

**POPULATION STATUS AND REPRODUCTIVE BIOLOGY OF *CLEMATIS*
MOREFIELDII, A FEDERALLY ENDANGERED PLANT**

by

Kyle Jordan Paris

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 14, 2013

Cumberland Plateau, herbivory, insecticide, limestone outcrop endemic, reproductive attrition,
seedling establishment

Copyright 2013 by Kyle Jordan Paris

Approved by

Robert S. Boyd, Chair, Professor of Biological Sciences, Auburn University
Kevin S. Burgess, Associate Professor of Biological Sciences, Columbus State University, GA
Amy N. Wright, Professor of Horticulture, Auburn University

Abstract

Clematis morefieldii is a federally endangered, perennial climbing vine. It inhabits limestone drains and outcrops on the Cumberland Plateau escarpments of northeast Alabama and south-central Tennessee. The NatureServe network ranks this species as imperiled in Alabama and critically imperiled in Tennessee. Given its habitat specificity, localized distribution, and rarity, it is of considerable interest to the conservation community and to the organizations obligated to manage it. Since its discovery over 30 years ago, there has been no in depth study of this species and pertinent information for management is absent. The goal of this thesis is to investigate the condition of current populations, document the species' biology and life history, and assess impacts of herbivory. To achieve this goal, a one-year population structure and herbivory survey, a four-year reproductive attrition study, and a three-year insecticide study were conducted between 2009-2012, sponsored, in part, by the U.S. Fish and Wildlife Service.

The population structure and herbivory survey estimated range-wide population numbers, described proportions of life history stages in each population sampled, and evaluated the extent of vegetative herbivory in each sampled population across the range of *C. morefieldii*. As of 2012, population numbers are estimated to be 8,868 in Alabama and 6,751 in Tennessee, totaling 15,619 plants across the range of the species. Non-reproductive plants made up the largest proportion of populations (~55%) and reproductive plants made up the smallest proportion (~10%). Alabama populations experienced significantly greater levels of vertebrate browse damage (3-fold) and piercing-sucking herbivore damage (2- to 3-fold) than Tennessee

populations. Seedling and non-reproductive plants experienced significantly greater levels of invertebrate browse damage (1.5- to 2-fold) than reproductive plants. These descriptive data provided a snapshot of the state of populations across the species' range.

Reproductive attrition, seedling establishment, and herbivory extent were studied at The Nature Conservancy's Keel Mountain Preserve in Madison County, Alabama. Study of reproductive attrition (2009-2012) found high levels of floral herbivory (30 to 70% loss attributed to herbivory each year) with the greatest damage occurring to flower buds in their smallest stage (15 to 50% attrition to herbivory). Post-dispersal predation of achenes (likely by rodents) was 15% after one week and slowly reached 30% by four months. Seeds failed to germinate the first year after planting, but germinated in significantly greater amounts the second year (23%) compared to the third (9%), indicating that a seed bank likely exists.

To estimate the impact of invertebrate vegetative and floral herbivory on reproduction, an insecticide study (2010-2012) was designed to exclude invertebrate herbivores from some plants and compare reproductive attrition to that of control plants sprayed with water. Insecticide applications increased flower bud survival and fruit production while decreasing floral herbivory. However, reproductive output was not affected by herbivory when peak flowering/fruitletting season rainfall was limiting.

While recruitment is occurring in surveyed *Clematis morefieldii* populations, herbivore exclusion could be a viable management option for increasing plant numbers in small populations if rainfall and suitable habitat are not limiting. High levels of vegetative herbivory

and low levels of florivory occurred across the species' range in 2012. Reproductive attrition studies documented high levels of florivory, and a bottleneck in reproductive unit maturation that could limit reproductive output. Rainfall was positively associated with reproductive output, but during high rainfall years invertebrate herbivory destroys a greater percentage of reproductive units, probably due to a combination of direct (attacks on flower buds and flowers) and indirect pathways (by vegetative herbivory). Insecticide treatment could be a useful option for managers seeking to increase recruitment to small populations. Despite moderately high post-dispersal achene predation levels and only a third of seeds germinating over three years, seedling recruitment was occurring across the range of *Clematis morefieldii*. I conclude that despite challenges by herbivores this species is not at imminent risk of extinction due to the probable longevity of individual plants, evidence of recruitment into existing populations, and the large sizes of some populations.

Acknowledgments

The author is much obliged for all the help and encouragement of his mentor Dr. Robert Boyd, whose humorous and enthusiastic approach to teaching and life have left a lasting impression. Appreciation is extended to Dr. Kevin Burgess and Dr. Amy Wright: their knowledge and patience with this project and the author's shortcomings were invaluable. Keith Tassin and The Nature Conservancy provided the best study site any researcher could ask for. Funding by the U.S. Fish and Wildlife Service was greatly appreciated and not spent all in one place. Geoff Call and Todd Crabtree are quality Tennessee naturalists, and their assistance significantly reduced the number of times I was lost in Tennessee. Hannah Gunter is a master of mouse identification. Dr. Charles Ray is an amazing entomologist, and his help was greatly appreciated. Scott Clem and Andrew Owens were a great help with field work, and always kept things interesting. To my brother and best friend Nate, I couldn't have done it without you fella. A special thanks to my parents, who cheerfully allowed me to use their house as base camp, eat their food, and never clean my room. To all the rest who helped me along the way, thank you so much. Most of all I thank God for all the good things in life, for His guidance and strength to face each new day and the challenges that come with it.

In dedication to Rena Miller, a fellow botanist and *Clematis morefieldii* enthusiast, you are missed.

Style manual or journal used: Castanea

Computer software used: Microsoft Word 2007, Microsoft Excel 2007, and StatView 5.0

TABLE OF CONTENTS

Abstract	ii
Acknowledgments.....	v
LIST OF TABLES	viii
LIST OF FIGURES	x
I. INTRODUCTION.....	1
II. POPULATION AND HERBIVORY SURVEY OF <i>CLEMATIS MOREFIELDII</i>	
Abstract	8
Introduction.....	9
Materials and methods	12
Results	16
Discussion	20
III. REPRODUCTIVE ATTRITION AND SEEDLING ESTABLISHMENT OF <i>CLEMATIS MOREFIELDII</i>	
Abstract	38
Introduction.....	39
Materials and Methods	41
Results	49
Discussion	51
IV. IMPACT OF INSECTICIDE TREATMENT ON HERBIVORY AND REPRODUCTIVE OUTPUT OF <i>CLEMATIS MOREFIELDII</i>	
Abstract	64
Introduction.....	65
Materials and Methods	69
Results	74
Discussion	76
LITERATURE CITED	95

LIST OF TABLES

Chapter II:

1. List of documented populations of *Clematis morefieldii*. Populations from Alabama (Weber 1991 and 1994; Emanuel 2000; Schotz 2007) and Tennessee (Bailey 2005; Crabtree 2011) are estimated to currently have at least 8868 and 6751 plants, respectively. Numbers in front of population names were used throughout this study to facilitate identification. EO Number is the element occurrence number given to population or subpopulation by the Alabama or Tennessee Natural Heritage Program. “Est.” refers to populations with estimated numbers and “subsample” surveys were incomplete plant counts.....29
2. Two-way ANOVA results for *Clematis morefieldii* herbivory category comparisons with significant differences defined as having a P-value ≤ 0.05 (asterisk denotes significance). Damage data for each herbivory category were analyzed for effect of region (Alabama and Tennessee), life history stage (seedling, non-reproductive, reproductive), and the region*life history stage interaction.....30
3. *Clematis morefieldii* Z-values from Z-test. Correlations are between stem length and degree of vegetative herbivory for each category of herbivory, with significant effects defined as those having a P-value ≤ 0.05 (asterisk denotes significance).....31

Chapter III:

1. Summary of 2009, 2010, 2011, and 2012 reproductive attrition data. Data were analyzed across years using ANOVA, with significant differences defined as having a P-value ≤ 0.05 . For each significant variable, mean annual values with the same superscripts do not differ significantly (Fisher’s PLSD test). Total florivory includes reproductive units destroyed by herbivores at any maturation stage.....58

Chapter IV:

1. Reproductive attrition results from insecticide study. Reproductive data were compared between treatments (insecticide and control) for 2010, 2011, and 2012. Mann-Whitney U tests compared treatment and control data within each year, with significant differences defined as $P \leq 0.05$ (asterisk denotes significance).....83
2. Control data (2010-2012) for the insecticide treatment study on *Clematis morefieldii*. Kruskal-Wallis test was used to collectively compare all three years, Mann-Whitney U tests were used to make pairwise comparisons between years for variables for which the Kruskal-Wallis test showed significant year effects. Significant differences were defined as $P \leq 0.05$. For each variable, mean annual values with the same superscripts do not differ significantly.....84

3. Effects of treatment (insecticide and control) and year on reproductive transitions of *Clematis morefieldii* using two-way ANOVA. Reproductive transitions were compared for effects of treatment (insecticide and water), year (2010-2012), and the treatment*year interaction. Significant effects were defined as $P \leq 0.05$ (asterisk denotes significance). “Intermediate” stage is a combination of “immature” and “medium” flower bud stages. “Fruit” stage is a combination of “tiny aggregate” and “immature aggregate” fruit stages.....85

LIST OF FIGURES

Chapter II:

1. Ecoregion map (Griffith *et al.* 2001) showing all *Clematis morefieldii* populations in Northeast Alabama (1-10) and South-Central Tennessee (11-22). Population numbers and names are: 1-Keel Mountain Preserve, 2-Deborah Drive, 3-Monte Sano Landtrust, 4-Rockslide, 5-Cramer Hollow, 6-Kennamer Preserve, 7-Pelletier Hollow, 8-Hale Mountain, 9-Moore Cove, 10-Honey Hollow, 11-Blooin Hollow, 12-Keith Cove, 13-Carter Cave, 14-War Memorial, 15-Snowbird Hollow, 16-Hawkins Cove, 17-Mill Creek, 18-The Narrows, 19-Miller Cove, 20-Kirby Smith Branch, 21-Sinking Cove, 22-Burrow Cove.....33
2. Mean proportions (bars are S.E.) of *Clematis morefieldii* life history stages (seedling, non-reproductive, and reproductive) in Alabama (N = 5) and Tennessee (N = 7) populations in 2012..34
3. Mean *Clematis morefieldii* stem length (cm) (bars are S.E.) for each life history stage (seedling, non-reproductive, and reproductive) from sampled Alabama (N = 5) and Tennessee (N = 7) populations in 2012.....35
4. Mean density of reproductive units and reproductive units destroyed by herbivores per meter of stem length (bars are S.E.) on *Clematis morefieldii* from Alabama (N = 5) and Tennessee (N = 7) populations in 2012.....36
5. Mean percent vegetative herbivory (bars are S.E.) for each *Clematis morefieldii* life history stage (seedling ☐, non-reproductive ◻, reproductive ◼) from Alabama (N = 5) and Tennessee (N = 7) populations in 2012: (A) vertebrate browse damage, (B) invertebrate browse damage, (C) piercing-sucking herbivory, (D) leaf miner herbivory.....37

Chapter III:

1. Percent departure of annual and April-June rainfall from average rainfall amounts (1983-2012) from the Redstone Technical Test Center (RTTC) Meteorological Team at Redstone Arsenal in Huntsville, AL. This weather station (N 34° 36' 0.8", W 86° 37' 49.7") is approximately 21 km from the Keel Mountain Preserve (N 34° 39' 27.8", W 86° 24' 42.3"). Snowfall (cm) was converted to rainfall (cm) by dividing snow depth by 10 cm.....60
2. Percent survival for buds in the 2009, 2010, 2011 and 2012 reproductive attrition study. Points signify percentages of reproductive units reaching each maturation stage.....61

3. Linear regressions of total annual fruit production (by all 40 plants) plotted with percent departure from annual average rainfall (Fig. 3A) and also peak flowering/fruitletting season rainfall (April-June) (Fig. 3B) using year (2009-2012) as the replicate. Rainfall data were acquired from the Redstone Arsenal weather station near Huntsville, AL (~ 21 km from study site).....62
4. Percent survival of achenes in the *Clematis* and low-*Clematis* zone. Achene survival did not significantly differ between zones. Study began September 24, 2011 and ended January 21, 2012. Diamond points represent the *Clematis* zone and square points represent the low-*Clematis* zone.....63

Chapter IV:

1. Linear regressions of insecticide effectiveness on *Clematis morefieldii* fruit production (total insecticide study fruit production minus control fruit production) plotted against total annual rainfall (Fig. 1A) and peak flowering/fruitletting season rainfall (April-June) (Fig. 1B). In each graph, each point represents the values from one year of the experiment. Rainfall data were acquired from the Redstone Arsenal weather station near Huntsville, AL (~ 21 km from study site).....88
2. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2010. Ranges for time periods are as follows: Time 1: 26 April – 23 June; Time 2: 24 June – 21 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **)......89
3. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2011. Ranges for time periods are as follows: Time 1: 16 April – 26 May; Time 2: 27 May – 10 July; Time 3: 11 July – 16 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **).....90
4. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2012. Time period ranges are as follows: Time 1: Plant emergence to 2 May; Time 2: 3 May to 23 May; Time 3: 24 May to 28 June; Time 4: 29 June to 19 July; Time 5: 20 July to 9 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **)......91
5. Herbivory occurrence on insecticide-treated \blacksquare and control \boxtimes plants during 2010. Time period ranges are as follows: T1: Plant emergence to 23 June. T2: 24 June to plant senescence. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks (P = 0.05 *, P = 0.01 **)......92

6. Herbivory occurrence on insecticide-treated and control plants during 2011. Time period ranges are as follows: T1: Plant emergence to 26 May. T2: 27 May to 10 July. T3: 11 July to plant senescence. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks (P = 0.05 *, P = 0.01 **)93
7. Herbivory occurrence on insecticide-treated and control plants during 2012. Time period ranges are as follows: T1: Plant emergence to 2 May; T2: 3 May to 23 May; T3: 24 May to 28 June; T4: 29 June to 19 July; T5: 20 July to 9 August. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks (P = 0.05 *, P = 0.01 **).....94

I. INTRODUCTION

Humanity relies on Earth's biodiversity to provide a wide array of environmental, economical, and recreational services (Myers 1996). The exponential increase in human population has put great stress on the world's ecosystems, and retaining the integrity of the world's biodiversity is of great importance (Pimm *et al.* 1995). By protecting and seeking to understand the Earth's remaining biodiversity, humans can not only protect the well-being of the organisms around them but also improve and enrich their own lives (Chapin *et al.* 2000).

Within the United States, the Southeast in particular has a high level of biodiversity, with many rare endemics (Estill and Cruzan 2001; Stein 2002). The southeast also has some of the highest rates of population growth and land development in the U.S. (U.S. Census Bureau 2011), threatening the future of many species. Stein (2002) analyzed all 50 states for plant species richness and plant risk/threat and found Alabama to be within the top 10 for species richness and in the top 20 for the number of plant species at risk or under threat. With many rare species of the Alabama flora in peril, it is important to not only protect species at risk of extinction, but also the ecosystems on which they depend (Schemske *et al.* 1994; Flather *et al.* 1998).

Rare plants are generally defined as being limited in abundance and distribution (Rabinowitz 1981). Natural rarity of a species can be due to multiple factors including habitat specialization, low reproductive success, poor dispersal ability, high levels of herbivory, or limitation by natural disturbances such as glaciations, sea level changes, climate change, etc. (Weller 1994; Kunin and Gaston 1997; Pärtel *et al.* 2005). While many species in the Southeast are considered naturally rare (Estill and Cruzan 2001), other species have recently become rare due to the constant encroachment of urbanization and anthropogenic land alterations. As a

consequence, habitat fragmentation may be causing reduced genetic diversity and fitness in remnant populations because of genetic drift, e.g. by producing fewer seeds, through less resilience to changes in abiotic and biotic factors, etc. (Ellstrand and Elam 1993), which can lead to an extinction vortex (Tanaka 2000). Smaller populations also may be less conspicuous to pollinators resulting in reduced floral visits and subsequent reduced outcrossing, which in turn can amplify the genetic ramifications of drift (Fischer and Matthies 1998). Natural rarity, direct and indirect anthropogenic disturbance, and loss of genetic diversity are the greatest threats to many rare plant species (Kunin and Gaston 1997; Estill and Cruzan 2001; Pärtel *et al.* 2005).

Determining the factors that limit species abundance and distribution is necessary to successfully manage a rare plant species (Louda 1994). Fenster and Dudash (1994) list three basic criteria for the successful recovery of a rare species: First, a sufficient amount of habitat must be protected for the continued existence of the species. Second, demographic information must be collected to understand what life history stages or ecological relationships (Wiser *et al.* 1998) hinder/benefit the persistence of the species. Third, genetic variation within and between populations must be documented and the existing genetic variation preserved.

The federally endangered plant *Clematis morefieldii* is the focus of this thesis. Multiple entities, including the U.S. Fish and Wildlife Service, Alabama and Tennessee Natural Heritage Programs, and The Nature Conservancy, have been working towards this species' recovery (Schotz 2007; USFWS 2010; Crabtree 2011). State Natural Heritage Programs have discovered new populations, with some on public lands or with landowners interested in protecting the populations (e.g. Bear Hollow Mountain WMA TN, The University of the South TN, Huntsville Landtrust AL, etc.) (Bailey 2005; Crabtree 2011). The site where the largest population is located was purchased by The Nature Conservancy to protect the population and its habitat.

Little life history and ecological data and no genetic data have been generated for this species. This thesis will add valuable data regarding the life history and ecology of *C. morefieldii*. Information on genetic variation within and among populations is still needed to provide managers with essential information to create the most effective recovery plan.

Study Species: *Clematis morefieldii* Kral (Ranunculaceae)

Judd *et al.* (2002) describe the Ranunculaceae as a relatively large family consisting of approximately 2000 species in 47 genera. The family has a circumglobal distribution and is found primarily in the temperate and boreal zones of the northern hemisphere; species in this family occupy a diverse array of both terrestrial and aquatic habitats (Judd *et al.* 2002). They also come in an equally diverse array of life forms ranging from annual to perennial, including herbs, vines, and lianas (Simpson 2006). A large genus within Ranunculaceae, *Clematis* contains approximately 295 species, with nine species native to Alabama (Weakley 2012). *Clematis* are shrubs or prostrate to climbing vines with a wide range of stem lengths, ranging from less than 0.2 m to over 30 m. They occupy a wide spectrum of habitats from swamps and woodlands to grasslands and alpine meadows (Grey-Wilson 2000). Found in tropical to boreal climates, their distribution encompasses every continent excluding Antarctica. There are 9 subgenera recognized within the genus, with representatives from two subgenera (*Clematis* and *Viorna*) in Alabama (Grey-Wilson 2000; Weakley 2012).

According to Grey-Wilson (2000), the subgenus *Viorna* contains the majority of *Clematis* species native to North America (23 species). Within the subgenus, there is also section *Viorna*, and subsection *Viorna*, the latter composed of nine species, all of which are found in the central and southeastern United States. Species within subsection *Viorna* have opposite decussate

leaves, urceolate flowers with thick petaloid sepals that vary in color from creamy green to pink to red, and peduncles subtended by leafy bracts (Erickson 1943; Kral 1987; Grey-Wilson 2000). Of the eight species occurring in the Southeast, Weakley (2012) recognizes four as rare throughout their range.

A relatively newly described species in the *Viorna* subsection is the Huntsville Vasevine (*Clematis morefieldii* Kral). This species was first discovered near Huntsville, AL by James Morefield in 1982 (Weber 1994), and was described as a new species of *Clematis* in the *Viorna* subsection by Kral (1987). *Clematis morefieldii* is a perennial climbing vine up to 5 m long, with 1-9 stems arising from the same rootstock, and in which the stems die back to the belowground rootstock every year (Weber 1994). The reddish-brown stems are usually no more than 6 mm in diameter and are easily broken (Paris, pers. obs.). Kral (1987) described *C. morefieldii* as follows: compound leaves are oppositely arranged with 9-11 leaflets, the last 1-3 being modified into tendrils. The light green to deep pink flowers arise from axillary positions towards the terminal end of the stem and are arranged in a cyme inflorescence. Inflorescences usually have from one to three flowers, although they can have as many as 13 (Paris, pers. obs.). A flower forms an aggregate fruit composed of multiple achenes. Each achene has a long persistent style which becomes covered in long trichomes, presumably to assist in wind dispersal (Kral 1987; Judd *et al.* 2002). According to Kral (1987), the main features that distinguish *C. morefieldii* from other species in the *Viorna* subsection are the dense white trichomes on the stem, the abaxial leaf surface being sericeous or pilose, and short, stout peduncles having sessile to nearly sessile bracts at the very base.

Clematis morefieldii is endemic to the Cumberland Plateau and is only known to occur in Jackson and Madison counties of northeast Alabama and in Franklin and Grundy counties of

south Tennessee. There are 25 current and historical populations scattered along the Western edge of the Cumberland Plateau (Weber 1991 and 1994; Emanuel 2000; Bailey 2005; Estes and Fleming 2006; Schotz 2007; USFWS 2010; Crabtree 2011). *Clematis morefieldii* is one of several narrow endemic *Clematis* species found in the Southeast (Platt 1951; Baskin and Baskin 1988; Braunschweig *et al.* 1999), and is restricted to limestone covered south to southwest facing slopes within the Plateau Escarpment physiographic ecoregion (Weber 1991; Griffith *et al.* 2001; Irene Miller pers. comm. 2010). Plants usually occur at elevations of 250 to 350 m and are found near seeps and springs in red cedar-hardwood forests (Kral 1987; USFWS 1994).

Clematis morefieldii was federally protected as an endangered species on June 19, 1992 (USFWS 1992). At time of listing, there were only five known populations and three extirpated (historical) populations, all of which were found in Madison County, AL. Also during that time, two of those five remaining sites were being threatened by residential development (USFWS 1992). Since then five new populations have been found in Alabama and 12 new populations have been found in Tennessee, increasing the total number of extant populations to 22 (Schotz 2007; USFWS 2010; Crabtree 2011).

While still federally endangered, the immediate future of *C. morefieldii* appears relatively secure compared to its perceived dire state 21 years ago. The current NatureServe (2013) conservation ranking for *C. morefieldii* is “G2” globally and state rankings are “S2” for Alabama and “S1” for Tennessee. NatureServe’s ranking system relies on state Natural Heritage Program data (species abundance, quality of populations, and threat assessment) and signifies the risk of extinction and state extirpation. The first part of the ranking system designates the area of the species’ range covered by the rank: “G” signifies a global rank that describes the status of the entire species’ range, while “S” signifies a state rank referring to the species’ conservation status

within a particular state. The second part of the ranking system determines the level of conservation concern using a numerical gradient with “5” designating a species of least conservation concern and “1” designating a species of greatest conservation concern. Globally and in Alabama *C. morefieldii* is considered “imperiled,” while in Tennessee it is considered “critically imperiled.” The Heritage ranking system (ANHP 2012) defines “imperiled” species as experiencing a high risk of extinction due to a restricted range, usually with 6 to 20 occurrences or few individuals overall, and undergoing steep declines or threats from other factors. “Critically Imperiled” species are defined as extremely rare with 5 or fewer occurrences and few remaining individuals while also experiencing other threats that make it especially vulnerable to extinction (ANHP 2012).

Some *Clematis morefieldii* populations are still threatened and those in residential areas tend to be in the most immediate danger. Schotz (2007) identifies development as the primary threat to some populations. All Alabama populations are within a 40 km radius of Huntsville (Schotz 2007). The Huntsville metro area has a growth rate of approximately 50,000 people every ten years (~19%) (U.S. Census Bureau 2009), and the need for more residential areas has pushed urbanization into less desirable building sites on mountainsides (i.e., into the habitat of *C. morefieldii*). Tennessee *C. morefieldii* populations are found in more rural areas, though most are within a 70 km radius of Chattanooga, TN (fourth largest city in TN), and an 80 km radius of Huntsville, AL (U.S. Census Bureau 2010).

Other threats to populations include exotic invasive species, incompatible land use, or habitat fragmentation. Schotz (2007) warns that two species of invasive, non-native honeysuckles (*Lonicera japonica* and *L. fragrantissima*) threaten the viability of some of the residential populations in Huntsville. Land use incompatible with population growth or stability

include detrimental forestry practices, quarrying, roadside maintenance, or any other habitat degrading practice. Incompatible forestry practices can threaten rural populations and include overharvesting of the overstory layer which can facilitate establishment and competition by invasive species (Schotz 2007). Current quarrying operations pose a threat to one population in Tennessee which occurs on land owned by Chattanooga Shale (Bailey 2005). Additional quarry operations are proposed to occur in Grundy and Franklin counties, Tennessee (TDEC 2010). Populations near roads may be threatened by road widening or roadside maintenance practices such as herbicide application or mowing (USFWS 2010). Habitat fragmentation can interrupt plant-pollinator interactions between populations, which can reduce genetic diversity within a population and thus decrease its viability, especially if it is small and/or far away from other populations (Honnay and Jacquemyn 2007).

