	#L	LL	LW	PA	PH	IL	#F
Fall 2015	PA	0.51**	0.30+	0.50**	0.34 +	0.28 ++	0.29 ++	0.14	0.19
	PH	0.39*	0.13	0.32 +	0.21	0.21	0.25 ++	0.13	0.03
	IH	0.47**	0.25 ++	0.33 +	0.25 ++	0.24	0.2	0.18	0.22
	#F	0.49**	0.21	0.43*	0.31 +	0.31 +	0.26 ++	0.29 ++	0.24
		(*n* = 51)				(*n* = 46)			

		Fall 2016				Spring 2017			
		PA	PH	IL	#F	LA	#L	LL	LW
Spring 2016	LA	0.71***	0.54***	0.69***	0.69***	0.79***	0.73***	0.77***	0.73***
	#L	0.54***	0.34**	0.53***	0.66***	0.69***	0.69***	0.62***	0.71***
	LL	0.64***	0.66***	0.64***	0.45***	0.70***	0.58***	0.75***	0.62***
	LW	0.54***	0.33*	0.58***	0.70***	0.66***	0.70***	0.59***	0.72***
		(*n* = 93)				(*n* = 108)			

		Spring 2017				Fall 2017			
		LA	#L	LL	LW	PA	PH	IL	#F
Fall 2016	PA	0.64***	0.54***	0.64***	0.52***	0.64***	0.63***	0.47**	0.39*
	PH	0.53***	0.39**	0.54***	0.39**	0.51**	0.62***	0.41*	0.28 +
	IH	0.63***	0.51***	0.54***	0.58***	0.51**	0.42*	0.38*	0.39*
	#F	0.54***	0.53***	0.39**	0.52***	0.39*	0.14	0.14	0.34 +
		(*n* = 92)				(*n* = 52)			

		Fall 2017				Spring 2018			
		PA	PH	IL	#F	LA	#L	LL	LW
Spring 2017	LA	0.62***	0.49**	0.45**	0.51***	0.72***	0.56***	0.66***	0.65***
	#L	0.55***	0.30 +	0.37*	0.43*	0.57***	0.52***	0.52***	0.55***
	LL	0.54***	0.53***	0.40*	0.46**	0.67***	0.47***	0.65***	0.58***
	LW	0.57***	0.33 +	0.41*	0.58***	0.69***	0.55***	0.62***	0.69***
		(*n* = 54)				(*n* = 96)			

		Spring 2018			
		LA	#L	LL	LW
Fall 2017	PA	0.60***	0.50**	0.57***	0.54***
	PH	0.44*	0.26 ++	0.48**	0.32 +
	IH	0.42*	0.28 +	0.39*	0.32 +
	#F	0.58***	0.45**	0.53***	0.59***
		(*n* = 52)			