Goal of Thesis

The goal of this thesis is to investigate the condition of current populations of the federally endangered plant *Clematis morefieldii*, document the species' biology and life history, and assess impacts of herbivory on reproductive success. Although *C. morefieldii* is federally endangered, little information is available on any aspect of its biology or the threats associated with the persistence of this unique and endangered species. Without this information, it is difficult to ascertain what factors could be contributing to the rarity of the species. Data obtained from these studies will help managers to better understand the status of known populations and give insight into *Clematis morefieldii*'s general life history, reproductive attrition, seedling establishment, the extent of herbivory, and herbivory's effects on reproductive output. This knowledge will be useful for creating effective management strategies that may facilitate the recovery of the species.

II. POPULATION AND HERBIVORY SURVEY OF *CLEMATIS MOREFIELDII* KRAL

Abstract

Comparisons between Alabama and Tennessee populations provided information on regional differences across the range of the endangered plant *Clematis morefieldii* Kral. In 2012 a survey was conducted of 12 populations to document population structure (categorizing plants into three life history stages: seedling, non-reproductive, and reproductive), plant size, reproductive output, and percent herbivore damage. The extent of vegetative herbivory (categorized as vertebrate browse, invertebrate browse, piercing-sucking damage, and leaf miner damage) and reproductive herbivory data were recorded. Two-way analysis of variance (ANOVA) used populations as replicates in analyses with regions (Alabama and Tennessee) and life history stages as factors. Life history stage composition was not significantly different between regions but varied significantly within populations, with non-reproductive plants making up the largest proportion of populations (62%), followed by seedlings (28%), and then reproductive plants (10%). Reproductive and non-reproductive plants in Alabama had significantly longer stems than those in Tennessee. Neither production nor herbivory of reproductive units differed significantly between regions. Alabama populations experienced 3-fold greater levels of vertebrate browse damage and piercing-sucking herbivore damage than Tennessee populations. Invertebrate browse damage differed significantly among life history stages with seedling and non-reproductive plants experiencing almost 2-fold greater levels of invertebrate browse damage than reproductive plants. There were significant correlations between stem length and vegetative herbivory in the range-wide analysis and in both regions (Alabama and Tennessee). This study documented high seedling proportions, high vegetative herbivory damage, low reproductive herbivory damage and

differences in some herbivore damage categories among life history stages and between regions. Alabama populations generally experienced greater vegetative herbivory than Tennessee populations, with urbanization around Alabama populations considered a possible source of the discrepancy.

Introduction

The Earth is currently experiencing a major anthropogenic extinction event, affecting biodiversity and the biological processes generated by biodiversity upon which humans rely (Chapin *et al.* 2000). Within the United States, the Southeast in particular has a high level of biodiversity, with many rare endemics (Estill and Cruzan 2001; Stein 2002). The southeast also has some of the highest rates of population growth and land development in the U.S. (U.S. Census Bureau 2011), threatening the future of many species. Stein (2002) analyzed all 50 states for plant species richness and plant risk/threat and ranked Alabama 9th and Tennessee 17th for species richness and both states in the top 20 for number of plant species at risk or under threat. With many rare Alabama and Tennessee plants in peril, efforts should primarily focus on protecting the most vulnerable species and their habitats first.

Many populations of rare plants are under threat due to a multitude of factors. Many species in the Southeast U.S. are considered naturally rare (Estill and Cruzan 2001). Natural rarity of a species can be due to multiple factors including habitat specialization, low reproductive success, poor dispersal, extensive herbivory, or reduction in range due to natural disturbances such as glaciations, sea level changes, climate change, etc. (Weller 1994; Kunin and Gaston 1997; Pärtel *et al.* 2005). Other species have recently become rare due to urbanization and general anthropogenic land alterations due to land use. Natural rarity, direct and indirect anthropogenic disturbance, and loss of genetic diversity are the greatest threats to the continued

existence of many rare plant species (Kunin and Gaston 1997; Estill and Cruzan 2001; Pärtel *et al.* 2005).

The federally endangered *Clematis morefieldii* is one of several narrow endemic *Clematis* species found in the Southeast (Platt 1951; Baskin and Baskin 1988; Braunschweig *et al.* 1999). Endemic to limestone outcrops of the Cumberland Plateau, *C. morefieldii* is only known from four counties in northeast Alabama and south-central Tennessee with 25 current and historical populations scattered along the Western edge of the Cumberland Plateau (Weber 1991 and 1994; Emanuel 2000; Bailey 2005; Estes and Fleming 2006; Schotz 2007; USFWS 2010; Crabtree 2011). When listed as federally endangered in 1992 (USFWS 1992), there were only five known populations and three extirpated (historical) populations, all in Madison County, AL. Also at that time, two of those five remaining sites were being threatened by residential development (USFWS 1992). Since then five new populations have been found in Alabama and 12 new populations have been found in Tennessee, increasing the total number of extant populations to 22 (Schotz 2007; Crabtree 2011; NatureServe 2013).

The increased number of *Clematis morefieldii* populations discovered in recent years has given new hope for this imperiled species. Surveys/censuses conducted by state Natural Heritage Programs (Weber 1991 and 1994; Emanuel 2000; Bailey 2005; Schotz 2007; Crabtree 2011) have provided valuable data on habitat descriptions and plant numbers across the range of the species. However few data are available on the demographic structure of these populations or the severity of herbivore damage across the species' range. These additional data could provide insight into the overall health of populations and any potential threats.

A population survey provides data essential for understanding reproductive potential, recruitment, plant size, etc. within populations. Proportion of reproductive plants found in populations can indicate the reproductive potential of populations within regions (Mavraganis and Eckert 2001) while seedling proportions reveal the recruitment success of those reproductive plants (García 2003). Proportions of non-reproductive plants can show the potential of well managed populations within a region to increase their numbers of reproductive plants and ultimately their reproductive output (Levey 1990). Plant size data can be used as a measurement of overall plant vigor within a life history stage (White and Harper 1970; Mitchell 1994).

Herbivory can threaten the survival of plant populations. Herbivore damage has the potential to decrease seed weight and total seed production. It can also reduce plant growth while increasing susceptibility to disease (Bevill *et al.* 1999). With consecutive years of herbivory, mortality can occur in mature plants and recruitment can be severely hindered (Louda and Potvin 1995). Herbivory can negatively affect the competitive ability of individual plants and thus impact a population's ability to sustain itself or expand its numbers (Crawley 1989). Herbivory survey data can reveal herbivory pressures at different *C. morefieldii* life history stages and across the range of the species. By knowing the extent of herbivore damage throughout the range of *Clematis morefieldii*, managers can decide if herbivory may threaten a populations and then act to reduce that damage (Mitchell 1994; García 2003; Kettenring *et al.* 2009).

The objective of this study is to investigate regional differences in population structure and severity of herbivory in *Clematis morefieldii* populations. Specifically, I will evaluate: a) life history stage proportions; b) stem length; c) herbivory damage by four categories of herbivores; d) how stem length and herbivory are affected by both region and life history stage; and e) correlations between stem length and extent of herbivory.

Materials and methods

Study Populations

Population and herbivory data were collected from all plants (or a subsample from large populations) at five Alabama populations (four in Madison County and one in Jackson County) and seven Tennessee populations (all in Franklin County) (Fig. 1) and comparisons were made between regions. Populations were selected for the survey based on population size from past surveys (larger populations were selected) and population accessibility (populations that were on public land, or if on private land, then landowner permission was granted). Populations were surveyed in 2012 from 8 June to 2 July, when herbivory damage nears its peak but the plants have not yet senesced (Paris pers. obs.). I divided plant size into three life history stage categories that were easily recognized in the field: seedling (no compound leaves present), non-reproductive (compound leaves present, no flower buds/flowers present), and reproductive (compound leaves and flower buds/flowers present). A single plant was defined as one stem or a tight bunching of stems (≤ 7 cm diameter grouping of stems) that presumably arose from a single rootstock.

For very large populations, where collecting population structure and herbivory data for all plants was not feasible, data were collected from a subsample. Subsampling covered the densest area of a population using transects laid out systematically perpendicular to the contour of the slope. Data were recorded for all plants located within 3 meters to either side of a transect line. Multiple transects were used for populations wider than 15 meters by establishing additional transects every 15 meters perpendicular to the contour.

Population Survey

The 2012 population surveys provided census, life history stage proportion, and plant size data. Complete censuses were conducted on five populations (three in Alabama, two in Tennessee) and incomplete censuses were conducted on seven populations (two in Alabama, five in Tennessee). Census data for populations that were not surveyed were used to estimate the total numbers of individuals in Alabama, Tennessee, and across the species' range. These additional data were acquired from U.S. Fish and Wildlife and Natural Heritage Program sources dating from 1990 to 2012 (including Weber 1991 and 1994; Emanuel 2000; Bailey 2005; Schotz 2007; Crabtree 2011). The newest and/or most extensive census was selected for each population, and the sum of selected censuses provided estimates for number of individuals in Alabama, Tennessee, and across the species' range.

Life history stage proportions and plant size data were also collected from each population surveyed. During the survey of each population the life history stage of each plant counted was recorded and the proportions of plants in each life history stage (seedling, non-reproductive, reproductive) were then determined for each population. Plant size data also were recorded at each population by measuring the total stem length (including branches) of each plant included in the survey. For multi-stemmed plants, the lengths of all stems were summed to calculate total stem length. To quantify their reproductive output at the time of the survey, both live and dead reproductive units (flower buds, flowers, clusters of achenes) were counted on all plants classified as being in the "reproductive" life history stage.

Herbivory Survey

Amounts of vegetative and reproductive herbivory were recorded from all sampled plants at each population. Vegetative herbivory was divided into four categories: invertebrate browse damage, vertebrate browse damage, piercing-sucking damage, or leaf miner damage.

Invertebrate browse damage was defined as damage consisting of holes or other evidence of folivorous invertebrate feeding. Vertebrate browse damage was defined as damage consisting of complete or partial leaf removal with no sign that insects were involved. Invertebrate browse herbivores included grasshoppers, caterpillars, gastropods, blister beetles (*Epicauta* sp.), etc., and vertebrate browse herbivores included white-tailed deer (*Odocoileus virginianus*). Piercing-sucking herbivore damage was defined as yellow speckling on the leaves. Piercing-sucking herbivores included mealy bugs (*Heliococcus* sp.), leafhoppers (*Acanalonia* sp. and a Flatidae species), and garden fleahoppers (*Halticus bractatus*). Leaf miner damage consisted of light brown lines (~3mm wide) that extended across the blade of a leaf. For each damage category, percent damage was visually estimated to the nearest 5% of the leaf surface that was assumed to be originally available.

Total reproductive units (flower buds, flowers, aggregate of achenes, etc.) were counted on all reproductive plants. For each plant surveyed, reproductive units destroyed by herbivores also were recorded. Reproductive units that were considered as destroyed by herbivores had obvious herbivory damage, including partial flower bud consumption and hollowing out of flower buds.

Data Analysis

Population data were analyzed for regional differences by classifying populations by region (Alabama or Tennessee) and using two-way analysis of variance (ANOVA) to determine if main effects (region and life history stage) and their interaction affected population composition. Populations were replicates, with 7 in Tennessee and 5 in Alabama. Proportion data for each life history stage category in each population were arcsine square root transformed to improve data structure and then analyzed with a two-way ANOVA using StatView 5.0 (SAS Institute, 2005).

Plant size data also were analyzed to determine the effects of region and life history stage. Plant size (total stem length) data were Log_{10} transformed and then analyzed as above using two-way ANOVA.

Reproductive plants varied greatly in stem length which affected total number of reproductive units produced. To quantify the reproductive output of plants in a way that accounted for differences in plant size, the total number of reproductive units produced by a plant was divided by total plant size (in meters). The resultant data (reproductive units per meter of stem length) reflected reproductive vigor and were analyzed by two 1-way ANOVAs. In the first, data from each plant were classified by region and in the second the data were classified by population to determine if either of these factors significantly affected reproductive vigor. Vegetative herbivory was analyzed for differences in severity as affected by region, life history stage and the interaction. Data for each of the four herbivory categories were arcsine square root transformed (to normalize data distribution) and then analyzed as above using two-way ANOVAs. Vertebrate browse damage was further analyzed among populations to elucidate the

possible effects of urbanization on increased herbivory, using Fisher's PLSD test to compare vertebrate browse damage among populations.

To identify correlations between plant size and extent of herbivory, stem length data were Log_{10} transformed while herbivory data were arcsine square root transformed and then analyzed with Z-tests using StatView 5.0 (SAS Institute, 2005). Three analyses were used for each herbivory category. Separate Z-tests were conducted on data from each region and then a third test was used for the entire dataset to determine if regional relationships extended across the species' range.

Herbivore damage to reproductive units (by definition occurring on reproductive plants only) was analyzed to determine if this damage varied between regions and for differences among populations. Florivory was analyzed for significant differences between regions. To quantify florivory on each plant and scale it to plant size, total reproductive units destroyed by herbivores was divided by plant stem length (in meters). The resultant data were analyzed to determine if herbivory varied between regions using a Mann-Whitney U test. To summarize florivory intensity per plant, the percent of total reproductive units that were destroyed by herbivores was also analyzed between regions using a Mann-Whitney U test in StatView 5.0 (SAS Institute, 2005).

Results

Population Survey

Locations of all known *Clematis morefieldii* populations are shown in Figure 1, including the locations of the 12 populations surveyed for this study. Alabama and Tennessee population sizes from my 2012 survey and previous surveys are listed in Table 1. There are 22 populations

of *Clematis morefieldii* in Alabama and Tennessee as of 2012, ranging in size from extremely small (8 plants) to very large (>7,000 plants). Alabama and Tennessee populations are currently estimated to have at least 8,868 and 6,751 plants, respectively (Table 1). As of 2012, there is an estimated total of 15,619 *Clematis morefieldii* plants in four counties, across two states.

The effects of life history stage, region (Alabama or Tennessee), and the life history stage * region interaction on population structure among surveyed populations were documented using two-way ANOVA. Life history stage was a significant factor (ANOVA: $F_{2,30} = 134$; $P < 0.0001$) as non-reproductive plants (Fig. 2) made up the largest proportion of plants in populations (~62%), followed by seedlings (~28%) and then reproductive plants (~10%). Life history stage proportions were not significantly affected by region (ANOVA: $F_{1,30} = 0.108$; $P = 0.74$) but there was a significant interaction between life history stage and region (ANOVA: $F_{2,30} = 7.27$; $P = 0.003$). Tennessee populations had greater proportions of non-reproductive plants and lesser proportions of seedling and reproductive plants than Alabama populations (Fig. 2).

A similar two-way ANOVA using plant size data showed significant effects of life history stage, region (Alabama or Tennessee), and the life history stage*region interaction. Figure 3 reveals expected differences in stem length among life history stages (ANOVA: $F_{2,30} = 448$; $P < 0.0001$), with reproductive plants being the largest (~161 cm), followed by non-reproductive plants (~54 cm) and then seedlings (~10 cm). Stem length differed significantly between regions with Alabama having larger plants (ANOVA: $F_{1,30} = 19.1$; $P = 0.0001$) (Fig. 3). The significant life history stage*region interaction (ANOVA: $F_{2,30} = 7.47$; $P = 0.002$) was due to the larger size of reproductive (22% longer) and non-reproductive (27% longer) plants in Alabama populations and the larger size of Tennessee population seedlings (7% longer) (Fig. 3).

Population and region had no effect on reproductive output relative to plant size. The number of reproductive plants in this analysis varied greatly among the 12 populations (from 3 to 63 individuals). Reproductive units per meter of stem length did not significantly differ among populations (ANOVA: $F_{11,207} = 1.73$; $P = 0.07$) nor between regions (ANOVA: $F_{1,207} = 2.07$; $P = 0.062$) (Fig. 4).

Herbivory Survey

Life history stages, regions, and populations all had significant effects on vertebrate browse damage patterns. Two-way ANOVA indicated significant differences among life history stages and regions but not the region*life history stage interaction (Table 2), while Fisher's PLSD test revealed significant differences among populations. Regionally, Alabama populations experienced 3-times greater levels of vertebrate browse damage (Fig. 5A). Reproductive and non-reproductive plants experienced greater levels of vertebrate browse damage than seedlings (12- to 13-fold). The region*life history stage interaction was not significant, indicating that regional patterns of vertebrate herbivory did not differ due to life history stage. Vertebrate browse damage was significantly different among populations with two Alabama populations (2-Deborah Drive and 3-Monte Sano Landtrust) experiencing the greatest severity of vegetative herbivory (24% and 32% mean browse damage, respectively). Fisher's PLSD test indicated that the damage levels in these populations differed from all others ($P < 0.05$).

Two-way ANOVA documented significant regional and life history stage effects on some invertebrate herbivory categories (Table 2). Invertebrate browse damage was significantly different among life history stages with seedling and non-reproductive plants experiencing almost 2-fold greater levels of invertebrate browse damage than reproductive plants (Fig. 5B).

As seen in Figure 5C, piercing-sucking herbivore damage was significantly different between regions. Alabama populations experienced over 2-fold greater piercing-sucking herbivore damage than Tennessee populations, and piercing-sucking herbivory was absent at over half of the surveyed Tennessee populations (4 populations: 11-Blooin Hollow, 13-Carter Cave, 16-Hawkins Cove, and 19-Miller Cove). Leaf miner herbivory was not significantly affected by any factors (Fig. 5D). The lack of significant region*life history stage interactions for invertebrate herbivore categories indicated that regional herbivory patterns did not vary among plants of differing life history stages.

Z-tests revealed significant correlations between stem length and herbivory categories in the combined range-wide analysis and in both regions (Alabama and Tennessee) (Table 3). At least some range-wide correlations between stem length and plant size occurred for plants in all three life history stages. For seedlings, stem length was positively correlated with damage by herbivores in all four herbivory categories. Stem length of non-reproductive plants was positively correlated with vertebrate and leaf miner herbivory but negatively correlated with invertebrate herbivory. Similar to non-reproductive plants, for reproductive plants stem length was negatively correlated with invertebrate herbivory but that was the only significant correlation found (Table 3).

Within regions, significant correlations were more frequent in Tennessee than Alabama (Table 3). Significant Tennessee correlations followed a similar pattern to rangewide correlations while Alabama had only one significant correlation, in which non-reproductive plant size positively correlated with vertebrate herbivory (as it did for Tennessee and for the rangewide analysis).

There was no significant difference between regions (Mann-Whitney U test, $P = 0.46$) for density of reproductive units destroyed by herbivores. Herbivore damage was low: out of all reproductive units counted only 1.9% were destroyed in Tennessee, 4% in Alabama, and 2.8% overall (Fig. 4).

Discussion

Population and herbivory surveys are useful for rare plant management by exposing population structure variation, plant size variation, herbivory severity, and interactions among factors across the range of a species (Mitchell 1994; García 2003; Kettenring *et al.* 2009). This study documented high seedling proportions, high vegetative herbivory damage, low reproductive herbivory damage and differences in some herbivore damage categories among life history stages and between regions. Alabama populations generally experienced greater vegetative herbivory than Tennessee populations, with urbanization around Alabama populations considered a possible source of the discrepancy. Subsequent regional surveys would allow comparisons across time to determine changes in population structure or herbivory severity that could result in population decline and extirpation.

Population structure varied across the range of *Clematis morefieldii*. Non-reproductive plants (62%) made up the largest proportion of plants, followed by seedlings (28%), then reproductive plants (10%). These life history stage proportions formed a steep J-shaped curve with juveniles (seedlings and non-reproductive plants) comprising the majority of populations (Fig. 2). The relatively large proportion of juvenile plants found in both regions implies abundant regeneration (Condit *et al.* 1998; Gómez-Aparicio *et al.* 2005). The low proportion of reproductive plants compared to juveniles would suggest slow maturation from juvenile plants to

reproductive plants (Condit *et al.* 1998). It should be noted that some seedlings and small non-reproductive plants could have been overlooked due to their size, therefore population structure data could under-represent actual life history stage proportions.

Progression of *Clematis morefieldii* from seedling stage to non-reproductive stage to reproductive stage may not be a one-way process: some plants have the ability to return to a previous life history stage if conditions are unfavorable (Kyle Paris, pers. obs.). Though I assume most plants follow the typical transformation from seedling to non-reproductive to reproductive, plants experiencing drought/herbivory stress have been observed returning to a previous life history stage (reproductive to non-reproductive, or non-reproductive to seedling) the following growing season. Similar life history stage dynamics occur in perennial understory plants such as *Trillium* spp. (Kawano *et al.* 1986), *Lathyrus vernus* (Ehrlen 1995), and *Actaea spicata* (Fröborg and Eriksson 2003). Dynamic changes for a long-lived species such as *C. morefieldii* could result in periods of population decline, interrupted by population growth that compensates for previous losses (Fröborg and Eriksson 2003). Since these plants are perennials that store energy in swollen roots, their life history stage may be dependent on energy stored from the previous season.

While plants in the non-reproductive and seedling life history stages made up the largest proportion of populations, many of these plants were observed in what is speculated to be lower quality habitat (heavily shaded). These suppressed plants may persist for years and only become reproductive if disturbance increases available light (Todd Crabtree, pers. comm. 2012). While many of these plants may never reach sexual maturity, disturbances such as individual treefalls, logging, high wind events, or fire could temporarily open the canopy and allow non-reproductive plants to become reproductive (Levey 1990). Disturbance events could produce spikes in

reproductive output for a population (Pascarella 1995), although competition by understory and midstory plants may reduce reproductive effort. As the overstory regenerates it is possible that plants would eventually become smaller and revert to the non-reproductive stage.

Stem length was significantly different among life history stages, regions, and the life history stage*region interaction. The significant difference among life history stages is expected as plants increase in size as they mature. Alabama was found to have significantly larger plants than Tennessee. The significant interaction between life history stage and region occurred because non-reproductive and reproductive life history stages in Alabama had greater stem lengths than those in Tennessee. However, mean stem length for the seedling life history stage was greater in Tennessee than Alabama. Larger plants can suffer greater herbivore damage due to greater conspicuousness (Sletvold and Grindeland 2008) and the plant-vigor hypothesis (stating that larger, vigorously growing plants attract more herbivores due to greater nutrient levels in tissues) (Price 1991), however, correlations between stem length and herbivory damage revealed only one positive correlation in Alabama (Table 3).

Reproductive unit production was low. Reproductive unit production was not significantly different among populations or between regions and averaged 4.5 reproductive units produced per meter of stem length. Over the entire surveyed range of *Clematis morefieldii*, an average of seven reproductive units were produced per plant (N = 220). Reproductive unit production for other surveyed populations in 2012 did not significantly differ from that at the 1-Keel Mountain Preserve study site. But 2012 reproductive unit production was extremely low at the 1-Keel Mountain Preserve: average reproductive units per plant (N = 40) were: 22.25 (SE = 5.28) in 2009, 19.33 (SE = 4.47) in 2010, 17.4 (SE = 4.01) in 2011, and 5.78 (SE = 1.4) in 2012

(Ch. III, Table 1). This suggests that 2012 reproductive unit production across the range of *C. morefieldii* is below average compared to 2009-2011.

Surveyed *C. morefieldii* populations were at risk from a variety of factors. Though limestone outcrops naturally reduced canopy cover, at least three Alabama populations (2-Deborah Drive, 3-Monte Sano Landtrust, 6-Kenamer Preserve) and three Tennessee populations (11-Blooin Hollow, 14-War Memorial, and 17-Mill Creek) surveyed still had a relatively heavy canopy cover which could potentially be reducing the reproductive output of these populations. Weber (1990) noted that fire historically occurred on the plateau escarpment around Huntsville, and this disturbance could reduce the canopy cover in *C. morefieldii* habitat. Recent practices of fire suppression could be having a negative impact on *C. morefieldii* populations. Invasive plant species (*Lonicera* spp.) were also present in potentially detrimental numbers at two Alabama populations (2-Deborah Drive and 3-Monte Sano Landtrust), both of which are in close proximity to Huntsville, AL. Anthropogenic changes to the watershed appeared to be affecting an Alabama population (2-Deborah Drive), as plants appeared to be more water-stressed than other populations during visits in 2010 and 2012.

Rainfall was low during the year of this survey and could have had an effect on all data collected. Rainfall was 51% lower than average (1983-2012) in Huntsville, AL during the 2012 growing season (April-June) (Ch. III, Fig. 1) and drought conditions (wilted vegetation, parched ground, etc.) were observed at all populations visited during the survey. Reproductive attrition studies (Ch. III) at the Keel Mountain Preserve population in Alabama found reproductive effort to be at its lowest during 2012 compared to the previous three years (2009-2011). This below average year of rainfall could have negatively affected the life history stage, stem length, and reproductive output of plants across the range of *C. morefieldii*. Therefore data collected during

the 2012 survey likely may not reflect population structure, plant size, or even herbivory levels during an average rainfall year.

Herbivory is another factor that could be having a detrimental effect on surveyed *C. morefieldii* populations (Crawley 1989; Louda and Potvin 1995). Vegetative and reproductive herbivory varied across the range of the species. Potentially detrimental levels of vegetative herbivory (negatively affecting growth, reproduction, or survival) occurred in both regions although Alabama experienced significantly greater levels of vertebrate and piercing-sucking damage. Life history stages received significantly different levels of vertebrate and invertebrate browse damage. Correlations between stem length and herbivory levels were both positive and negative and were primarily restricted to Tennessee and the smaller life history stages. The general lack of reproductive herbivory across the range was unsurprising considering the lack of reproductive output during this drought year.

Many *Clematis morefieldii* populations are located in a fragmented landscape. The species is associated with forested mountain slopes, areas that are poorly suited for urbanization or traditional agriculture. While flatter areas above and below the mountainsides are highly utilized, the mountainsides remain wooded and are generally far less altered. Fragmentation of habitat lowers species richness of invertebrates, potentially resulting in a simplified suite of invertebrate herbivores and predators (Didham *et al.* 1996; Kolb 2008). Woodlots in suburban areas can be affected by deer overbrowsing, exotic invasive plants, and anthropogenic disturbances (Waller and Alverson 1997).

Deer are often considered keystone herbivores capable of directly affecting overstory recruitment and understory diversity while also indirectly producing a trophic cascade through the oversimplified ecosystems brought on by overgrazing (Waller and Alverson 1997; Augustine

and Frelich 1998; Rooney and Waller 2003). Augustine and Frelich (1998) found that deer had significant effects on growth and reproduction of understory forbs (*Trillium* spp.) in high densities. Natural areas in highly fragmented, highly altered habitat could attract deer because these fragmented woodlands can be used by deer for cover, travel corridors, and forage (Nixon *et al.* 1991). Without natural predators or hunters, deer populations may be concentrated in these scattered natural areas and consume palatable vegetation. Conversely, areas difficult to access by deer (cliff edges, steep slopes, within fallen tree branches, etc.) could become refuges for plants, allowing plants to experience little or no vertebrate browse damage (Rooney 1997). Many of the largest reproductive *C. morefieldii* (above average stem length and reproductive unit production) were observed in hazardous and difficult to access areas (Kyle Paris personal obs.).

Vertebrate herbivory levels were significantly higher in Alabama than in Tennessee. Two populations in Alabama (2-Deborah Drive and 3-Monte Sano Landtrust) experienced significantly greater vertebrate herbivory than the other 10 populations surveyed. Both of these populations are within the city limits of Huntsville, Alabama; an area with highly fragmented habitat and little if any hunting pressure. Deer sign was visible at both populations and much of the understory was open and appeared to be heavily browsed.

Vertebrate herbivory levels were significantly different between life history stages. Reproductive and non-reproductive plants experienced significantly greater levels of vertebrate herbivory than seedlings (Fig. 5A). Augustine and Frelich (1998) found that deer focused on larger reproductive understory plants (*Trillium* spp.) while ignoring smaller seedlings. The plant-vigor hypothesis (Price 1991) suggests that herbivores should prefer larger or more robust plants due to the greater amount of resources those plants offer. Heard and Cox (2009) suggest that similar results could occur because larger plants are simply more apparent to herbivores.

Reproductive and non-reproductive *C. morefieldii* plants are relatively large and could easily be located by vertebrate herbivores, while seedlings can be very small (<10 cm) and easily overlooked. However, there would likely be little left from a seedling eaten by a large herbivore, and so highly browsed seedlings could easily have been overlooked during the herbivory survey.

Invertebrate herbivory was found to be significantly different among life history stages. There was significantly more invertebrate browse damage on seedling and non-reproductive plants than on reproductive plants. These findings contradict the predictions of Price's (1991) plant-vigor hypothesis. The plant-stress hypothesis (White 1969, 1984) links stressed plants to higher nitrogen levels in their tissues which results in a better food source for invertebrate herbivores. Rhoades (1979) adds that stressed plants are less likely to produce herbivore defenses. Many seedling and non-reproductive plants were observed in lower quality habitat (closed canopy) and therefore could be stressed by the heavy shading. Also, equal absolute amounts of invertebrate browse damage would remove a greater percentage of the total leaf area on seedling and non-reproductive plants compared to reproductive plants. Reproductive plants' large size could result in invertebrate herbivore satiation before plants were heavily defoliated. The ubiquitous nature of generalist invertebrate herbivores could help to explain the lack of significant difference between regions.

Piercing-sucking herbivore damage was significantly greater in Alabama than in Tennessee. Four Tennessee populations had no piercing-sucking herbivore damage present. All animals in this herbivory category observed on *Clematis morefieldii* were very small and could be poor dispersers. Two of these populations (11-Blooin Hollow and 13-Carter Cave) are isolated from other populations and piercing-sucking herbivores may not have dispersed to them.

However, the two other populations (16-Hawkins Cove and 19-Miller Cove) are relatively close to populations with piercing-sucking herbivore damage present.

Leaf miner damage did not differ significantly between regions or among life history stages. While common across the range of *C. morefieldii*, leaf miner damage was typically minor and very rarely damaged more than 5% of the total leaf surface area of a plant.

Significant positive and negative correlations were observed for stem length and vegetative herbivore damage in the overall population analysis and in both regions (Alabama and Tennessee) (Table 3). Positive correlations were present for damage by all four herbivory categories on seedlings but only for vertebrate and leaf miner herbivory on non-reproductive plants. The plant-vigor hypothesis (Price 1991) could explain increased herbivory on larger plants. As plants become larger they could also become more conspicuous, thus attracting more herbivores (Sletvold and Grindeland 2008). Reproductive plants are larger than seedlings and most non-reproductive plants and therefore are more conspicuous. It is possible that size did not influence herbivore damage on reproductive plants because those relatively large plants were conspicuous enough to receive similar levels of herbivore damage.

Of all the correlations found to be significant, only three were negative. Negative correlations were found for invertebrate herbivory on non-reproductive and reproductive plants. The ubiquitous nature of insects would suggest that a plant would not necessarily have to be large and conspicuous to receive invertebrate browse damage: Sessions and Kelly (2001) found invertebrate herbivory homogeneously distributed throughout populations of a rare plant.

Reproductive herbivory was found to be extremely low across the range of *Clematis morefieldii*. With less than 3% of reproductive units destroyed by herbivores across the species' range, it would seem that reproductive unit herbivory is of little importance. However, the survey

was conducted in a relatively dry year and lack of rainfall is probably responsible for low reproductive effort in 2012 (Ch. III, Fig. 1). Many of the flower buds which were produced appeared to have been aborted before herbivory could take place. Therefore these data likely are not representative of a survey during a growing season with increased rainfall. Reproductive attrition studies at an Alabama population (Ch. III) found much higher levels of reproductive herbivory over a four-year period.

Information on the state of *Clematis morefieldii* populations and herbivory severity across its range can facilitate effective monitoring of populations and identification of factors affecting population structure and size dynamics. Censuses revealed a greater number of plants at some populations than previously recorded, and also documented the largest known population at the Keel Mountain Preserve in Alabama. The high proportion of juvenile plants (seedling and non-reproductive) in populations suggests the occurrence of recruitment and slow maturation of plants to the reproductive life history stage (Condit *et al.* 1998; Gómez-Aparicio *et al.* 2005). Populations should continue to be monitored for declines in population size or changes in population structure (reduced proportions of seedlings and reproductive plants), with greater vigilance for smaller populations that could easily become extirpated (Matthies *et al.* 2004). Severe vegetative herbivory could have impacts on population size and should be investigated if a population is declining (Augustine and Frelich 1998). Deer overpopulation and habitat fragmentation could be primary factors driving high levels of vertebrate browse damage on some populations, and steps to mitigate that damage may be required if populations show signs of decline. Vertebrate, invertebrate, and piercing-sucking herbivore damage are capable of severely reducing leaf surface area available to photosynthesize and could detrimentally affect reproductive output and population dynamics.

Table 1. List of documented populations of *Clematis morefieldii*. Populations from Alabama (Weber 1991 and 1994; Emanuel 2000; Schotz 2007) and Tennessee (Bailey 2005; Crabtree 2011) are estimated to currently have at least 8868 and 6751 plants, respectively. Numbers in front of population names were used throughout this study to facilitate identification. EO Number is the element occurrence number given to population or subpopulation by the Alabama or Tennessee Natural Heritage Program. “Est.” refers to populations with estimated numbers and “subsample” surveys were incomplete plant counts.

Population (EO#)	State	County	Last Documented Plant Numbers	2012 Survey Plant Numbers
1-Keel Mountain Preserve (1)	AL	Madison	500 (est. 1999)	7085*
2-Deborah Drive (2)	AL	Madison	550 (est. 1999)	63 (subsample)
3-Monte Sano Landtrust (4,5)	AL	Madison	122 (est. 1990/1999)	159
4-Rockslide (9)	AL	Madison	150 (est. 1999)	
5-Cramer Hollow (10)	AL	Madison	45 (2002)	
6-Kenamer Preserve (11)	AL	Jackson	41 (2002)	100
7-Pelletier Hollow (12)	AL	Madison	47 (2003)	282 (subsample)
8-Hale Mountain (13)	AL	Madison	50-60 (2004)	
9-Moore Cove (14)	AL	Madison	17 (2005)	
10-Honey Hollow (15)	AL	Jackson	300 (est. 2007)	
11-Blooin Hollow (1)	TN	Franklin	101 (2009)	91 (subsample)
12-Keith Cove (2,3,18)	TN	Franklin	37 (2009)	
13-Carter Cave (4)	TN	Franklin	92 (2009)	408
14-War Memorial (5)	TN	Franklin	84 (2009)	87
15-Snowbird Hollow (9)	TN	Franklin	4494 (2009)	568 (subsample)
16-Hawkins Cove (10)	TN	Franklin	748 (2009)	313 (subsample)
17-Mill Creek (13)	TN	Franklin	126 (2009)	192 (subsample)
18-The Narrows (12, 14)	TN	Franklin	21 (2009-2010)	
19-Miller Cove (15)	TN	Franklin	174 (2009)	154 (subsample)
20-Kirby Smith Branch (16)	TN	Franklin	36 (2009)	
21-Sinking Cove (17)	TN	Franklin	8 (2009)	
22-Burrow Cove (19)	TN	Grundy	445 (2009)	

* Note that the 2012 census for Keel Mountain Preserve covered the majority of the population, but the population was so extensive that a complete census was impractical. Thus the total population at this site was even greater than reported here.

Table 2. Two-way ANOVA results for *Clematis morefieldii* herbivory category comparisons with significant differences defined as having a P-value ≤ 0.05 (asterisk denotes significance). Damage data for each herbivory category were analyzed for effect of region (Alabama and Tennessee), life history stage (seedling, non-reproductive, reproductive), and the region*life history stage interaction.

Factor	Herbivory Category	d.f.	F	P
Region	Vertebrate	1	11	0.002*
Region	Invertebrate	1	1.8	0.19
Region	Piercing-sucking	1	5.4	0.03*
Region	Leaf Miner	1	2.5	0.13
Life history stage	Vertebrate	2	8.6	0.01*
Life history stage	Invertebrate	2	13	<0.001*
Life history stage	Piercing-sucking	2	1.8	0.19
Life history stage	Leaf Miner	2	2.8	0.08
Region*Life history stage	Vertebrate	2	2.3	0.11
Region*Life history stage	Invertebrate	2	0.91	0.41
Region*Life history stage	Piercing-sucking	2	1.2	0.30
Region*Life history stage	Leaf Miner	2	0.02	0.98

Table 3. *Clematis morefieldii* Z-values from Z-test. Correlations are between stem length and degree of vegetative herbivory for each category of herbivory, with significant effects defined as those having a P-value ≤ 0.05 (asterisk denotes significance).

Life history stage category	Correlation between stem length and herbivory category	Range-wide	AL	TN
Seedling	Vertebrate	0.093*	0.065	0.1
Seedling	Invertebrate	0.134*	0.074	0.151*
Seedling	Piercing-sucking	0.141*	0.165	0.185*
Seedling	Leaf Miner	0.162*	0.093	0.184*
Non-reproductive	Vertebrate	0.274*	0.328*	0.198*
Non-reproductive	Invertebrate	-0.107*	-0.058	-0.106*
Non-reproductive	Piercing-sucking	0.056	-0.028	0.048
Non-reproductive	Leaf Miner	0.147*	0.097	0.183*
Reproductive	Vertebrate	-0.028	-0.116	-0.099
Reproductive	Invertebrate	-0.168*	-0.086	-0.101
Reproductive	Piercing-sucking	0.067	-0.018	0.032
Reproductive	Leaf Miner	0.021	0.178	-0.06

Figure Legends

Figure 1. Ecoregion map (Griffith *et al.* 2001) showing all *Clematis morefieldii* populations in Northeast Alabama (1-10) and South-Central Tennessee (11-22). Population numbers and names are: 1-Keel Mountain Preserve, 2-Deborah Drive, 3-Monte Sano Landtrust, 4-Rockslide, 5-Cramer Hollow, 6-Kennamer Preserve, 7-Pelletier Hollow, 8-Hale Mountain, 9-Moore Cove, 10-Honey Hollow, 11-Blooin Hollow, 12-Keith Cove, 13-Carter Cave, 14-War Memorial, 15-Snowbird Hollow, 16-Hawkins Cove, 17-Mill Creek, 18-The Narrows, 19-Miller Cove, 20-Kirby Smith Branch, 21-Sinking Cove, 22-Burrow Cove.

Figure 2. Mean proportions (bars are S.E.) of *Clematis morefieldii* life history stages (seedling, non-reproductive, and reproductive) in Alabama (N = 5) and Tennessee (N = 7) populations in 2012.

Figure 3. Mean *Clematis morefieldii* stem length (cm) (bars are S.E.) for each life history stage (seedling, non-reproductive, and reproductive) from sampled Alabama (N = 5) and Tennessee (N = 7) populations in 2012.

Figure 4. Mean density of reproductive units and reproductive units destroyed by herbivores per meter of stem length (bars are S.E.) on *Clematis morefieldii* from Alabama (N = 5) and Tennessee (N = 7) populations in 2012.

Figure 5. Mean percent vegetative herbivory (bars are S.E.) for each *Clematis morefieldii* life history stage (seedling ☐, non-reproductive ■, reproductive ▣) from Alabama (N = 5) and Tennessee (N = 7) populations in 2012: (A) vertebrate browse damage, (B) invertebrate browse damage, (C) piercing-sucking herbivory, (D) leaf miner herbivory.

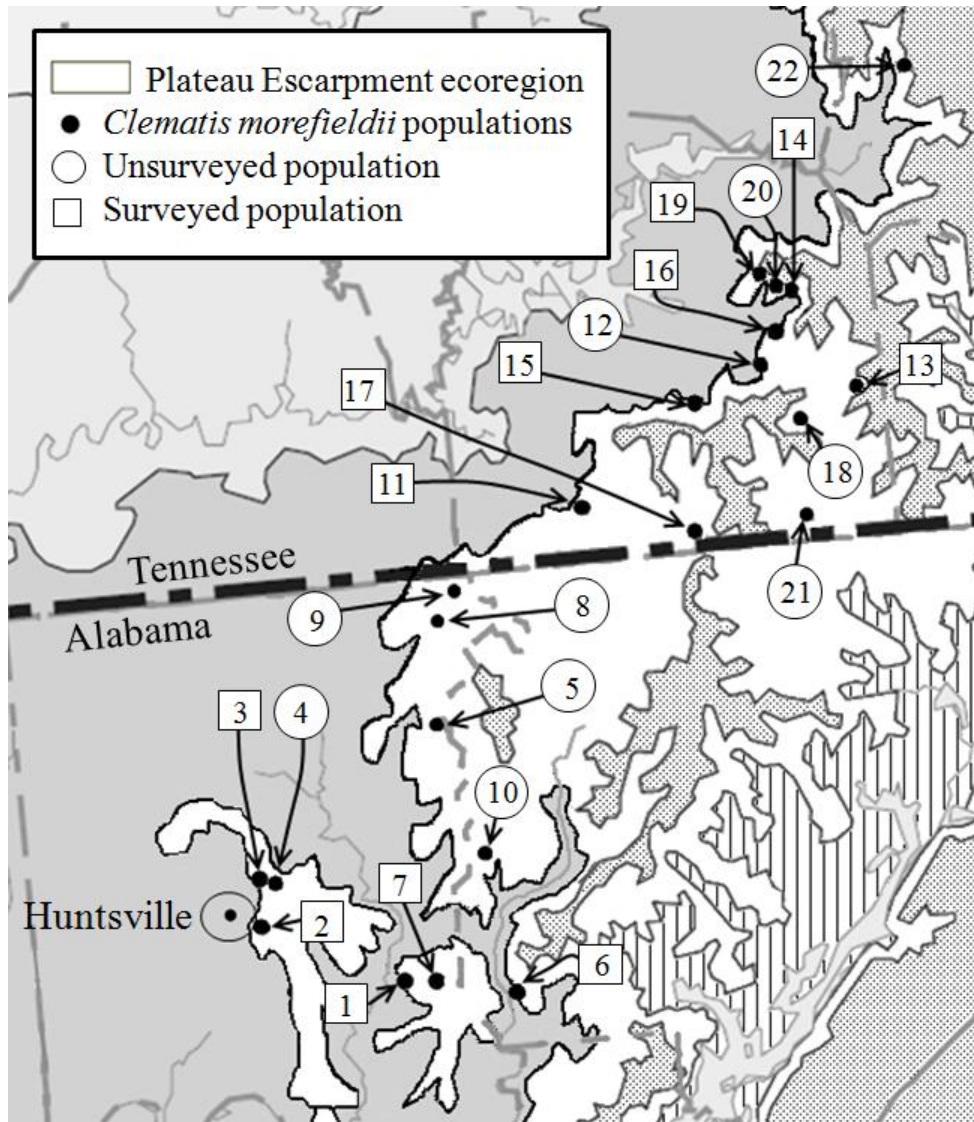


Figure 1

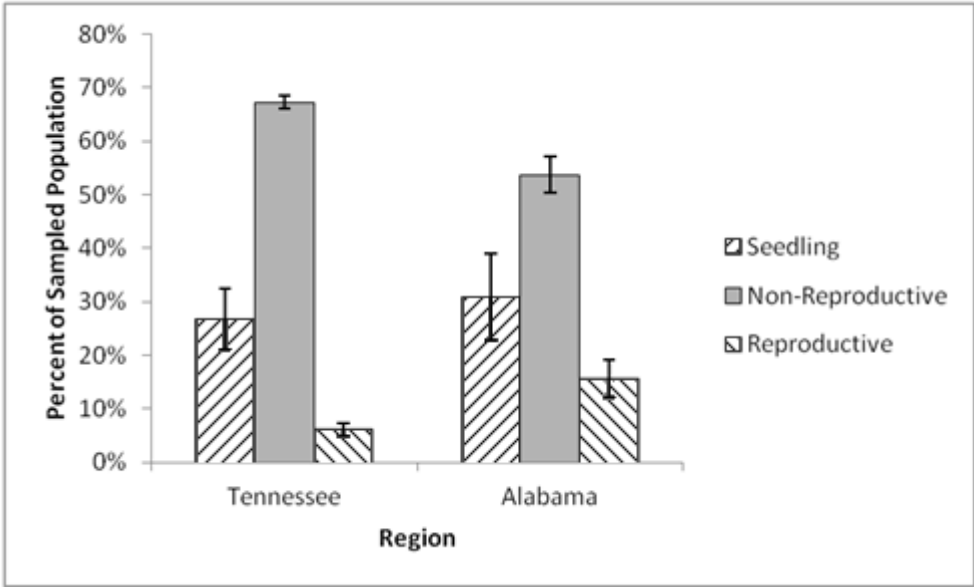


Figure 2

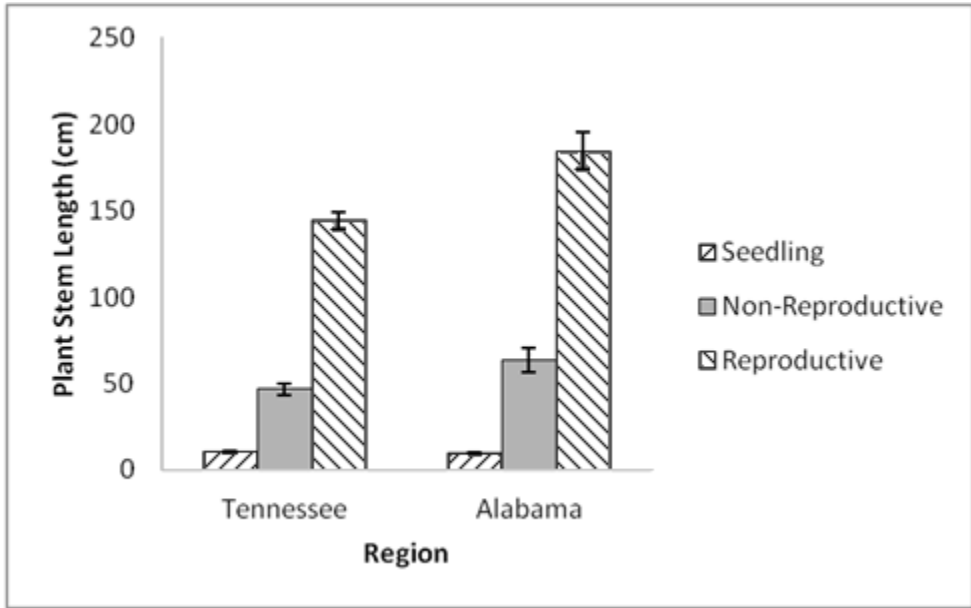


Figure 3

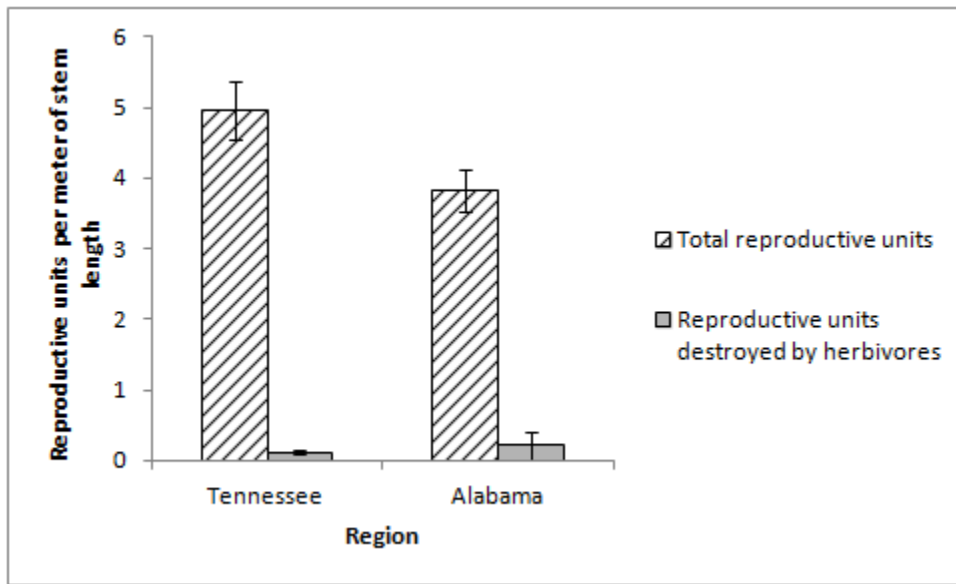


Figure 4

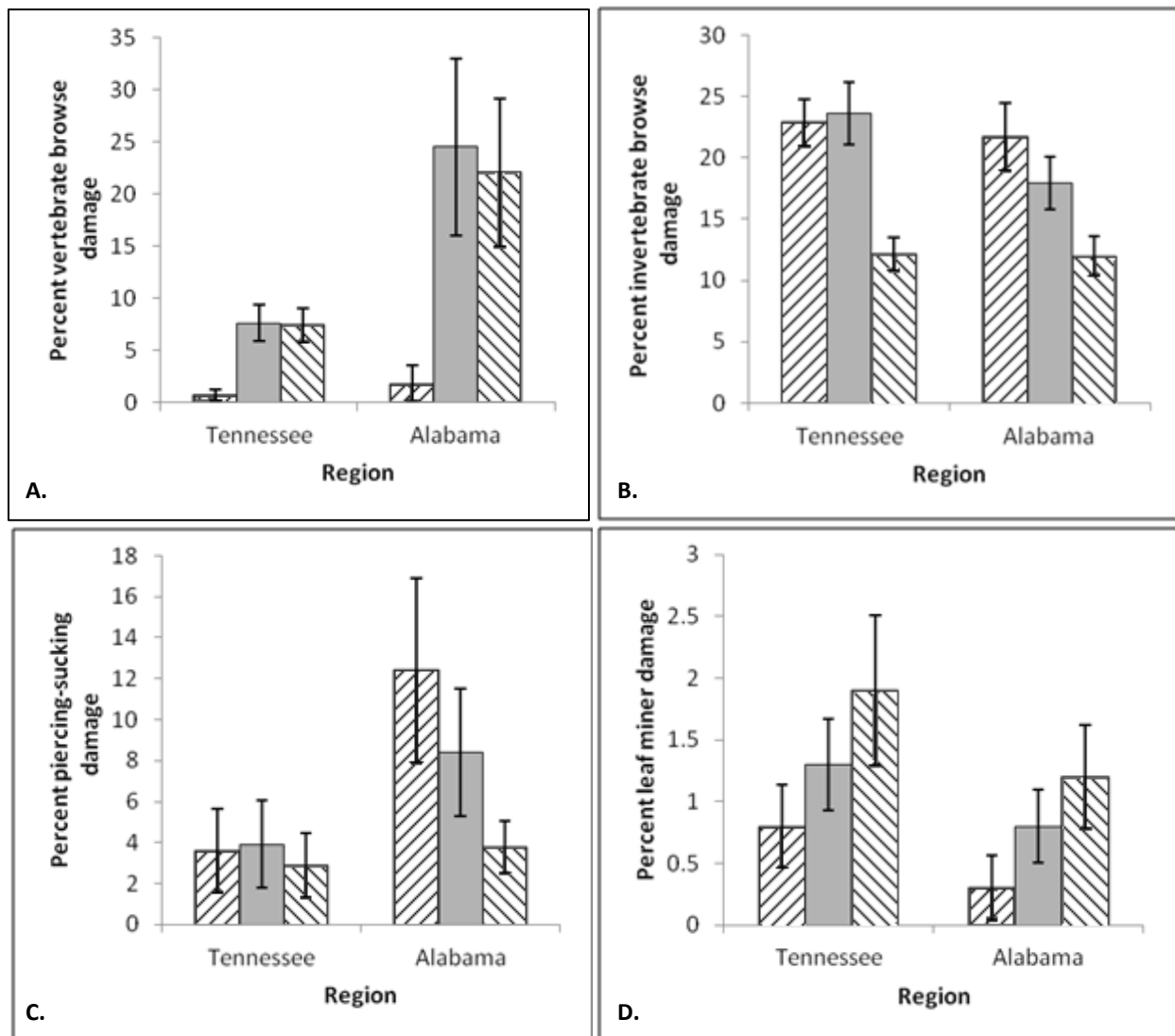


Figure 5 (A-D)

III. REPRODUCTIVE ATTRITION AND SEEDLING ESTABLISHMENT OF *CLEMATIS* *MOREFIELDII* KRAL

Abstract

Key components for successful management of rare plant species include an understanding of reproductive life history and knowledge of reproductive attrition and seedling establishment. To assess reproductive attrition, the status of all reproductive units (expressed in seven stages) on 40 plants located at The Nature Conservancy's Keel Mountain Preserve in Madison County, AL was recorded throughout four growing seasons (2009-2012). Floral herbivory by lepidopteran larvae (Geometridae) and abortion of reproductive units was common, with few flower buds (8% average across four years) producing mature fruit and the greatest average attrition rate (59%) occurring during the smallest flower bud stage. Reproductive output was positively correlated with peak flowering/fruitlet rainfall (April-June) with plants producing more fruits in rainy years (2009 and 2011) than in drought years (2010 and 2012). Post-dispersal achene predation assessed in *Clematis* (areas with high plant densities) and low-*Clematis* (areas with low plant densities) zones reached 15% after 1 week and slowly increased to 30% over a 4-month period. Achene predation was not significantly different between *Clematis* and low-*Clematis* zones. *In situ* germination was documented by planting achenes in cages in *Clematis* and low-*Clematis* zones. Seeds remained dormant the first year of the study, but germinated in significantly greater amounts the second year (23%) compared to the third year (9%) after planting. There was no significant difference in seed germination between *Clematis* and low-*Clematis* zones. This study documented high levels of floral herbivory, a bottleneck in reproductive unit maturation that limits reproductive output, a positive correlation of reproductive output with peak flowering/fruitlet rainfall, moderately high post-dispersal achene predation levels, one year

delayed germination, and a third of seeds germinating in three years. Managers may be able to use these results to design management strategies that can boost reproductive output of plants in declining or small populations.

Introduction

Key components for successful management of rare plant species include an understanding of reproductive life history and knowledge of any factors that may be inhibiting successful reproductive output and seedling establishment. Plants that do not reproduce vegetatively rely on sexual reproduction to produce seeds for generating new individuals in a population (Kaye 1999). Sexual reproduction not only sustains population numbers via seedling recruitment but also increases genetic variation within a population (Fischer and Matthies 1998). Small population size is a common problem with rare plants and can lead to reduced genetic diversity and fitness in remnant populations because of genetic drift, e.g. by producing fewer seeds, through less resilience to changes in abiotic and biotic factors, etc. (Ellstrand and Elam 1993), which can lead to an extinction vortex (Tanaka 2000). Reproductive output is a critical part of maintaining or increasing population size, and reproductive attrition studies allow a better understanding of how effective reproductive units are at producing fruit and what factors may be reducing fruit production (e.g. Boyd and Brum 1983; Boyd and Serafini 1992; Kaye 1999). Seedling establishment is also crucial to maintaining populations and studies revealing post-dispersal seed predation and *in situ* germination can provide data for effective management of a species (e.g. Maron and Simms 1997; Wenny 2000).

Reproductive attrition studies document the fate of reproductive units through time from initiation of flower buds to maturation of fruit (e.g. Boyd and Brum 1983; Boyd and Serafini 1992; Kaye 1999). Data are collected on reproductive units as they mature, documenting in

which maturation stage they are destroyed or aborted. If reproductive units successfully produce mature fruits, fruit numbers are recorded. These studies provide data for determining how successful reproductive units are at producing fruit, and also identify stages in the maturation process with high levels of abortion/herbivory. These data are useful for identifying factors that reduce reproductive output.

Abortion of a reproductive unit by a plant is one of the major factors affecting the proportion of reproductive units successfully producing mature fruit. Pollination of flowers usually exceeds fruit set in most species (Guth and Weller 1986; Kokubun *et al.* 2001) although plants may abort some flowers due to a lack of effective pollination (e.g. Timmerman-Erskine and Boyd 1999; Mattila and Kuitunen 2000). A lack of resources (rainfall, nutrients, sunlight, etc.) available for maturation and fruit production by a reproductive unit also can cause abortion (Stephenson 1981; Bookman 1983; Niesenbaum 1996; Kokubun *et al.* 2001; Fang *et al.* 2009). Abiotic (temperature) and biotic (florivory) damage to reproductive units are also major factors affecting the proportion of reproductive units successfully producing fruits (Stephenson 1981; McIntosh 2002; Nayyar *et al.* 2005). Many plants produce an overabundance of flower buds, ensuring enough will survive to generate maximum fruit production with available resources (Stephenson 1981; Bookman 1983).

Seed predation can be a severe threat to the proportion of reproductive output available for germination (e.g. Maron and Simms 1997; Wenny 2000). Severity of seed predation depends on type of predator, dispersal distance from parent plant, size and conspicuousness of seed/fruit, palatability, etc. (Janzen 1971; Guimarães *et al.* 2003; Chauvet *et al.* 2004; Nathan and Casagrandi 2004). Seed predation can also restrict seedling establishment to certain habitats and microsites (e.g. Boyd 1988; Smith 1987). Viable seeds not consumed by seed predators must

successfully germinate to establish new seedlings. Many species delay germination, creating seed banks that can protect populations from extirpation due to climatic variation and catastrophic events (Satterthwaite *et al.* 2007). However, the longer seeds remain dormant, the greater the chance of seed predation and other factors that destroy seeds or reduce viability. Documentation of the process from seed dispersal to seedling establishment can predict the proportion of reproductive output that generates seedlings.

The federally endangered *Clematis morefieldii* is one of several narrow endemic *Clematis* species found in the Southeast (Platt 1951; Baskin and Baskin 1988; Braunschweig *et al.* 1999). Endemic to limestone outcrops of the Cumberland Plateau, *C. morefieldii* is only known from four counties in northeast Alabama and south-central Tennessee. Little is known about *Clematis morefieldii*'s reproductive biology including reproductive output and the proportion of seeds that successfully establish seedlings. Objectives of this study were 1) to document pre-dispersal attrition of reproductive units among years, and 2) to compare post-dispersal seed predation and *in situ* germination rates between habitat types. In addition to providing basic information on *C. morefieldii* biology and ecology, these studies can also aid managers of this rare species. For example, with knowledge of factors inhibiting maturation of reproductive units, managers can implement strategies for increasing successful fruit set and ultimately boost reproductive output (e.g. Boyd and Brum 1983; Boyd and Serafini 1992; Kaye 1999). Additionally, studies of post-dispersal seed predation and seed germination rates can elucidate factors retarding seedling establishment and provide managers with the knowledge to boost seedling recruitment (e.g. Maron and Simms 1997; Wenny 2000).

Materials and Methods

Study Site

The study site is located on The Nature Conservancy's Keel Mountain Preserve near Gurley, AL in Madison County (N 34° 39' 27.8", W 86° 24' 42.3"). This preserve is located in Esslinger Hollow on the south facing slope of Keel Mountain. Part of the Cumberland Plateau, Keel Mountain's slopes are rugged and covered in limestone. The slopes are arranged into terraces with steeply sloping areas abundant in exposed limestone followed by gently sloping areas with little exposed limestone. Limestone boulders, cliff-faces, and exposed bedrock are primarily found in the steeply sloping areas though smaller limestone rocks are nearly ubiquitous across the mountainside. The entire mountainside is forested but canopy cover is noticeably reduced in the steeply sloping areas due to the abundance of exposed limestone and shallow soil depth.

The vegetation is typical for Cumberland Plateau escarpments and understory density varies widely from impenetrable to open. Overstory composition is primarily oak (*Quercus* spp.), hickory (*Carya* spp.), ash (*Fraxinus* spp.), Eastern redcedar (*Juniperus virginiana*), and smoketree (*Cotinus obovatus*). The midstory and shrub layer are primarily made up of St. John's wort (*Hypericum frondosum*), blackhaw (*Viburnum prunifolium*), beauty berry (*Callicarpa americana*), Buckthorn (*Frangula caroliniana*), upland swamp-privet (*Forestiera ligustrina*), and fragrant sumac (*Rhus aromatica*). A diversity of understory herbs can be found growing on the slopes including prickly pear (*Opuntia humifusa*), Cumberland rosinweed (*Silphium brachiatum*), eared goldenrod (*Solidago auriculata*), mercury spurge (*Euphorbia mercurialina*), indian pinkroot (*Spigelia marilandica*), downy agrimony (*Agrimonia pubescens* var. *pubescens*)

and many other asters (Asteraceae), grasses (Poaceae), and sedges (Cyperaceae). Several vines are commonly found on the mountainside including cross vine (*Bignonia capreolata*), Alabama supplejack (*Berchemia scandens*), greenbriar (*Smilax* spp.), poison ivy (*Toxicodendron radicans*), muscadine (*Vitis rotundifolia*), and Virginia creeper (*Parthenocissus quinquefolia*).

Past anthropogenic disturbances on the plateau escarpment (including the preserve) include fire and logging. Lightning ignition is rare in the Southern Appalachians and pre-Colombian fires were thought to be infrequent to common (depending on the area) and primarily from anthropogenic ignition for maintenance of hunting and gathering areas (Delcourt and Delcourt 1997). Ison (2000) found that within the Cumberland Plateau, the plateau escarpments were thought to be the most frequently burned by Native Americans. Post-Colombian fires also occurred on the southern Cumberland Plateau escarpments due to anthropogenic ignitions (escaped campfires, embers from trains, etc.) and were frequent on the mountains surrounding Huntsville, AL (Weber 1990). Fire scars can be seen on old cedar stumps at the Keel Mountain Preserve so fire was historically present (Kyle Paris pers. obs.).

Logging is common practice on the Cumberland Plateau escarpment on land usually considered unsuitable for residential or agriculture land uses. Logging of the Cumberland Plateau forest began during the War Between the States (1860s) and peaked in 1920 (Hinkle *et al.* 1993). Most mature forests in the Cumberland Plateau are now secondary stands or stands that have developed following the high levels of forest harvesting in the late 1800s and early 1900s (Hart and Grissino-Mayer 2008). While clear-cutting on the Cumberland Plateau escarpment is still practiced, sustainable forestry management by selectively harvesting desirable trees over time is also prevalent (Scott Helms pers. comm.). The forest on the preserve is mature though old Eastern redcedar slash piles and stumps from past logging are still evident.

Reproductive Attrition

A sample of 40 plants was selected at the Keel Mountain Preserve population from four sites (~25 to 50 m apart) with high densities of reproductive *C. morefieldii*. At each site 10 reproductive plants in close proximity to one another (~10 m) were selected for the study. Each plant was marked with a numbered metal tag and then every node that produced at least one flower bud was marked with a colored pigeon band. Data were then recorded approximately bimonthly over the course of each of four growing seasons (2009, 2010, 2011, 2012) for all flowers and fruits produced by the study plants. The stage at which flowers and fruits were destroyed was recorded and, if they did not produce mature fruits, how the reproductive unit died (abortion or herbivory). As the aggregate fruits matured, all of their achenes were counted and observed until fully mature and dispersed.

To collect data on reproductive units (flower buds, flowers, and developing achenes), seven stages were used to describe the maturation process from newly initiated flower buds to mature fruits. Flower buds in the “tiny” stage were 1-3 mm in length and sessile. Flower buds with a width of 3-6 mm and a developing pedicel were designated as the “immature” stage. Flower buds greater than 6 mm, still green in color, and with sepals firmly closed were designated as the “medium” stage. The “flower” stage referred to reproductive units that usually had pink sepals which were partially or fully opened. The “tiny aggregate” stage designated flowers that were transitioning into aggregate fruits that had senesced/fallen sepals, but with ovaries that had not begun to enlarge. The “immature aggregate” stage referred to aggregate fruits with enlarging ovaries no greater than 4 mm in width, green in coloration, and without long trichomes on the persistent styles. The “mature aggregate” stage referred to matured fruits,

usually with a width of ~6 mm, green to brown in coloration, and with persistent styles covered in ~4 mm long trichomes.

Rainfall data were acquired from Redstone Technical Test Center (RTTC) Meteorological Team at Redstone Arsenal in Huntsville, AL. This weather station (N 34° 36' 0.8", W 86° 37' 49.7") is approximately 21 km from the Keel Mountain Preserve (N 34° 39' 27.8", W 86° 24' 42.3"). Snowfall data (cm) were converted to rainfall data (cm) by dividing snow depth by 10 cm (the common method for converting snow depth to rainfall is dividing snow depth by 10). Average rainfall was calculated from records spanning 30 years (1983-2012) and was used to calculate departures from average for the years of the reproductive attrition study.

Post-Dispersal Achene Predation

Seed predation by vertebrates could be a bottleneck in recruitment and was therefore documented and analyzed for differences in predation rates between *Clematis* and low-*Clematis* zones. The *Clematis* zone is the primary area where *C. morefieldii* plants occur and is characterized by an abundance of limestone outcrops and reduced canopy cover compared to the surrounding area. The low-*Clematis* zone has fewer *C. morefieldii* plants (most are non-reproductive or seedlings) and is characterized by infrequent exposed limestone and thicker canopy cover than the *Clematis* zone.

Achene predation was studied using the technique that Timmerman-Erskine and Boyd (1999) used to document achene predation of another narrow endemic *Clematis* species (*Clematis socialis*). Mature achenes were harvested from the site the previous year. I separated these into two categories: those likely to contain a viable seed (judged to be "healthy") and those

that were relatively light weight and which I suspected contained a nonviable seed (judged to be “unhealthy”). I tested these judgments by arbitrarily selecting and cutting open a subsample of 15 healthy achenes and 15 unhealthy achenes. All viable achenes contained a seed that was whitish in color and appeared fully developed, whereas 75% of the unhealthy achenes contained a dark-colored or shrunken seed. Healthy achenes were used for the following predation experiment, as well as the germination experiment described below.

In the predation experiment, seven achenes were tethered by several centimeters of cotton thread to each of 50 square pieces of wire mesh (1.25 cm mesh hardware cloth; 10 cm by 10 cm). Mesh squares were staked to the ground using thick copper wire on 24 September 2012. Sets of achenes were placed in the field in a paired fashion. One member of each pair was placed in the *Clematis* zone and the other in the low-*Clematis* zone: each member was placed perpendicular to the contour approximately 9-13 m apart. Subsequent visits to the achenes revealed if any had been eaten or taken. Besides documenting achene predation (as shown by removal or chewing damage) rate, this pairing of plots allowed comparison of achene predation between the *Clematis* and low-*Clematis* zones since predation rates may differ in those locations.

Small rodent species found on the study site were documented by deploying live traps. Sherman live traps were set around the achene predation plots during three trapping nights during 28 January to 5 February 2012. Approximately 20 traps were baited with a peanut butter/oat mix and left overnight. All traps were checked the following day and any rodents captured were identified.

In Situ Germination

Information on *in situ* germination rate, and differences in germination between *Clematis* and low-*Clematis* zones, could be useful in understanding seed dormancy and germination. To document this, healthy achenes were placed on the soil surface and lightly covered in leaf litter under screen cages on 12 February 2011. The cages (15 x 15 x 15 cm) were made from hardware cloth (1.25 cm mesh) and staked to the ground using thick copper wire. Subsequent visits to the cages were made during the springs of 2011 through 2013 to document achene germination and stem lengths of all germinants in each cage were measured on 29 April 2013. Twenty-five sets of cages were placed in the field in a paired fashion (50 cages total). One member of each pair was placed in the *Clematis* zone (where *C. morefieldii* is in abundance) and the other member in the low-*Clematis* zone (area with low *C. morefieldii* density). Each pair was placed perpendicular to the contour approximately 9-13 m apart. This pairing of plots allowed a comparison of achene germination between the *Clematis* and low-*Clematis* zones since conditions affecting germination may differ in those locations.

Data Analysis

For each variable in the reproductive attrition study, data per plant were Log₁₀ transformed and then one-way ANOVA (Analysis of Variance) was used to compare data among years using StatView 5.0 (SAS Institute, 2005). Variables evaluated included numbers of flower buds, flowers, aggregate fruits, flower buds successfully producing fruits, achene production, as well as flower buds that were aborted or destroyed by herbivores. Some of these variables were also combined to create additional variables such as achenes/bud, achenes/flower, and flowers/bud to document achene and flower production per flower bud and achene production per flower.

Plants producing zero flower buds were omitted from analyses so that comparisons could be made between reproductive plants among years. There were no plants to omit in 2009 because all plants chosen were required to be reproductive. Out of 40 reproductive plants selected in 2009, 32 were reproductive in 2010, 27 in 2011, and 18 in 2012.

The relationship between achene production and rainfall was analyzed using simple linear regressions. Achene production values represented total achene production for all 40 plants in a given year. The departure from average total annual rainfall, and the departure from peak flowering/fruitleting season (April-June) rainfall, were calculated as the percent difference from average values (1983-2012) for each year of the study. Linear regressions were used to identify relationships between achene production and departure from annual rainfall averages using year as a replicate.

Paired-sign tests were used to analyze numbers of remaining achenes from the post-dispersal achene predation experiment. Since plots were placed in pairs (one in *Clematis* zone and one in low-*Clematis* zone), a paired sign test analyzed differences between locations to reveal if the zones significantly differed in numbers of achenes remaining at each date. I also analyzed the proportion of plots that had been discovered by predators in each zone (by having one or more achenes damaged or removed) at the end of study, using contingency table analysis in StatView 5.0 (SAS Institute, 2005).

Paired sign tests also were used to analyze numbers of germinated seedlings from the *in situ* germination study. Since plots were placed in pairs (one in *Clematis* zone and one in low-*Clematis* zone), the paired sign tests analyzed differences between locations for each year (and total germination over all years) to compare germination between *Clematis* and low-*Clematis*

zones. Germination rates were also compared by a paired sign test for each plot using 2012 and 2013 germination counts to determine if germination varied between those years.

Results

Reproductive Attrition

Reproductive attrition results differed significantly among years for multiple variables (Table 1). Significantly more buds were destroyed by herbivores in 2010 (2- to 4-fold) and 2011 (1.5- to 3-fold) compared to other years, although total bud production did not differ significantly among years (Table 1). Total achene production was not significantly different among years, despite high variability among yearly means. The percent of flower buds that reached the flower stage was highest in 2009 (48%) while 2012 had the lowest value (0.2%). Mean achene to flower bud ratio was highest in 2009, with significantly more achenes produced per flower than in 2010 (3.7-fold) or 2012 (935-fold).

Data in Table 1 can also be assessed visually: Figure 2 shows reproductive attrition for each year as the percentage of initial flower buds that survived to each developmental stage. In all years, the greatest drop in number of flower buds occurred between the “tiny” and “immature” stages. Many buds in the “tiny” stage were destroyed or aborted, with 43% (at least 15% destroyed by herbivores) dying in 2009, 59% (at least 49% destroyed by herbivores) in 2010, 53% (at least 38% destroyed by herbivores) in 2011, and 82% (at least 41% destroyed by herbivores) in 2012. This reveals a major potential bottleneck impeding fruit production, which can in large part be attributed to herbivory.

Linear regressions revealed a positive relationship between achene production and rainfall (Fig. 3). The linear fit for reproductive output data was much better for rainfall during the

peak flowering/fruitleing season ($R^2 = 0.946$; $P = 0.03$) (Fig. 3A) than for total annual rainfall ($R^2 = 0.551$; $P = 0.26$) (Fig. 3B).

Post-Dispersal Achene Predation

Achene predation did not differ significantly between *Clematis* and low-*Clematis* zones. Achene predation (i.e. removal or chewing damage) averaged 15% at 7 days, increased to 20% by 26 days, and by the conclusion of the study at 119 days a mean of 30% predation occurred (Fig. 4). At the end of the study, 60% of *Clematis* zone sets of achenes had been discovered by predators, while 76% of low-*Clematis* zone sets had at least one achene damaged or removed. These proportions were not statistically different (Contingency table analysis, Chi-square = 1.47, $df = 1$, $P = 0.23$). Damage to achenes was thought to be primarily by small rodents due to many predated achenes having small rodent-like chew marks on them. Differences in achene predation between the *Clematis* and low-*Clematis* zone were not significant at any point (paired-sign test $P > 0.05$). Small rodent trapping around achene predation plots during three overnight sets resulted in the capture of four white-footed mice (*Peromyscus leucopus*) with a trapping success of ~7%.

In Situ Germination

Achenes were planted in February 2011 but no germination was observed until spring of 2012. During 2012 and 2013 some cages were lost (i.e. washed away, disturbed by animals, etc.) from the study resulting in only 15 intact pairs. Data from these 15 pairs were analyzed for differences between *Clematis* and low-*Clematis* zones overall and during 2012, 2013. The mean germination rate for the entire study period was 32% (averaging 2.44 achenes/cage). Mean germination rates for cages in the *Clematis* zone and low-*Clematis* zone were 26% (1.83 achenes/cage; $N = 15$; $SE = 0.55$) and 42% (2.95 achenes per cage; $N = 15$; $SE = 0.33$),

respectively. There were no significant differences between *Clematis* and low-*Clematis* zone germination rates, either overall ($P = 0.092$) or during 2012 ($P = 0.057$) or 2013 ($P > 0.99$). A paired sign test was also used to analyze differences in mean germination rates between 2012 and 2013. Germination was significantly greater in 2012 than in 2013 ($P = 0.035$). Mean germination rate since planting for cages during the second (2012) and third (2013) springs were 23% (1.6 achenes per cage; $N = 30$; $SE = 0.31$) and 9% (0.63 achenes per cage; $N = 30$; $SE = 0.17$), respectively.

All germinants remained in the seedling stage (no compound leaves) through the duration of the study. Mean stem length in late April 2013 for all seedlings was only 6.6 cm ($S.E. = 0.28$; $N = 70$). No seedlings had transitioned into the non-reproductive life history stage, and the largest seedling had a stem length of 15.5 cm.

Discussion

Key components for successful management of rare plant species include an understanding of reproductive life history and knowledge of any factors that may inhibit seed production and seedling establishment. Reproductive attrition records over a four year period provided a detailed description of reproductive output changes through time in the Keel Mountain population. Consistent rainfall during the peak flowering/fruitletting period (April through June) appeared to be a major contributor to flower bud maturation and subsequent fruit set. Severe summer droughts occurred in 2010 and 2012 (Fig. 1), with approximately half as much rainfall during April – June than in an average year (average of 1983-2012). This growing season drought was reflected in the greatly reduced reproductive output in those years, and total achene production was positively associated with flowering/fruitletting season rainfall (Fig. 3).

Reproductive attrition variables experienced high variation among plants yet some differed significantly among years (Table 1). Total flower bud production did not differ significantly among years, but 2012 experienced very low flower bud production, which I expect was attributable to the flowering/fruitleting season drought. Drought can significantly reduce flower production in some species (e.g. Milton 1992; Harrison 2000) which could in turn reduce reproductive output. Reproductive units successfully producing fruit and total achene production varied widely among years but did not differ significantly among years. The lack of significant difference among years for these variables may be due to great variation in plant size (verified in the large standard errors in Table 1). Thus, a larger sample size may have made it possible to identify more significant differences among years for more variables. Achenes produced per flower bud (achenes/total buds) differed significantly among years. The lowest achene output per flower bud was in 2012 while 2009 and 2011 experienced the greatest. While not all variables differed significantly among years, a positive association between flowering/fruitleting season rainfall and total achene production was evident (Fig. 3).

Clematis morefieldii experienced a severe bottleneck during fruit production, in part attributed to herbivory. Over the course of this study relatively few buds produced mature fruit (Fig. 1; Table 1). The major herbivore contributing to this profound lack of reproductive success appeared to be a lepidopteran larva (Geometridae) which consumed flower buds in all maturation stages. Buds destroyed by herbivores were found to be significantly different among years (Table 1). Herbivory was lowest during 2009 and 2012, years with the greatest and least reproductive output, respectively. Lower floral herbivory in these extremes could be due to the overabundance of buds in 2009, and the general lack of available buds in 2012. Many buds during 2012 are thought to have aborted without herbivore damage due drought stress. Fang *et al.* (2009) found

drought conditions on *Cicer arietinum* increased flower and immature fruit abortion. The transition of flower buds to flowers is critical for reproductive units to successfully produce a fruit. Significantly fewer reproductive units reached the flower stage in 2012 than in the other years (Table 1). Even during 2009, a year of exceptional reproductive output, only 48% of reproductive units matured to flowers. This attests to the generally low success of reproductive units to survive the flower bud maturation process due to herbivory and/or drought (Fang *et al.* 2009; Martin 2010).

The post-dispersal achene predation study documented that seed predation occurred, but there was no significant difference between the *Clematis* zone and the low-*Clematis* zone. With 30% achene predation during late fall and early winter (119 days), small rodents could have affect seedling recruitment. In smaller populations with limited achene production, seed predation could threaten population persistence. Timmerman-Erskine and Boyd (1999) found rodent seed predation was even greater (30% destroyed in 1 week) for the federally endangered Alabama Leatherflower (*Clematis socialis*). It should be noted that seeds dispersed naturally could experience less seed predation than when placed in artificial plots due to lower seed densities and settling of seeds into the leaf litter in nature (e.g. Myster and Pickett 1993; Cintra 1997).

Herbivory and seed predation have the potential to restrict seedling recruitment to particular habitats (e.g. Boyd 1988; Rooney 1997). Habitats differed between the *Clematis* and low-*Clematis* zones, suggesting that some factors associated with habitat could be affecting seedling establishment and growth in the low-*Clematis* zone (heavily shaded areas without limestone outcrops that are predominantly devoid of *C. morefieldii*). I hypothesized that seed predators may have a habitat preference which would affect achene predation within zones.

Dispersal distance and density of seeds can affect predation rates (Nathan and Casagrandi 2004), with the escape hypothesis predicting reduced predation on seeds dispersed farther from parent plants and the satiation hypothesis predicting less seed predation on seeds closer to parent plants (McCanny and Cavers 1987; Chauvet *et al.* 2004). I found no significant difference in predation levels between the *Clematis* and low-*Clematis* zones. Seed predation is therefore not likely to be the factor hindering recruitment in low-*Clematis* zones. Timmerman-Erskine and Boyd (1999) also found similar non-significant results for *C. socialis* post-dispersal achene predation between the zone where it occurs along a creek and the surrounding woodlands.

The *in situ* germination study provided seedling establishment data describing the rate of germination of apparently viable achenes that were protected from rodent predation by cages. Lack of germination during 2011 and subsequent germination in 2012 and 2013 suggest existence of a seed bank, with seeds having at least a one year dormancy period. A third of the planted seeds germinated. Seeds that did not germinate could have been nonviable, may have washed away in heavy rains, may have been destroyed by small invertebrate herbivores, or may have remained dormant. Because germination significantly decreased from 23% in 2012 to 9% in 2013, it seems likely that germination rates might continue to decrease in subsequent years. Germination rates did not significantly differ between the *Clematis* and low-*Clematis* zones during 2012 or 2013. Germination does not appear to be influenced by zone, as seeds germinated equally well in both *Clematis* and low-*Clematis* zones.

Models created by Satterthwaite *et al.* (2007) found that species with a seed bank had a small but significant reduction in the likelihood of extinction compared to species without a seed bank. The presence of a seed bank can increase a population's ability to persist following traumatic events or periods of climatic extremes that might kill established plants. A seed bank

can also contain greater levels of genetic diversity than established plants in a population and could buffer the effects of small population size on genetic diversity (McCue and Holtsford 1998). The seed bank shown by my study, along with the potential longevity of established *C. morefieldii* plants, provides two mechanisms for perennation of populations that may be important for this rare species.

Plants germinating from planted achenes remained in the seedling stage (no compound leaves) through the duration of the study, showing that the transition from seedling to non-reproductive stages (defined as having compound leaves) in nature takes at least two years. Average stem length for all seedlings in 2013 was 6.6 cm, with two-thirds of seedlings germinating in 2012 and one-third germinating in 2013. Average stem length of seedlings during the range-wide survey (Ch. II) was 10.4 cm. The below average stem length of *in situ* planted seedlings in 2013 would suggest that it could take a few more years for most of them to reach the non-reproductive stage. Seedlings grown in containers and provided with ample nutrients, water, and sunlight are capable of transitioning to the non-reproductive stage by the second growing season (Kyle Paris pers. obs.), suggesting that unfavorable growth conditions in nature markedly slow seedling development.

Post-dispersal achene predation and *in situ* seed germination data can provide a general estimate for the proportion of dispersed seeds that can potentially establish seedlings. It should be noted that seeds were protected from vertebrate seed predators during the three year study while the post-dispersal achene predation study only measured predation rates for four months. Achene predation of un-germinated achenes could be considerably higher over a longer period of time. Regardless, if all seemingly viable achenes are assumed equally likely to be eaten by seed predators (30% predation over four months, leaving 70% surviving) and then germinate at the

rate documented here (32%), a maximum of 22.4% of achenes would germinate into seedlings over three years' time.

Seed predation and germination were not significantly different between *Clematis* and low-*Clematis* zones at the Keel Mountain Preserve. Therefore habitat differences should not affect establishment of plants in low-*Clematis* zones. A factor that was not studied but is believed to be limiting growth and reproduction for plants in these areas is sunlight. Baskin and Baskin (1988) examined why endemism is present in the rock outcrops of the unglaciated eastern North America. Many of these endemic plants were capable of growing in a variety of soil types suggesting that minerals in the outcrop soil were not essential for the plants' survival. When these plants were exposed to competition for light under greenhouse conditions, however, they were found to be poor competitors. Baskin and Baskin (1988) observed that these outcrop endemics languish or are absent in woodlands surrounding outcrops. This same pattern has been observed for the limestone outcrop endemic *Clematis morefieldii* (Ch. II). These outcrops usually have a greater percentage of exposed limestone than the surrounding area. This results in a reduction in canopy cover, allowing increased light and precipitation throughfall (Anderson *et al.* 1969). Though no studies have been done on *C. morefieldii*'s soil or light preferences, it is assumed that light levels could be a major factor explaining the plant's affinity for outcrops.

The effects of drought and florivory on reproductive output, and the effects of seed predation and germination on seedling establishment, point to potential strategies that managers may consider to boost reproduction and recruitment of this rare plant species. Managers seeking to boost flower and fruit production may treat plants with insecticide (Bevill *et al.* 1999) during flower bud initiation (~April) and provide supplemental water during the peak flowering/fruited period in years of drought. Although boosting reproductive success would not be needed in a

relatively large population (such as at Keel Mountain Preserve), populations with small numbers of flowering plants might benefit from herbivore exclusion/water supplementation during drought years to boost achene production. According to my seed predation and germination data, at most a quarter of achenes produced may establish seedlings by three years. Seedling recruitment during rainy years could initially be very high, though it is assumed that higher rates of achene predation will be occurring over the long period of delayed germination. These approaches to management could help to continue the recovery of this endangered species, increasing recruitment in small populations or producing seeds for use in *ex situ* conservation.

Table 1. Summary of 2009, 2010, 2011, and 2012 reproductive attrition data. Data were analyzed across years using ANOVA, with significant differences defined as having a P-value ≤ 0.05 . For each significant variable, mean annual values with the same superscripts do not differ significantly (Fisher's PLSD test). Total florivory includes reproductive units destroyed by herbivores at any maturation stage.

Variable (per plant)	2009 (SE)	2010 (SE)	2011 (SE)	2012 (SE)	P-value
Total buds	22.3 (5.28)	19.3 (4.47)	17.4 (4.01)	5.78 (1.4)	0.19
Total florivory	6.48 ^a (1.69)	13.5 ^b (3.25)	9.48 ^b (2.37)	2.98 (0.74) ^a	0.007
Successful buds	2.7 (0.76)	1.05 (0.37)	2.25 (0.73)	0.03 (0.03)	0.72
Total achenes	36.6 (10.28)	12.3 (5.2)	31.6 (14)	0.03 (0.03)	0.13
Flowers/Total buds	0.48 ^c (0.05)	0.14 ^b (0.03)	0.27 ^b (0.05)	0.002 ^a (0.002)	<0.001
Achenes/Total buds	1.87 ^c (0.49)	0.5 ^{a,b} (0.2)	1.31 ^{b,c} (0.4)	0.002 ^a (0.002)	0.01

Figure Legends

Figure 1. Percent departure of annual and April-June rainfall from average rainfall amounts (1983-2012) from the Redstone Technical Test Center (RTTC) Meteorological Team at Redstone Arsenal in Huntsville, AL. This weather station (N 34° 36' 0.8", W 86° 37' 49.7") is approximately 21 km from the Keel Mountain Preserve (N 34° 39' 27.8", W 86° 24' 42.3"). Snowfall (cm) was converted to rainfall (cm) by dividing snow depth by 10 cm.

Figure 2. Percent survival for buds in the 2009, 2010, 2011 and 2012 reproductive attrition study. Points signify percentages of reproductive units reaching each maturation stage.

Figure 3. Linear regressions of total annual fruit production (by all 40 plants) plotted with percent departure from annual average rainfall (Fig. 3A) and also peak flowering/fruited season rainfall (April-June) (Fig. 3B) using year (2009-2012) as the replicate. Rainfall data were acquired from the Redstone Arsenal weather station near Huntsville, AL (~ 21 km from study site).

Figure 4. Percent survival of achenes in the *Clematis* and low-*Clematis* zone. Achene survival did not significantly differ between zones. Study began September 24, 2011 and ended January 21, 2012. Diamond points represent the *Clematis* zone and square points represent the low-*Clematis* zone.

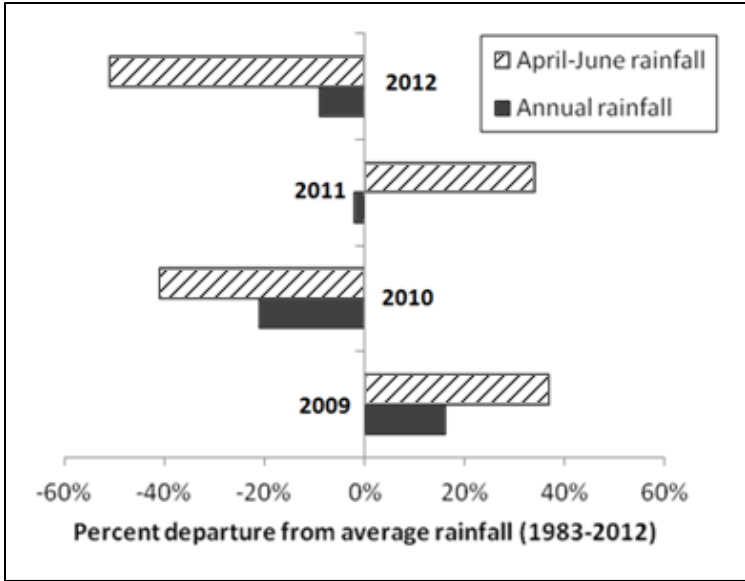


Figure 1

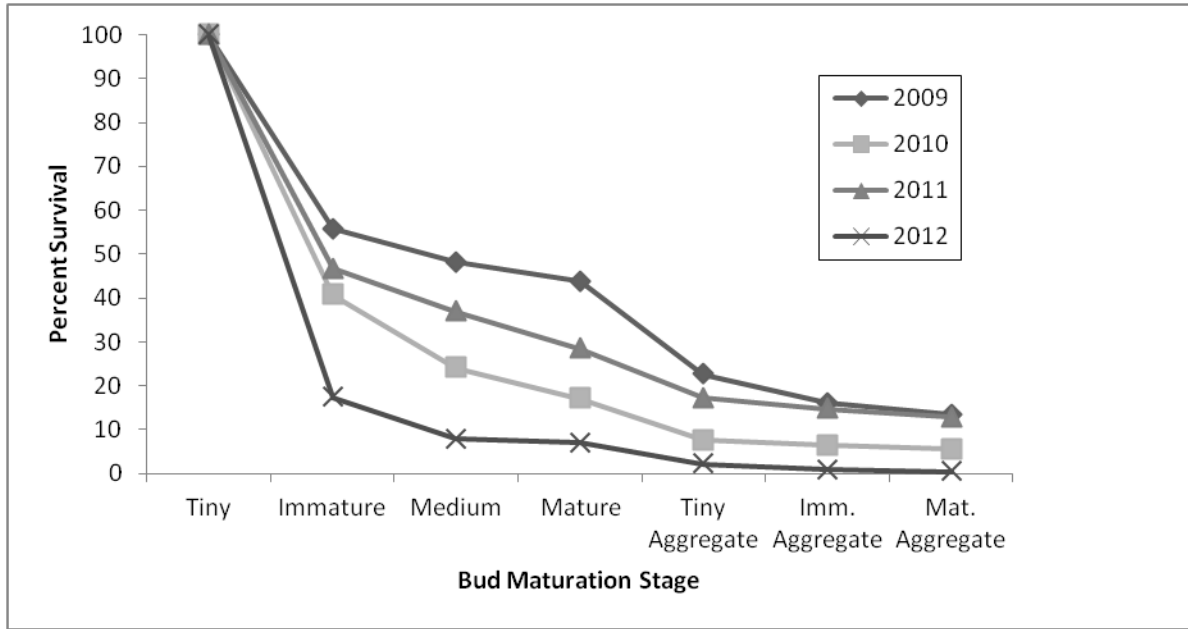


Figure 2

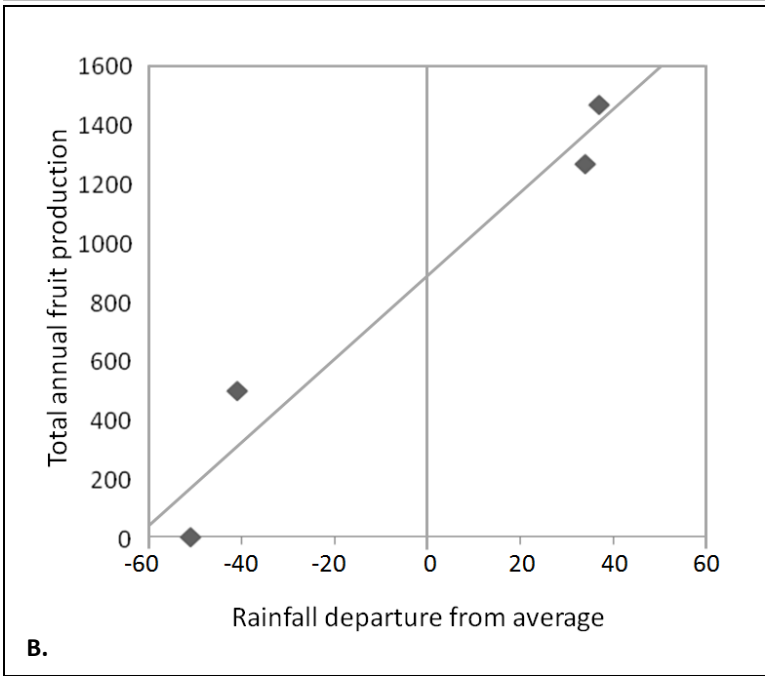
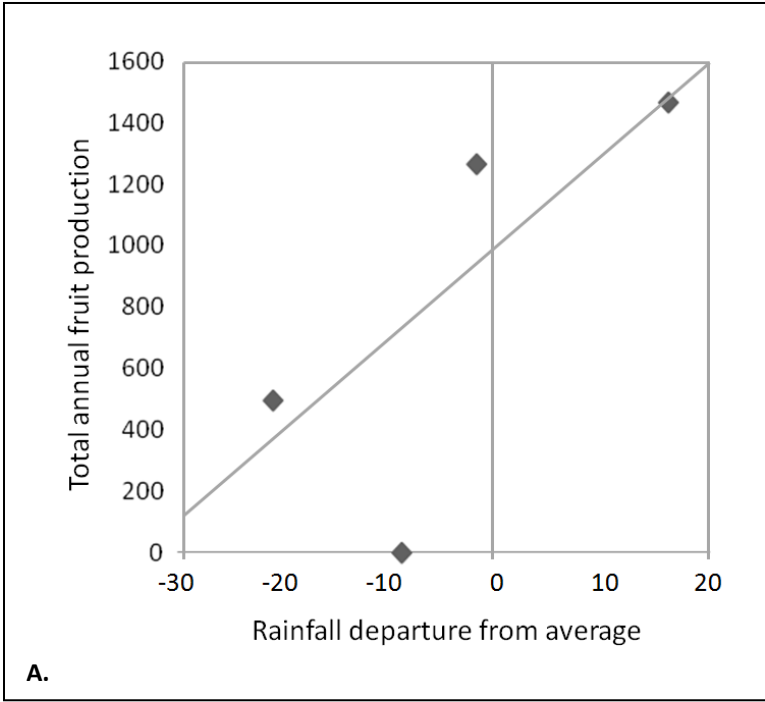


Figure 3 (A and B)

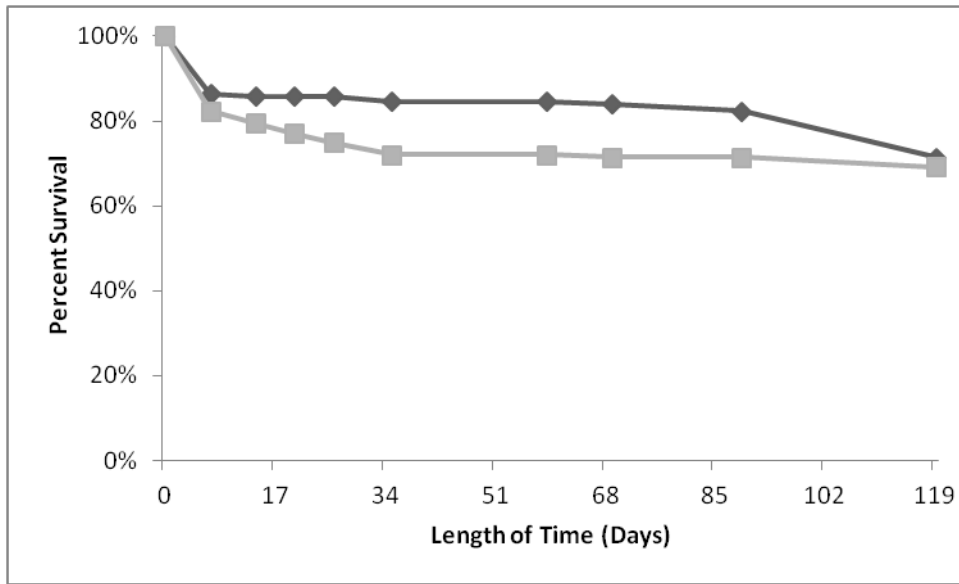


Figure 4

IV. IMPACT OF INSECTICIDE TREATMENT ON HERBIVORY AND REPRODUCTIVE OUTPUT OF *CLEMATIS MOREFIELDII* KRAL

Abstract

Herbivory can have a major effect on plant health and can directly and indirectly affect reproductive output and subsequent seedling recruitment. Herbivory is common on the federally endangered *Clematis morefieldii*, although relatively little is known about its effect on reproductive output. The insecticide Sevin (0.26% carbaryl) or water (as a control) were applied to 40 plants (20 per treatment) every three weeks to test the effects of reduced herbivory on reproductive attrition/output. Plants treated with insecticide had significantly fewer buds destroyed by herbivory (18.4% less in 2010, 45.6% in 2011 and 39.7% in 2012), but only 2011 had significantly greater fruit production (3.5-fold) on plants treated with insecticide. Control plants were compared among the three growing seasons (2010-2012) for total buds, dead buds, buds destroyed by herbivores, fruiting buds, achene number, and fruiting bud proportion. For all variables, means were significantly reduced in 2012 compared to other years. Rainfall during peak flowering/fruitleting (April-June) influenced the effectiveness of treatments by boosting achene production in 2011. Vegetative herbivory was classified as vertebrate browse damage, invertebrate browse damage, piercing-sucking herbivore damage, or leaf miner damage, and incidence was compared between treatments within each year. Both vertebrate and invertebrate browse damage were significantly greater (1.2- to 2.2-fold) on control plants than treated plants for all three years. Piercing-sucking herbivory was greater on control plants than treated plants during most of the growing season for all three years (25 to 40% difference). Leafhopper nymph damage frequency was significantly greater on control plants than treated plants during 2012 (30 to 45% difference). Vertebrates were attracted to insecticide treated plants with a significantly

higher occurrence (25 to 40% difference) of browse damage during 2012. During high rainfall years invertebrate herbivory appears to reduce reproductive output whether directly (by attacking buds and flowers) or indirectly (by vegetative herbivory) and insecticide use during those years could be a viable option for managers seeking to increase recruitment.

Introduction

Endangered species management requires a holistic approach to effectively recover a species from endangered status. Effective management not only includes protection and restoration of habitat, but also addressing the many other factors that may contribute to species rarity (Schemske *et al.* 1994). Herbivory (Fletcher *et al.* 2001), pathogens (Alexander and Antonovics 1988), competition (Walck *et al.* 1999), pollen limitation (Bierzychudek 1981), dispersal limitation (Primack and Miao 1992), low genetic diversity (Reed and Frankham 2003), and climate change (Wall *et al.* 2003) are major factors that can limit population growth and range expansion. These factors can reduce population size, reproductive output, seedling recruitment, and successful establishment of new populations, thereby contributing to plant rarity. By identifying and understanding factors causing rarity of a species, rare plant managers can be better equipped to effectively manage for its recovery.

Reproductive output is an important aspect of a species' life history that can be affected by multiple variables. Herbivory is one of the primary contributors to reduced reproductive output directly through florivory (herbivory of reproductive structures) (McCall and Irwin 2006) and indirectly through vegetative herbivory (Strauss *et al.* 1996; Mothershead and Marquis 2000). Herbivory can threaten the survival and long term success of plant populations, therefore understanding the effects of herbivore damage on reproductive output can provide insight into the health of a population (Ancheta and Heard 2011).

Florivory is common in natural systems and can have significant direct and indirect effects on reproductive output (McCall and Irwin 2006). Florivory directly affects male and female reproductive success by consumption of pistils and anthers (Krupnick and Weis 1999). Florivory can also directly affect resource availability in plants due to the removal of resources by consumption of floral parts, especially late in the maturation process when valuable resources have been committed to seed and fruit production (Lowenberg 1994; McCall and Irwin 2006). Florivore damage to non-sexual flower parts (i.e. sepals, petals, bracts) can reduce flower attractiveness to pollinators and disrupt pollen transfer from pollinators to stigmas, significantly decreasing successful fruit production (Leavitt and Robertson 2006). Floral herbivory can also reduce pollinator service to undamaged flowers on the same plant and could therefore indirectly affect outcrossing and reproductive output (Krupnick *et al.* 1999).

Vegetative herbivory can directly and indirectly affect reproductive output and overall plant health. Reduction in leaf surface area and subsequent photosynthesis can affect resource availability thus directly reducing seed numbers or seed weight (Beville *et al.* 1999; Mothershead and Marquis 2000). Vegetative herbivory can also have an indirect, negative effect on fruit set; decreasing flower size and pollen production which can affect pollinator preference and effectiveness (Strauss *et al.* 1996; Mothershead and Marquis 2000). Vegetative herbivory can reduce overall plant growth, while increasing susceptibility to diseases (Beville *et al.* 1999). Consecutive years of herbivory can severely reduce recruitment and can cause mortality in mature plants (Louda and Potvin 1995). Herbivory also can negatively affect the competitive ability of individual plants, thus impacting a population's ability to sustain itself or expand its numbers (Crawley 1989).

Climate change can have a significant impact on plant growth, survival, and reproductive output, which in turn affect recruitment and population dynamics. Change in temperature has the potential to create mismatches between plant flowering phenology and activity of pollinating mutualists, thus reducing or eliminating effective pollination (Wall *et al.* 2003; Hegland *et al.* 2009). Changes in rainfall can affect plant growth (Fisher *et al.* 1988; Kirnak *et al.* 2001), reproductive unit production (Milton 1992; Fang *et al.* 2009), reproductive unit abortion (Fang *et al.* 2009), and survival of seedlings/seedling establishment (Lieberman and Li 1992; League and Veblen 2006).

Variation in rainfall can also affect herbivory severity, which can in turn have significant effects on reproductive output. Studies of herbivory severity during stress (such as drought stress) have supported contradictory patterns (plant-stress or plant vigor hypotheses) or showed more complex relationships (English-Loeb 1990; Schowalter *et al.* 1999; Staley *et al.* 2006). The plant-stress hypothesis predicts herbivore populations will increase and perform better when environmental stresses such as drought occur for plant populations (White 1969 and 1984). Drought stress can increase vulnerability to herbivory and reduce reproductive output by reducing compensatory responses to herbivore damage (Fox *et al.* 1999; Levine and Paige 2004). On the other hand, the plant vigor hypothesis (Price 1991) states that healthier, vigorously growing plants should be more nutritious and thus be more attractive to herbivores. For example, above average growing season rainfall can increase herbivore density and damage (Masters *et al.* 1998; Schowalter *et al.* 1999; Staley *et al.* 2006). Herbivory severity can also vary in more complex ways with respect to water availability (English-Loeb 1990; Schowalter *et al.* 1999; Staley *et al.* 2006).

The absence of herbivores from plants allows for maximum reproductive output with available resources. Manipulation of herbivory levels by killing herbivores/reducing palatability of plant tissue with an insecticide is an uncommon method of invertebrate herbivore exclusion on rare plants utilized by researchers (Louda and Potvin 1995; Boyd 2003; MacDonald and Kotanen 2010; Ancheta and Heard 2011). Invertebrate herbivore exclusion can elucidate the effects of invertebrate herbivory on attrition of reproductive unit maturation stages, and reproductive output. If data are collected over several years that vary in rainfall, interactions between water availability and herbivory and their effects on reproductive output can be revealed.

A rare plant species impacted by herbivores is the Huntsville Vasevine (*Clematis morefieldii* Kral), a federally endangered perennial vine endemic to limestone outcrops of the Cumberland Plateau in Alabama and Tennessee. Many aspects of this rare species' biology are unknown, and information on factors contributing to its rarity would be beneficial to managing its recovery. Severe vegetative herbivory was recorded across the range of *C. morefieldii* (Ch. II) and severe floral herbivory by a lepidopteran larva (Geometridae) and abortion of reproductive units were recorded during an in-depth study of a population at the Keel Mountain Preserve (Ch. III), with the greatest attrition rate occurring in the smallest flower bud stage (averaging 59% attrition among four years). Few flower buds at the Keel Mountain population produced mature fruits, with only 8% (average among four years) of buds successful. Study of the Keel Mountain population also revealed a positive linear relationship between reproductive output and peak flowering/fruitlet rainfall (April-June), with plants producing many more fruits in rainy years (2009 and 2011) than in drought years (2010 and 2012) (Ch. III; Fig. 3b). By quantifying and analyzing the extent of herbivory and its impacts on the reproductive output of *C. morefieldii*, managers can understand how herbivory affects reproductive output and act accordingly.

The objective of this study is to determine how variation in rainfall and insect herbivory affect reproductive output in a *Clematis morefieldii* population. Specifically, the effects of insecticide and control treatments on reproductive attrition and output were compared. The study was repeated for three years so that reproductive output could be evaluated for differences between treatments under varying rainfall regimes.

Materials and Methods

The study site is located on The Nature Conservancy's Keel Mountain Preserve near Gurley, AL in Madison County (N 34° 39' 27.8", W 86° 24' 42.3"). Keel Mountain is part of the Cumberland Plateau, and the site is primarily located on its South-facing slope. The forested mountainside has moderate to steeply sloping areas abundant in exposed limestone bedrock, boulders, and cliff-faces: the primary habitat of *Clematis morefieldii*.

This manipulative study used insecticide and control treatments to test the effects of invertebrate herbivory on reproductive output. The insecticide Sevin (active ingredient 0.26 % Carbaryl in the diluted spray) was used to experimentally reduce insect damage and a water treatment was used as a control. At the beginning of the 2010 growing season 40 reproductive plants were haphazardly selected and then randomly assigned to either the treatment or control group. Spraying began in May during 2010 and in mid-April (when plants had just begun to produce flower buds) for 2011 and 2012 in order to protect the "tiny" stage flower buds that are initiated at about that time. Both insecticide and water treated plants were sprayed every three weeks during the growing season. The amount of insecticide or water sprayed on each plant was only enough to wet all aboveground plant parts.

Rainfall data were acquired from Redstone Technical Test Center (RTTC) Meteorological Team at Redstone Arsenal in Huntsville, AL. This weather station (N 34° 36' 0.8", W 86° 37' 49.7") is approximately 21 km from the Keel Mountain Preserve (N 34° 39' 27.8", W 86° 24' 42.3"). Any snowfall data (cm) were converted to rainfall data (cm) by dividing snow depth by 10 cm (the common method for converting snow depth to rainfall is dividing snow depth by 10) and then added to rainfall totals. Average rainfall was calculated from 30 years of data (1983-2012). The data were used to calculate departure from average rainfall during each year of this study (2010-2012) to identify changes that may affect treatment success.

Reproductive Attrition

Reproductive attrition data were collected for all 40 plants used in this study. Each plant was marked with a numbered metal tag and then every node that produced at least one flower bud was marked with a colored pigeon band. Data were then recorded approximately bimonthly over the course of each of three growing seasons (2010-2012) for all reproductive units produced by the selected plants. The stage at which flowers and fruits were destroyed was recorded, and if they did not produce mature fruits, how they died (abortion or herbivory) was also recorded. As the aggregate fruits matured, all of their achenes were counted and observed until fully mature and dispersed.

Vegetative Herbivory

Vegetative herbivory has the potential to directly and indirectly affect the reproductive output of a plant, making it integral to reproductive success in *C. morefieldii*. Vegetative herbivory was divided into four categories (invertebrate browse damage, vertebrate browse damage, piercing-sucking damage, or leaf miner damage) and the extent of damage in each category was quantified. Invertebrate browse damage was defined as damage consisting of holes or other obvious evidence of invertebrate feeding. Invertebrate browse herbivores included grasshoppers, caterpillars, gastropods, and blister beetles (*Epicauta* sp.). Vertebrate browse damage was defined as damage consisting of complete or partial leaf removal with no sign that insects were involved: the most likely vertebrate browse herbivore on the study site was white-tailed deer. Total herbivore browse damage was calculated by summing the percent of the plant's vegetative tissue removed by both browsing vertebrate and invertebrate herbivores. Piercing-sucking damage was defined as yellow speckling on the leaves. Piercing-sucking herbivores included mealy bugs (*Heliococcus* sp.), leafhoppers (*Acanalonia* sp. and a Flatidae species), and garden flea beetles (*Halticus bractatus*). Leaf miner damage was defined as small light brown lines (~3mm wide) that wandered across a leaf. For all categories of damage, percent damage was visually estimated to the nearest 5% (or to the nearest 1% if herbivory was less than 5%) relative to the leaf surface that was originally produced.

Data Analysis

Variables compared between treatments included total bud production, percent florivory, percent of buds successful, total achene production, percent of buds flowering, and achenes per flower. The Mann-Whitney U test was used to compare variables between insecticide treatment

and control plants for each year. Data from plants that produced no flower buds were omitted because their inclusion would greatly increase variability of the data. There were no buds to omit in 2010 because all plants chosen were required to be reproductive. Some of those plants were not reproductive in 2011 or 2012, usually due to unknown causes.

Variation of control data among years was also analyzed to compare the effects of year on reproductive attrition variables. Only control plant data were analyzed because of differences in the timing of insecticide treatment between 2010 (insecticide application began in early May) and 2011/2012 (insecticide application began in mid-April). The Kruskal-Wallis Test was used to compare variables (total bud production, dead buds, florivory, buds successful, total achene production, percent of buds successful, and percent florivory) among the three years. The Mann-Whitney U Test was used for pairwise comparisons among years for variables that differed significantly among years.

Transitions between reproductive unit maturation stages were analyzed between treatments and among years with a two-way analysis of variance (ANOVA) using StatView 5.0 (SAS Institute 2005). Reproductive unit maturation was divided into five stages as follows: tiny bud (unopened flower buds 1-3 mm in length and sessile), intermediate bud (unopened flower buds ≥ 3 mm with a pedicel), flower (open flowers), immature fruit (post sepal/stamen abscission), and mature fruit (brown woody achenes ~ 5 mm wide). The four transitions analyzed were: 1) buds surviving from the tiny to intermediate stage, 2) intermediate buds that survived to flower, 3) flowers that became immature fruits, and 4) immature fruits that becomes mature fruits. Transition data, expressed as proportions, were arcsine square root transformed and then analyzed using a two-way ANOVA to determine if year, treatment, or the year*treatment interaction significantly affected them. For factors with significant P-values, a Fisher's Protected

Least Significant Difference (PLSD) post hoc test was used to identify differences among years (SAS Institute 2005).

The relationship between insecticide effectiveness and rainfall was analyzed with simple linear regression to determine how herbivory and rainfall affect reproductive output. Insecticide effectiveness was defined as total number of achenes produced by all insecticide treatment plants minus achenes produced all water treatment (control) plants. There was no significant difference in flower bud production between treatments for all three years so it was assumed that variation in fruit production among years was due to insecticide effectiveness. Using StatView 5.0 (SAS Institute 2005), a linear regression was used to investigate the relationship between insecticide effectiveness and departure from average (1983-2012) total annual rainfall. A second linear regression was used to investigate the relationship between insecticide effectiveness and peak flowering/fruitletting season (April-June) rainfall.

Herbivore damage data were analyzed to determine if treatments influenced herbivory severity or frequency. Herbivory damage data were compared between treatments two ways: as the percent of the total leaf surface area damaged (percent browse damage) and by the frequency of occurrence of herbivory damage (piercing-sucking, leafhopper nymph, leaf miner, and vertebrate) on plants. Mann-Whitney U tests in StatView 5.0 (SAS Institute 2005) were used to compare percent browse damage between insecticide and control treatments for each of three years. Using StatView 5.0 (SAS Institute 2005), Chi-square analysis was used to determine significance differences in occurrence of damage to individual plants between insecticide and control treatments.

Results

Reproductive Herbivory

Insecticide application decreased florivory and positively affected reproductive output in most years (Table 1). Total flower buds produced did not differ significantly between control and insecticide treatments for all three years. Percent florivory of flower buds was significantly greater in the control treatment for all three years: 2010 (1.4-fold), 2011 (5.1-fold), and 2012 (3.1-fold). In 2010 and 2012, there was no significant difference between treatments concerning the percent of flower buds that successfully produced mature achenes. However, in 2011 the insecticide treatment had a greater proportion of buds that successfully produced achenes (4.6-fold). Achene numbers were not significantly different between treatments in 2010 or 2012, but achene numbers in the 2012 insecticide treatment were significantly greater than the control (3.7-fold). A high standard error for this variable (Table 1) attests to the great variation in reproductive output between study plants. Despite these statistics, 2010 achene production was four times greater in the insecticide treatment compared to the control. Significantly greater amounts of flower buds reached the flowering stage in the insecticide treatment during all three years. However, only insecticide treated plants in 2011 produced greater numbers of achenes per flower than the control (2.2-fold).

Two-way ANOVA of transitions between reproductive maturation stages revealed significant differences between treatments, among years, and for the treatment*year interaction (Table 3). Significantly more flower buds treated with insecticide survived transitions between tiny buds to intermediate buds (1.1- to 1.9-fold), intermediate buds to flowers (1.8- to 1.9-fold), and flowers to fruits (2.4- to 3.2-fold). Transition from fruits to mature fruits was not significantly affected by treatment. Significant differences among years were experienced for

transitions between tiny to intermediate flower buds and intermediate flower buds to flowers. Fisher's PLSD test revealed significant differences among all three years for the tiny to intermediate transition, and significant differences between 2011 and 2012 only for the intermediate flower buds to flowers transition. Treatment*year interactions were significant for transitions from flowers to mature fruits.

Control data were used to compare variation among years (Table 2). There was a significant effect of year on every variable except percent florivory. Pairwise comparisons between years revealed that 2012 had significantly lower means than the previous two years but that 2010 and 2011 were statistically similar.

Linear regressions revealed a positive linear relationship between insecticide effectiveness and rainfall, with the best linear fit for peak flowering/fruitlet season rainfall data. Variation of insecticide effectiveness on reproductive output was much more completely explained by rainfall during the peak flowering/fruitlet season ($R^2 = 0.938$; $P = 0.16$) (Fig. 1A) than by total annual rainfall ($R^2 = 0.262$; $P = 0.66$) (Fig. 1B).

Vegetative Herbivory

Multiple herbivores contributed to moderate levels of vegetative herbivory on *C. morefieldii*, with greater levels of invertebrate herbivory on control plants and greater levels of vertebrate herbivory on insecticide treated plants. Herbivore browse damage increased as the growing seasons progressed and mean browse damage for all three years was significantly greater (1.2- to 2.2-fold) on control compared to insecticide treated plants (Figs. 2, 3, and 4). Occurrence of herbivory damage categories varied between treatments and as the growing seasons progressed. Piercing-sucking damage was significantly more prevalent in control plants

than insecticide treated plants during 2010 (25% difference) (Fig. 5) and 2011 (38 to 42% difference) (Fig. 6). Significantly greater occurrences of piercing-sucking herbivore damage (30% difference) and leafhopper nymph damage (30 to 44% difference) were observed sporadically throughout the 2012 growing season (Fig. 7). Vertebrate browse damage was significantly more prevalent (25 to 40% difference) on insecticide treated plants during the beginning of the 2012 growing season (Fig. 7).

Discussion

Insecticide treatment decreased florivory and vegetative herbivory levels while increasing reproductive output. Insecticide treated plants experienced significantly less (1.4- to 5.1-fold) florivory on reproductive units than control plants (Table 1). Florivory intensity can negatively affect pollinator visitation (Krupnick *et al.* 1999) and seed production (Söber *et al.* 2009). Reduced florivory on insecticide protected plants would allow more reproductive units the opportunity to produce fruit. With reduced florivory on insecticide treated plants, significantly more reproductive units (1.4- to 3.8-fold) successfully produced mature flowers. Plants with larger floral displays can attract more pollinators resulting in greater pollen load and reproductive output (Klinkhamer *et al.* 1989; Ohara and Higashi 1994). Decreased florivory and a greater percentage of reproductive units producing flowers on insecticide treated plants allowed a greater percentage of reproductive units to successfully produce fruits (1.8- to 4.6-fold), though only one year (2011) was statistically significant. Achene production between treatments varied from no difference to 4.2-fold more achenes produced on insecticide treated plants, although only one year (2011) was significantly different. Greater reproductive output on insecticide treated plants can be attributed in part to protection from florivory throughout maturation stages.

Herbivory not only affected reproductive output, but also transitions between maturation stages of reproductive units. Insecticide treatment significantly increased reproductive units' successful transitions from tiny flower buds to fruit initiation (Table 3). Reproductive herbivory was primarily observed on flower buds and flowers (Ch. III), and these results confirm that exclusion of herbivores only affected transitions between these stages. Oguro and Sakai (2009) found herbivory on *Iris gracilipes* flower bud and fruit stages had negative effects on seed production while herbivory on the flower stage usually did not negatively affect seed production due to a lower loss of reproductive units in this stage. In my study, some reproductive variables were unaffected by insecticide treatments (Table 1). Total reproductive units produced per plant was not significantly different between treatments. Lack of significant difference between treatments signifies bud production was not affected.

Some categories of vegetative herbivory were reduced by insecticide treatments which could have positively affected plant health and reproductive output. Percent browse damage (invertebrate + vertebrate) was significantly reduced on insecticide treated plants (Figs. 2, 3, and 4). Occurrence of damage by leafhopper nymphs and other piercing-sucking herbivores was also reduced by insecticide (Figs. 5, 6, and 7). Reduction in photosynthesis due to leaf removal/damage from vegetative herbivory can negatively affect resource availability thus directly reducing seed weight and reproductive output (Beville *et al.* 1999; Mothershead and Marquis 2000). Vegetative herbivory can decrease flower size and pollen production which can affect pollinator preference and effectiveness (Strauss *et al.* 1996; Mothershead and Marquis 2000). Vegetative herbivory can also increase susceptibility to diseases (Beville *et al.* 1999). Piercing-sucking herbivores are major vectors for disease transmission in plants (Brown and Bird 1992; Almeida and Purcell 2003) and some species have been shown to transmit diseases

(phytoplasmas) to and from *Clematis vitalba* in Europe (Filippin *et al.* 2009; Mehle *et al.* 2011). Controlling vegetative herbivores via insecticide has the potential to reduce infection of host plants (Perring *et al.* 1999) and increase reproductive output (Beville *et al.* 1999).

Insecticide application did not reduce the occurrence of leaf miner or vertebrate herbivory on plants (Figs. 5, 6, and 7). Leaf miner herbivory was common but usually only damaged a small percentage of leaf surface area (Ch. II, Fig. 5D). Occurrence of vertebrate herbivory was significantly greater on insecticide treated plants. The plant vigor hypothesis (Price 1991) states that healthier, vigorously growing plants should be more nutritious and thus more attractive to herbivores. Reduction of invertebrate herbivory on insecticide treated plants would result in more leaf tissue available for photosynthesis, allowing increased growth compared to control plants (Beville *et al.* 1999). Vertebrate herbivores could have been attracted to the undamaged insecticide treated plants and were not dissuaded by the insecticide.

Effect of insecticide treatment on florivory, vegetative herbivory, and reproductive output also varied among years and was more significant in 2011 than in other years. Rainfall during the peak flowering/fruitletting season (April-June) in 2011 was 34% greater than average, whereas severe droughts during peak flowering/fruitletting season (April-June) occurred in 2010 and 2012 with 41% and 51% less rainfall than average, respectively (Ch. III, Fig. 1). While 2010 and 2012 had significantly less florivory on insecticide plants, achene production was not significantly increased. Though significantly less herbivory occurred on flower buds in both years, lack of water availability prevented fruit production. Insecticide treated plants in 2011, however, experienced reduced herbivory and increased achene production. Increased rainfall during the peak flowering/fruitletting period (Fig. 4, Ch. III) allowed insecticide treated plants to utilize the greater number of surviving buds to increase achene production. Insecticide treated plants during

all three years also had significantly greater levels of buds reaching the flower stage. This verifies that the insecticide treatment was protecting flower buds in their most vulnerable stage to florivores, allowing significantly more buds to mature to flowers. Rainfall limitations in 2010 and 2012 however, restricted the amount of resources available to produce fruits.

Most reproductive variables from control plants significantly differed among years (Table 2). Only control data were analyzed because insecticide treatments were applied differently in different years (most notably the later initiation of insecticide application in 2010). Comparisons of reproductive variables from control plants among years revealed 2010 and 2011 were not significantly different but both differed significantly from 2012 for almost all variables. For total flower buds produced, buds successful, proportion of buds successful, and achene production a general trend can be observed with 2011 experiencing the greatest reproductive success, 2012 experiencing the least, and 2010 experiencing medial levels. Florivory proportion was similar among years despite significant differences in flower bud production among years.

Transitions of maturation stages were also affected by year, with fewer reproductive units transitioning to flowers and fruits during drought years. Transitions from tiny flower buds to flowers were significantly different among years (Table 3) with 2011 having a greater proportion of flower buds successfully maturing to flowers (Table 1). Increased rainfall in 2011 is thought to have an effect on reducing abortion among flower buds, and Milton (1992) documented similar results for *Osteospermum sinuatum*. The few buds that matured to the flower stage without being aborted or destroyed by herbivores during 2010 and 2012 drought years were allocated enough resources to produce mature seeds. Therefore there was no significant difference among years for transitions between flowers to fruit and fruit to mature fruit. Fang *et al.* (2009) found drought conditions on *Cicer arietinum* reduced flower bud production and

increased flower and immature fruit abortion. Drought conditions also greatly decreased pollen viability and reduced fertilization rates. The treatment*year interaction was significant only for transitions between flowers to fruits and fruits to mature fruits (Table 3). Successful transitions from flowers to mature fruits were not affected by year and only flowers to immature fruits were affected by treatment: reasons for the significant interactions are unknown.

The effects of herbivory on plants are primarily determined by the period in a plant's phenology when herbivory occurs and the extent of damage. While many plants are capable of compensating for low levels of herbivory, high levels of herbivory can have a detrimental impact on growth, reproduction, and survival. Maschinski (2001) found that early season herbivory on the rare Arizona Leatherflower (*Clematis hirsutissima* var. *arizonica*) resulted in a significant reduction of reproductive output during the current and subsequent growing seasons whereas late season herbivory had no significant effect. While vegetative herbivory on *C. morefieldii* was usually low at the start of a growing season and accumulated over time, florivory was observed to be greatest on the smallest flower buds which were most abundant at the beginning of the growing season.

Effect of insecticide on achene production was positively associated with departure from average rainfall during peak flowering/fruitletting season (Fig. 1B). When rainfall was below average during the peak flowering/fruitletting season (2010 and 2012), lack of water resources restricted any increase in reproductive output of insecticide protected plants. However when water was not a limiting factor (2011), significantly greater numbers of flower buds produced fruits. While other studies also documented drought (e.g. Milton 1992; Fang *et al.* 2009) and florivory's (e.g. Lowenberg 1994; Krupnick and Weis 1999; McCall and Irwin 2006) negative

effects on reproductive output, no literature could be found that documented the hierarchical relationship between drought and florivory.

Results of this insecticide study suggest that an insecticide treatment can significantly reduce herbivory damage and boost reproductive output. Thus insecticide treatment is a possible management approach (Beville *et al.* 1999) that may be especially attractive to boost reproduction in small populations. The success of this tactic, however, is likely dependent upon water availability from rainfall during peak flowering/fruitletting period which provides resources for flower bud survival and ultimately achene maturation.

For *ex situ* conservation efforts targeting small populations, insecticide application could be a viable option to boost achene production to allow enough to be produced for collecting and storage. Menges *et al.* (2004) used stochastic simulations to predict risk of extinction by varying seed collecting frequency and intensity on different sized populations. Collection of 10% of total seeds with a 90% collection frequency resulted in a very low chance of population extinction (0 to 10%) for populations with 50 or more individuals. For populations smaller than 50 individuals, reduction in collection frequency should reduce any negative impacts that could result in population extinction. Since seed collection guidelines generally limit collected seeds to 10% or less of the total seeds produced in a year (Menges *et al.* 2004), boosting achene production with insecticide may be worthwhile as a tactic to allow seed collecting from small populations. If minimal insecticide application is desired, application during peak flower bud initiation (early- to mid-April) could protect flower buds during the stage of greatest herbivory damage while also minimizing the potential negative effect of insecticide on pollinators.

Lesica and Atthowe (2000) warn that insecticide use as a management strategy for increasing reproductive output and ultimately greater recruitment should be considered carefully. Interactions between rare plants and their herbivores have likely been ongoing for hundreds of generations and these interactions may not be the cause of a plant's rarity. Beneficial plant-animal interactions such as pollination and seed dispersal could be negatively affected due to insecticide application. Also without identifying all herbivores on a rare plant, it is possible to threaten the existence of an equally rare invertebrate. Boyd (2003) conducted an insecticide study on the endangered *Fremontodendron californicum* subsp. *decumbens* and found flower buds were attacked by an unknown and possibly undescribed *Decodes* sp. larva. The primary florivore on *C. morefieldii* was an unknown Geometridae larva. A better understanding of the taxonomy and ecology of rare plant herbivores is crucial to avoid possibly endangering the existence of one organism by managing for the recovery of another (e.g. Simon *et al.* 2001). All known factors contributing to a plant's rarity should be considered before designing a management plan that could affect the ecological balance of the rare species' ecosystem.

Table 1. Reproductive attrition results from insecticide study. Reproductive data were compared between treatments (insecticide and control) for 2010, 2011, and 2012. Mann-Whitney U tests compared treatment and control data within each year, with significant differences defined as $P \leq 0.05$ (asterisk denotes significance).

Year	Treatment	Total buds	Percent florivory	Percent buds successful	Total achenes	Percent buds flowering	Achenes per flower
2010	Insecticide mean (SE)	33.2 (5.9)	46.5 (5.6)	16.6 (2.8)	97.8 (30)	32.1 (3.5)	5.46 (0.98)
	Control mean (SE)	27.1 (5.2)	64.9 (5.3)	9.2 (1.8)	23.3 (5.2)	22.4 (3.9)	4.86 (1.03)
	P-value	0.55	0.03*	0.08	0.19	0.03*	0.64
2011	Insecticide mean (SE)	33.7 (7.1)	11 (2.8)	44 (3.6)	258 (58.5)	57 (4.3)	13.6 (1.4)
	Control mean (SE)	38 (12)	56.6 (7.5)	9.5 (2.3)	70.4 (33.2)	24 (2.9)	6.23 (2.2)
	P-value	0.71	<0.001*	<0.001*	0.01*	<0.001*	0.003*
2012	Insecticide mean (SE)	7.1 (1.4)	18.7 (4.6)	9.5 (3.9)	6.4 (2.3)	30 (6)	2.9 (1.01)
	Control mean (SE)	10.9 (3.2)	58.4 (8.9)	2.5 (1.9)	6.6 (5.9)	8 (4)	3 (2.2)
	P-value	0.57	0.003*	0.08	0.25	0.007*	0.82

Table 2. Control data (2010-2012) for the insecticide treatment study on *Clematis morefieldii*.

Kruskal-Wallis test was used to collectively compare all three years, Mann-Whitney U tests were used to make pairwise comparisons between years for variables for which the Kruskal-Wallis test showed significant year effects. Significant differences were defined as $P \leq 0.05$. For each variable, mean annual values with the same superscripts do not differ significantly.

Variable (per plant)	2010 (SE)	2011 (SE)	2012 (SE)	Kruskal-Wallis P-Value
Total buds	27.1 ^a (5.22)	38 ^a (12)	10.9 ^b (3.23)	0.008
Dead buds	24.8 ^a (4.98)	34.2 ^a (10.5)	10.3 ^b (3.07)	0.013
Total florivory	17.6 ^a (3.71)	16.8 ^a (3.79)	5.95 ^b (1.75)	0.011
Buds successful	2.35 ^a (0.53)	3.9 ^a (1.76)	0.55 ^b (0.45)	0.005
Achene number	23.3 ^a (5.18)	70.4 ^a (33.2)	6.6 ^b (5.89)	0.006
Percent buds successful	9.2 ^a (1.8)	9.5 ^a (2.3)	2.5 ^b (1.9)	0.011
Percent florivory	64.9 (5.3)	56.6 (7.5)	58.4 (8.9)	0.72

Table 3. Effects of treatment (insecticide and control) and year on reproductive transitions of *Clematis morefieldii* using two-way ANOVA. Reproductive transitions were compared for effects of treatment (insecticide and water), year (2010-2012), and the treatment*year interaction. Significant effects were defined as $P \leq 0.05$ (asterisk denotes significance).

“Intermediate” stage is a combination of “immature” and “medium” flower bud stages. “Fruit” stage is a combination of “tiny aggregate” and “immature aggregate” fruit stages.

Variable	Transition	Degrees of freedom	F-Value	P-Value
Treatment	Tiny - Intermediate	1	15	0.001*
Treatment	Intermediate - Flower	1	14.6	0.001*
Treatment	Flower - Fruit	1	10.8	0.002*
Treatment	Fruit - Mature Fruit	1	0.08	0.78
Year	Tiny - Intermediate	2	10.3	<0.001*
Year	Intermediate - Flower	2	3.16	0.047*
Year	Flower - Fruit	2	0.49	0.62
Year	Fruit - Mature Fruit	2	2.21	0.118
Treatment*Year	Tiny - Intermediate	2	1.38	0.26
Treatment*Year	Intermediate - Flower	2	2.83	0.064
Treatment*Year	Flower - Fruit	2	3.2	0.047*
Treatment*Year	Fruit - Mature Fruit	2	3.21	0.047*

Figure Legends

Figure 1. Linear regressions of insecticide effectiveness on *Clematis morefieldii* fruit production (total insecticide study fruit production minus control fruit production) plotted against total annual rainfall (Fig. 1A) and peak flowering/fruitletting season rainfall (April-June) (Fig. 1B). In each graph, each point represents the values from one year of the experiment. Rainfall data were acquired from the Redstone Arsenal weather station near Huntsville, AL (~ 21 km from study site).

Figure 2. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2010. Ranges for time periods are as follows: Time 1: 26 April – 23 June; Time 2: 24 June – 21 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **).

Figure 3. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2011. Ranges for time periods are as follows: Time 1: 16 April – 26 May; Time 2: 27 May – 10 July; Time 3: 11 July – 16 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **).

Figure 4. Mean percent browse damage (\pm SE) on insecticide study plants by herbivores in 2012. Time period ranges are as follows: Time 1: Plant emergence to 2 May; Time 2: 3 May to 23 May; Time 3: 24 May to 28 June; Time 4: 29 June to 19 July; Time 5: 20 July to 9 August. Data were analyzed using a Mann-Whitney U test and any control treatment with significantly greater leaf herbivory than the insecticide treatment is denoted with asterisks (P = 0.05 *, P = 0.01 **).

Figure 5. Herbivory occurrence on insecticide-treated ■ and control ☒ plants during 2010.

Time period ranges are as follows: T1: Plant emergence to 23 June. T2: 24 June to plant senescence. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks ($P = 0.05$ *, $P = 0.01$ **).

Figure 6. Herbivory occurrence on insecticide-treated ■ and control ☒ plants during 2011.

Time period ranges are as follows: T1: Plant emergence to 26 May. T2: 27 May to 10 July. T3: 11 July to plant senescence. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks ($P = 0.05$ *, $P = 0.01$ **).

Figure 7. Herbivory occurrence on insecticide-treated ■ and control ☒ plants during 2012.

Time period ranges are as follows: T1: Plant emergence to 2 May; T2: 3 May to 23 May; T3: 24 May to 28 June; T4: 29 June to 19 July; T5: 20 July to 9 August. Data were analyzed using Chi-square analysis and control treatments with significantly greater leaf herbivory than the corresponding insecticide treatment are denoted with asterisks ($P = 0.05$ *, $P = 0.01$ **).

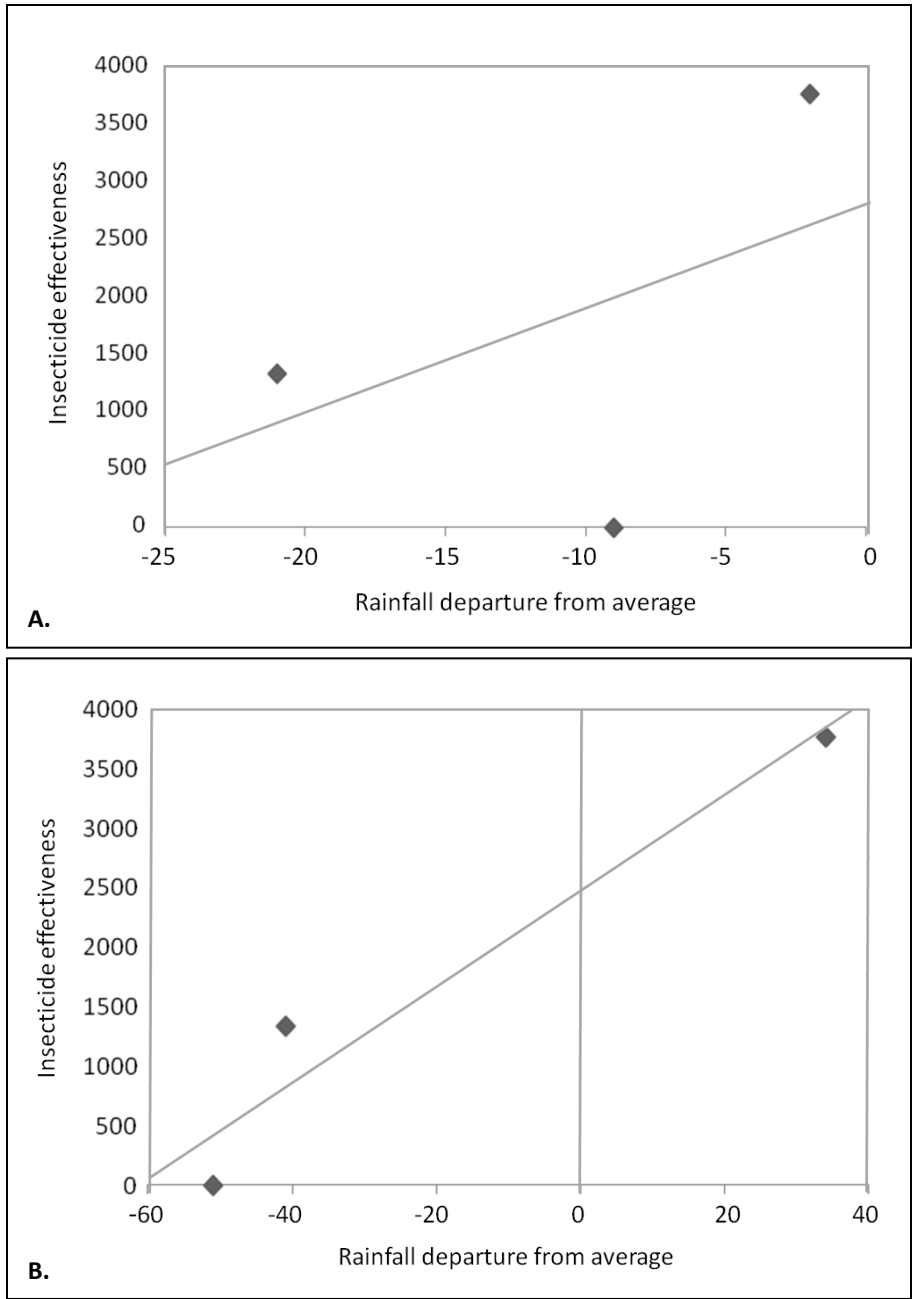


Figure 1 (A and B)

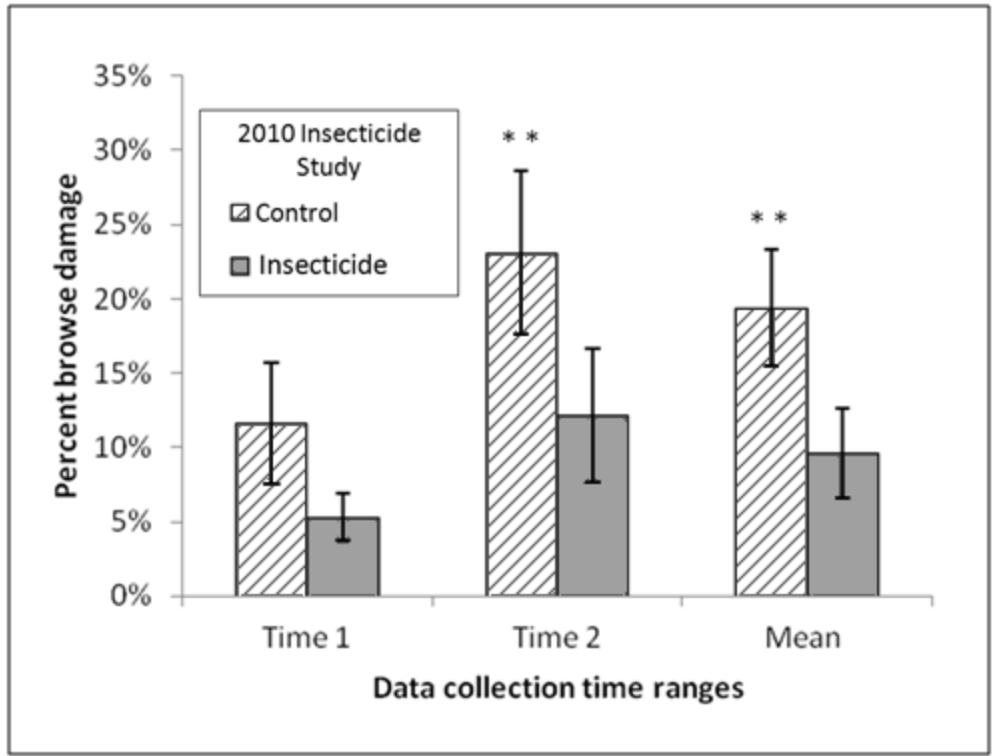


Figure 2

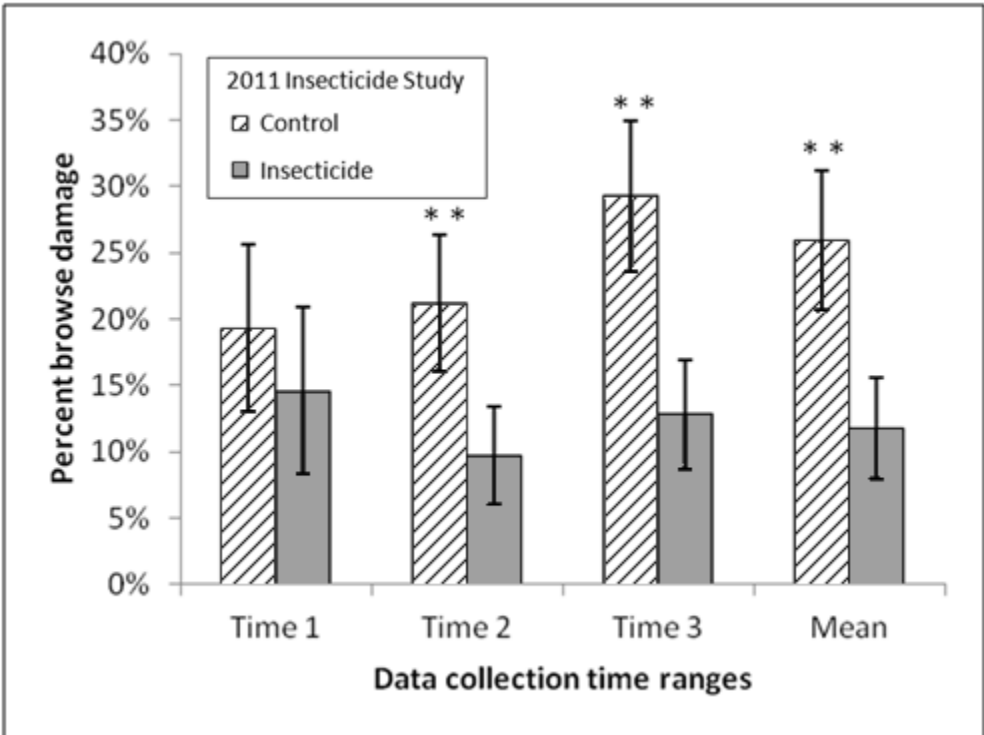


Figure 3

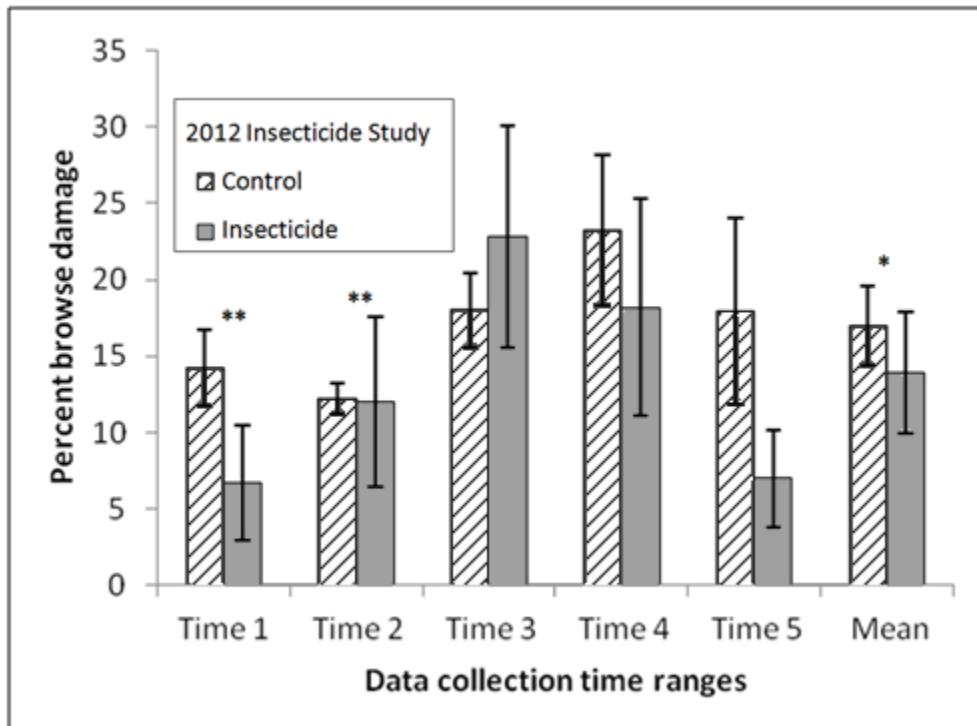


Figure 4

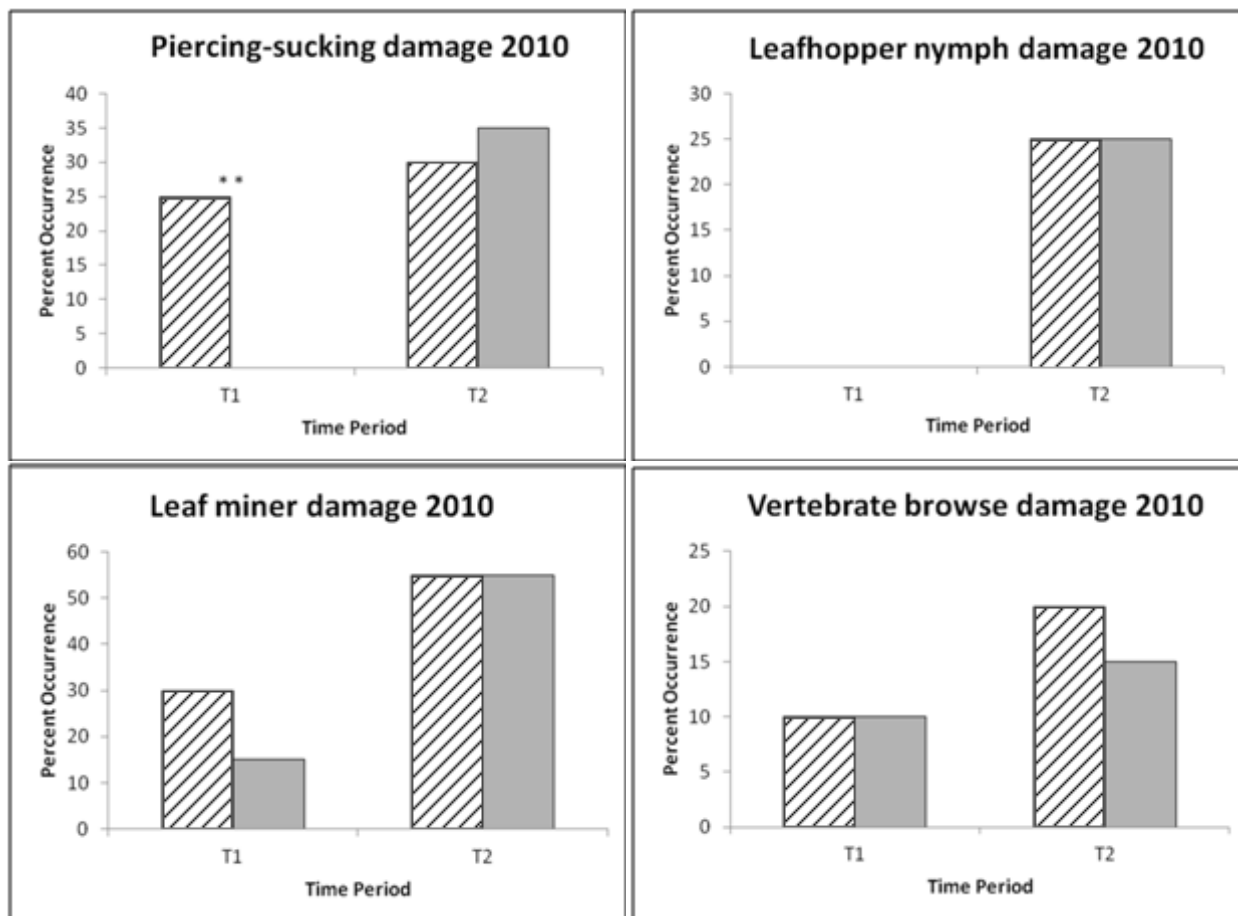


Figure 5

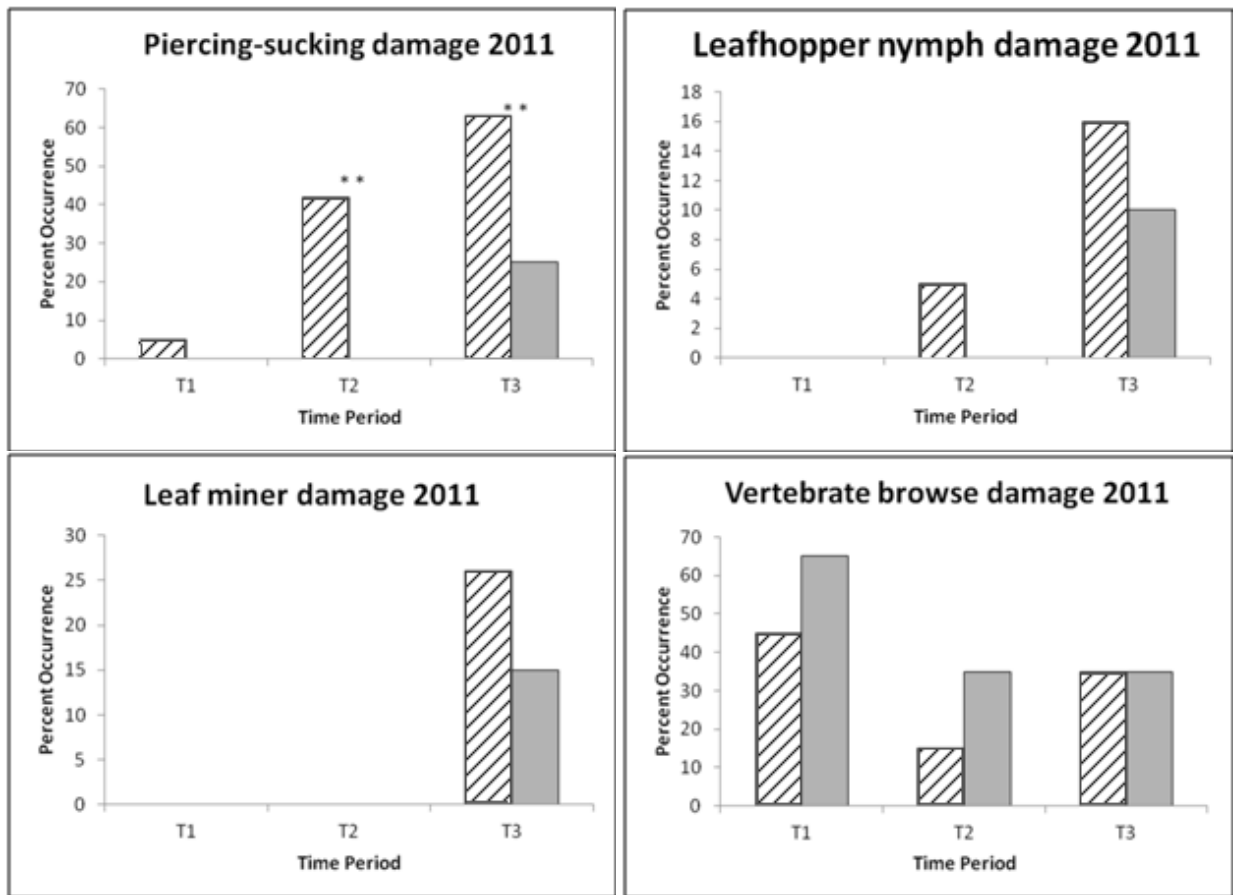


Figure 6

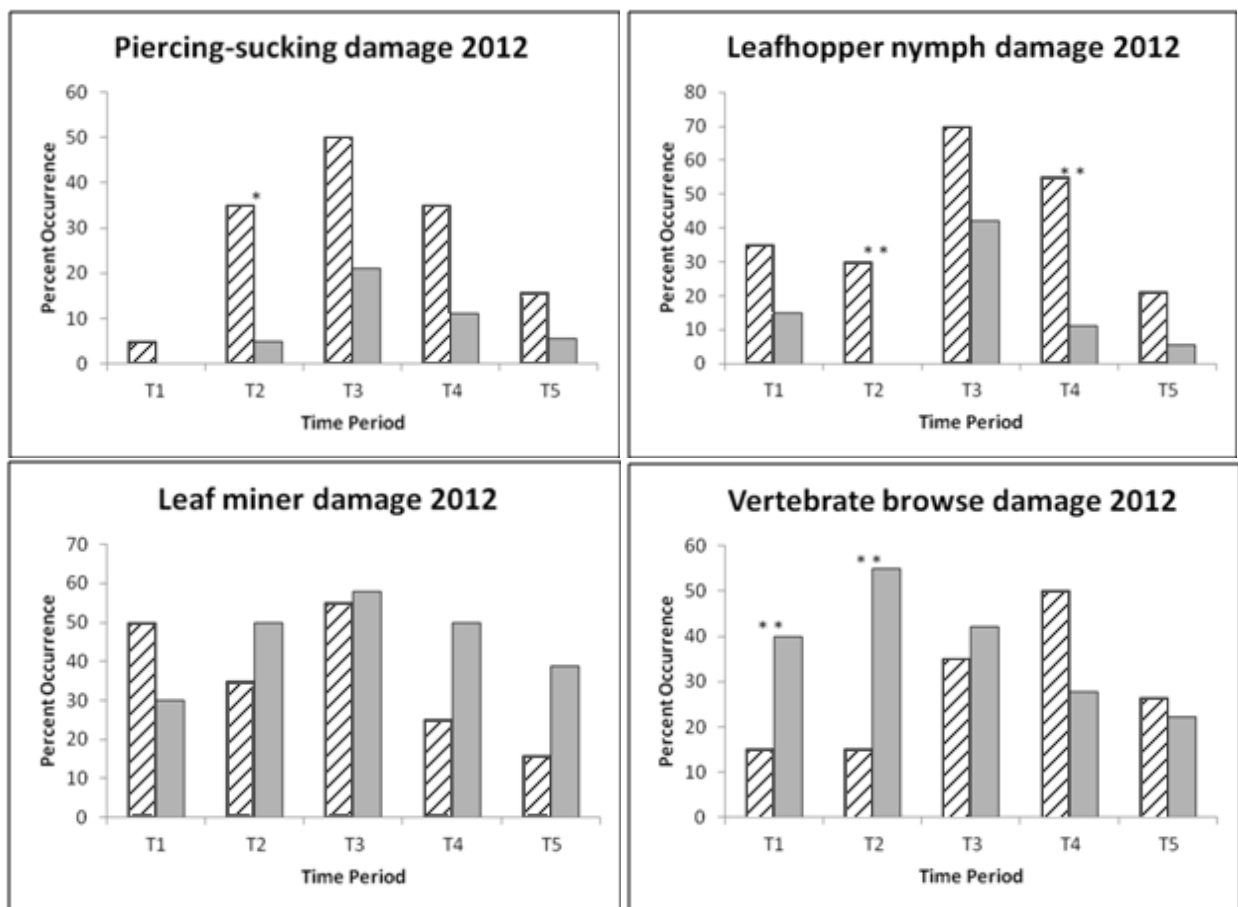


Figure 7

LITERATURE CITED

- Alabama Natural Heritage Program (ANHP). 2012. Alabama inventory list: the rare, threatened and endangered plants & animals of Alabama. Privately printed by the Alabama Natural Heritage Program, 1090 South Donahue Drive, Auburn University, AL 36849.
- Alexander, H.M. and J. Antonovics. 1988. Disease spread and population dynamics of anther-smut infection of *Silene alba* caused by the fungus *Ustilago violacea*. *Journal of Ecology* 76: 91-104.
- Almeida, R.P.P. and A.H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). *Journal of Economic Entomology* 96(2): 264-271.
- Ancheta, J. and S.B. Heard. 2011. Impacts of insect herbivores on rare plant populations. *Biological Conservation* 144: 2395-2402.
- Anderson, R.C., O.L. Loucks, and A.M. Swain. 1969. Herbaceous response to canopy cover, light intensity, and throughfall precipitation in coniferous forests. *Ecology* 50 (2): 255-263.
- Augustine, D.J. and L.E. Frelich. 1998. Effects of white-tailed deer on populations of an understory forb in fragmented deciduous forests. *Conservation Biology* 12 (5): 995-1004.
- Bailey, C. 2005. Survey for Morefield's leather flower (*Clematis morefieldii*) in Southeastern Tennessee. Unpublished report to U.S. Fish and Wildlife Service. Atlanta, Georgia. 14 pp.

- Baskin, J.M. and C.C. Baskin. 1988. Endemism in rock outcrop plant communities of unglaciated eastern United States: an evaluation of the roles of the edaphic, genetic and light factors. *Journal of Biogeography* 15: 829-840.
- Bevill, R.L. and S.M. Louda. 1999. Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology* 13(3): 493-498.
- Bevill, R.L., S.M. Louda, and L.M. Stanforth. 1999. Protection from natural enemies in managing rare plant species. *Conservation Biology* 13:1323-1331.
- Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. *American Naturalist* 117: 838-840.
- Bookman, S.S. 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. *Ecology* 64(2): 264-273.
- Boyd, R.S. 1988. Microdistribution of the beach plant *Cakile maritima* (Brassicaceae) as influenced by a rodent herbivore. *American Journal of Botany* 75: 1540-1548.
- Boyd, R.S. 2003. Factors affecting seed production by the endangered chaparral shrub *Fremontodendron californicum* subsp. *decumbens* (Sterculiaceae). *Madroño* 50(4): 232-242.
- Boyd, R.S. and G.D. Brum. 1983. Predispersal reproductive attrition in a Mojave Desert population of *Larrea tridentata* (Zygophyllaceae). *American Midland Naturalist* 110(1): 14-24.

- Boyd, R.S. and L.L. Serafini. 1992. Reproductive attrition in the rare chaparral shrub *Fremontodendron decumbens* Lloyd (Sterculiaceae). *American Journal of Botany* 79(11): 1264-1272.
- Braunschweig, S.H., E.T. Nilsen, and T.F. Wieboldt. 1999. The Mid-Appalachian shale barrens. p. 83-98. *In*: Anderson, R.C., J.S. Fralish, and J.M. Baskin (eds.). *Savannas, Barrens, and Rock Outcrop Plant Communities of North America*. Cambridge University Press. Cambridge, UK.
- Brown, J.K. and J. Bird. 1992. Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. *Plant Diseases* 76(3): 220-225.
- Chapin III, F.S., E.S. Zavaleta, V.T. Eviner, R.L. Naylor, P.M. Vitousek, H.L. Reynolds, D.U. Hooper, S. Lavorel, O.E. Sala, S.E. Hobbie, M.C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature* 405: 234-242.
- Chauvet, S., F. Feer, and P. Forget. 2004. Seed fate of two Sapotaceae species in a Guianan rain forest in the context of escape and satiation hypotheses. *Journal of Tropical Ecology* 20: 1-9.
- Cintra, R. 1997. Leaf litter effects on seed and seedling predation of the palm *Astrocaryum murumuru* and the legume tree *Dipteryx micrantha* in Amazonian forest. *Journal of Tropical Ecology* 13(5): 709-725.
- Condit, R., R. Sukumar, S.P. Hubbell, and R.B. Foster. 1998. Predicting population trends from size distributions: A direct test in a tropical tree community. *The American Naturalist* 152(4): 495-509.

- Crabtree, T. 2011. Monitoring of *Clematis morefieldii* Kral in Tennessee. Tennessee Natural Heritage Program, Nashville, TN. Unpublished report for the U.S. Fish and Wildlife Service. 5 pp. + 2 Appendices.
- Crawley, M.J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34: 531-564.
- Delcourt, H.R. and P.A. Delcourt. 1997. Pre-Columbian Native American use of fire on Southern Appalachian landscapes. *Conservation Biology* 11 (4): 1010-1014.
- Didham, R.K., J. Ghazoul, N.E. Stork, and A.J. Davis. 1996. Insects in fragmented forests: a functional approach. *Trends in Ecology and Evolution* 11 (6): 255-260.
- Ehrlen, J. 1995. Demography of the perennial herb *Lathyrus vernus*. II. Herbivory and population dynamics. *Journal of Ecology* 83(2): 297-308.
- Ellstrand, N.C. and D.R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-243.
- Emanuel, C. 2000. Monitoring report for Morefield's leather flower, *Clematis morefieldii*. U.S. Fish and Wildlife Service, Jackson, MS. 8 pp.
- English-Loeb, G.M. 1990. Plant drought stress and outbreaks of spider mites: a field test. *Ecology* 71(4): 1401-1411.
- Erickson, R.O. 1943. Taxonomy of *Clematis* section *Viorna*. *Annals of the Missouri Botanical Garden* 30(1): 1-15.

- Estes, D. and C. Fleming. 2006. *Clematis morefieldii* (Ranunculaceae) new to Tennessee. *SIDA* 22(1): 821-824.
- Estill, J.C. and M.B. Cruzan. 2001. Phylogeography of rare plant species endemic to the southeastern United States. *Castanea* 66(1-2): 3-23.
- Fang, X., N.C. Turner, G. Yan, F. Li, and K.H.M. Siddique. 2009. Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. *Journal of Experimental Botany* 61(2): 335-345.
- Fenster, C.B. and M.R. Dudash. 1994. Genetic considerations for plant population restoration and conservation. p. 34-62. *In*: M.L. Bowles and C.J. Whelan (eds.). *Restoration of Endangered Species*. Cambridge University Press, Cambridge, Massachusetts.
- Filippin, L., J. Jović, T. Cvrković, V. Forte¹, D. Clair, I. Toševski, E. Boudon-Padieu, M. Borgo¹, and E. Angelini. 2009. Molecular characteristics of phytoplasmas associated with *Flavescence dorée* in *Clematis* and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector. *Plant Pathology* 58(5): 826-837.
- Fischer, M. and D. Matthies. 1998. RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *American Journal of Botany* 85(6): 811-819.
- Fisher, F.M., J.C. Zak, G.L. Cunningham, and W.G. Whitford. 1988. Water and nitrogen effects on growth and allocation patterns of creosotebush in the northern Chihuahuan Desert. *Journal of Range Management* 41(5): 387-391.
- Flather, C.H., M.S. Knowles, and I.A. Kendall. 1998. Threatened and endangered species geography. *BioScience* 48(5): 365-376.

- Fletcher, J.D., L.A. Shipley, W.J. McShea, and D.L. Shumway. 2001. Wildlife herbivory and rare plants: the effects of white-tailed deer, rodents, and insects on growth and survival of Turk's cap lily. *Biological Conservation* 101: 229-238.
- Fox, L.R., S.P. Ribeiro, V.K. Brown, G.J. Masters, I.P. Clarke. 1999. Direct and indirect effects of climate change on St John's wort, *Hypericum perforatum* L. (Hypericaceae). *Oecologia* 120(1): 113-122.
- Fröberg, H. and O. Eriksson. 2003. Predispersal seed predation and population dynamics in the perennial understorey herb *Actaea spicata*. *Canadian Journal of Botany* 81(11): 1058-1069.
- García, M.B. 2003. Demographic viability of a relict population of the critically endangered plant *Borderea chouardii*. *Conservation Biology* 17(6): 1672-1680.
- Gómez-Aparicio, L., R. Zamora, and J.M. Gómez. 2005. The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. *Biological Conservation* 121(2): 195–206.
- Grey-Wilson, C. 2000. *Clematis* the genus: A comprehensive guide for gardeners, horticulturists and botanists. Timber Press, Inc., Portland, OR.
- Griffith, G.E, J.M. Omernik, J.A. Comstock, G. Martin, A. Goddard, and V.J. Hulcher. 2001. Ecoregions of Alabama. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, OR.
- Guimarães, P.R., J. José, M. Galetti, and J.R. Trigo. 2003. Quinolizidine alkaloids in *Ormosia arborea* seeds inhibit predation but not hoarding by Agoutis (*Dasyprocta leporina*). *Journal of Chemical Ecology* 29(5): 1065-1072.

- Guth, C.J. and S.G. Weller. 1986. Pollination, fertilization and ovule abortion in *Oxalis magnifica*. *American Journal of Botany* 73(2): 246-253.
- Harrison, R.D. 2000. Repercussions of El Niño: drought causes extinction and breakdown of mutualism in Borneo. *Proceedings of the Royal Society of London B* 267: 911-915.
- Hart, J.L. and H.D. Grissino-Mayer. 2008. Vegetation patterns and dendroecology of a mixed hardwood forest on the Cumberland Plateau: Implications for stand development. *Forest Ecology and Management* 255: 1960–1975.
- Harvell, C.D., C.E. Mitchell, J.R. Ward, S. Altizer, A.P. Dobson, R.S. Ostfeld, and M.D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296(5576): 2158-2162.
- Heard, S.B. and G.H. Cox. 2009. Plant module size and attack by the goldenrod spindle-gall moth. *The Canadian Entomologist* 141(4): 406-414.
- Hegland, S.J., A. Nielsen, A. Lázaro, A. Bjerknes, and Ø. Totland. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12(2): 184-195.
- Hinkle, C.R., W.C. McComb, J.M. Safely Jr. and P.A. Schmalzer. 1993. Mixed mesophytic forests. p. 203–253. *In*: Martin, W.H., Boyce, S.G., Echternacht, A.C. (eds.). *Biodiversity of the Southeastern United States: Upland Terrestrial Communities*. John Wiley and Sons, New York.
- Honnay, O. and H. Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21(3): 823–831.

- Ison, C.R. 2000. Fire on the edge: Prehistoric fire along the escarpment zone of the Cumberland Plateau. p. 36-45. *In*: Yaussy, D.A. (ed.). Proceedings: Workshop on Fire, People, and the Central Hardwoods Landscape. Richmond, Kentucky. USDA Forest Service: General Technical Report NE-274.
- Janzen, D.H. 1971. Seed predation by animals. *Annual Review of Ecology and Systematics* 2: 465-492.
- Judd, W.S, C.S. Campbell, E.A. Kellogg, P.F. Stevens, and M.J. Donaghue. 2002. *Plant systematics: A phylogenetic approach*. Sinauer Associates, Inc., Sunderland, MA, U.S.A.
- Kawano, S., M. Ohara, and F.H. Utech. 1986. Life history studies on the genus *Trillium* (Liliaceae) II. Reproductive biology and survivorship of four eastern North American species. *Plant Species Biology* 1(1): 47-58.
- Kaye, T.N. 1999. From flowering to dispersal: reproductive ecology of an endemic plant, *Astragalus australis* var. *olympicus* (Fabaceae). *American Journal of Botany* 86(9): 1248-1256.
- Kettenring, K.M., C.W. Weakley, and E.S. Menges. 2009. Herbivory delays flowering and reduces fecundity of *Liatris ohlingerae* (Asteraceae), an endangered, endemic plant of the Florida scrub. *The Journal of the Torrey Botanical Society* 136(3): 350-362.
- Kirnak, H., C. Kaya, I. Tas, and D. Higgs. 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in eggplants. *Bulgarian Journal of Plant Physiology* 27(3-4): 34-46.

- Klinkhamer, P.G.L., T.J. de Jong, and G. de Bruyn. 1989. Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* 54: 201-204.
- Kokubun, M., S. Shimada, and M. Takahashi. 2001. Flower abortion caused by preanthesis water deficit is not attributed to impairment of pollen in soybean. *Crop Science* 41: 1517–1521.
- Kolb, A. 2008. Habitat fragmentation reduces plant fitness by disturbing pollination and modifying response to herbivory. *Biological Conservation* 141 (10): 2540-2549.
- Kral, R. 1987. A new “Viorna” *Clematis* from northern Alabama. *Annals Missouri Botanical Gardens* 74: 665-669.
- Krupnick, G.A. and A.E. Weis. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* 80: 135–149.
- Krupnick, G.A., A.E. Weis, and D.R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* 80: 125–134.
- Kunin, W.E. and K.J. Gaston. 1997. *The biology of rarity: causes and consequences of rare–common differences*. Chapman & Hall, London.
- League, K. and T. Veblen. 2006. Climatic variability and episodic *Pinus ponderosa* establishment along the forest-grassland ecotones of Colorado. *Forest Ecology and Management* 228: 98-107.
- Leavitt, H. and I.C. Robertson. 2006. Petal herbivory by chrysomelid beetles (*Phyllotreta* sp.) is detrimental to pollination and seed production in *Lepidium papilliferum* (Brassicaceae). *Ecological Entomology* 31(6): 657-660.

- Lesica, P. and H.E. Atthowe. 2000. Should we use pesticides to conserve rare plants?
Conservation Biology 14: 1549-1550.
- Levey, D.J. 1990. Habitat-dependent fruiting behaviour of an understory tree, *Miconia centrodesma*, and tropical treefall gaps as keystone habitats for frugivores in Costa Rica.
Journal of Tropical Ecology 6(4): 409-420.
- Levine, M.T. and K.N. Paige. 2004. Direct and indirect effects of drought on compensation following herbivory in Scarlet Gilia. Ecology 85(12): 3185-3191.
- Lieberman, D. and M. Li. 1992. Seedling recruitment patterns in a tropical dry forest in Ghana.
Journal of Vegetation Science 3(3): 375-382.
- Louda, S. M. 1994. Experimental evidence for insect impact on populations of short-lived, perennial plants, and its application in restoration ecology. p. 118-138. *In*: M. L. Bowles and C. J. Whelan (eds.). Restoration of endangered species: Conceptual issues, planning, and implementation. Cambridge University Press, Cambridge, Massachusetts.
- Louda, S.M. and M.A. Potvin. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. Ecology 76: 229-245.
- Lowenberg, G.J. 1994. Effects of floral herbivory on maternal reproduction in *Sanicula arctopoides* (Apiaceae). Ecology 75(2): 359-369.
- MacDonald, A.A.M. and P.M. Kotanen. 2010. The effects of disturbance and enemy exclusion on performance of an invasive species, common ragweed, in its native range. Oecologia 162: 977-986.

- Maron, J.L. and E.L. Simms. 1997. Effect of seed predation on seed bank size and seedling recruitment of bush lupine (*Lupinus arboreus*). *Oecologia* 111(1): 76-83.
- Martin, E.F. 2010. Reproduction, demography, and habitat characterization of *Astragalus peckii* (Fabaceae), a rare central Oregon endemic. PhD dissertation. Oregon State University.
- Maschinski, J. 2001. Implications of managing the timing and intensity of herbivory for conservation of Arizona leatherflower. p. 128-138. *In*: Maschinski, J., Holter, Louella, (eds.). Southwestern rare and endangered plants: Proceedings of the Third Conference; 2000 September 25-28; Flagstaff, AZ. Proceedings RMRS.
- Masters, G.J., V.K. Brown, I.P. Clarke, J.B. Whittaker, and J.A. Hollier. 1998. Direct and indirect effects of climate change on insect herbivores: *Auchenorrhyncha* (Homoptera). *Ecological Entomology* 23: 45-52.
- Matthies, D., I. Bräuer, W. Maibom, and T. Tschardt. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. *Oikos* 105(3): 481-488.
- Mattila, E. and M.T. Kuitunen. 2000. Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata* (Orchidaceae). *Oikos* 89(2): 360-366.
- Mavraganis, K. and C.G. Eckert. 2001. Effects of population size and isolation on reproductive output in *Aquilegia canadensis* (Ranunculaceae). *Oikos* 95(2): 300-310.
- McCall, A.C. and R.E. Irwin. 2006. Florivory: the intersection of pollination and herbivory. *Ecology Letters* 9: 1351-1365.
- McCanny, S.J. and P.B. Cavers. 1987. The escape hypothesis: a test involving a temperate, annual grass. *Oikos* 49: 67-76.

- McCue, K.A. and T.P. Holtsford. 1998. Seed bank influences on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *American Journal of Botany* 85(1): 30-36.
- McIntosh, M.E. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). 162(2): 273-288.
- Mehle, N., M. Ravnkar, G. Seljak, V. Knapiè, and M. Dermastia. 2011. The most widespread phytoplasmas, vectors and measures for disease control in Slovenia. *Phytopathogenic Mollicutes* 1(2): 1-12.
- Menges, E.S., E.O. Guerrant Jr., and S. Hamzé. 2004. Effects of seed collection on the extinction risk of perennial plants. p. 305-324. *In*: Guerrant, O., K. Lavens, and M. Maunder (eds.). *Ex situ plant conservation: Supporting species survival in the wild*. Island Press, 1718 Connecticut Avenue, NW, Suite 300, Washington, DC.
- Milton, S.J. 1992. Effects of rainfall, competition, and grazing on flowering of *Osteospermum sinuatum* (Asteraceae) in arid Karoo rangeland. *Journal of the Grassland Society of Southern Africa* 9(4): 158-164.
- Mitchell, R.J. 1994. Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *The American Naturalist* 143(5): 870-889.
- Mothershead, K. and R.J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* 81: 30-40.
- Myers, N. 1996. Environmental services of biodiversity. *Ecology* 93: 2764-2769.
- Myster, R.W. and S.T.A. Pickett. 1993. Effects of litter, distance, density and vegetation patch type on postdispersal tree seed predation in old fields. *Oikos* 66: 381-388.

- Nathan, R. and R. Casagrandi. 2004. A simple mechanistic model of seed dispersal, predation and plant establishment: Janzen-Connell and beyond. *Journal of Ecology* 92(5): 733-746.
- NatureServe. 2013. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available <http://www.natureserve.org/explorer>. (Accessed: August 29, 2013)
- Nayyar, H., T. Bains, and S. Kumar. 2005. Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. *Environmental and Experimental Botany* 53(1): 39-47.
- Niesenbaum, R.A. 1996. Linking herbivory and pollination: defoliation and selective fruit abortion in *Lindera benzoin*. *Ecology* 77(8): 2324-2331.
- Nixon, C.M., L.P. Hansen, P.A. Brewer, and J.E. Chelvig. 1991. Ecology of white-tailed deer in an intensively farmed region of Illinois. *Wildlife Monographs* 118: 3-77.
- Oguro, M. and S. Sakai. 2009. Floral herbivory at different stages of flower development changes reproduction in *Iris gracilipes* (Iridaceae). *Plant Ecology* 202: 221-234.
- Ohara, M. and S. Higashi. 1994. Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). *Oecologia* 98(1): 25-30.
- Pärtel, M., R. Kalamees, Ü. Reier, E. Tuvi, E. Roosaluuste, A. Vellak, and M. Zobel. 2005. Grouping and prioritization of vascular plant species for conservation: combining natural rarity and management need. *Biological Conservation* 123: 271-278.

- Pascarella, J.B. 1995. The effects of Hurricane Andrew on the population dynamics and the mating system of the tropical understory shrub *Ardisia escallonioides* (Myrsinaceae). Dissertation. University of Miami, Coral Gables, Florida, USA.
- Perring, T.M., N.M. Gruenhagen, and C.A. Farrar. 1999. Management of plant viral diseases through chemical control of insect vectors. *Annual Review of Entomology* 44: 457-481.
- Pimm, S.L., G.J. Russell, J.L. Gittleman, and T.M. Brooks. 1995. The future of biodiversity. *Science* 269: 347-350.
- Platt, R.B. 1951. An ecological study of the Mid-Appalachian shale barrens and of the plants endemic to them. *Ecological Monographs* 21 (4): 269-300.
- Price, P.W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251.
- Primack, R.B. and S.L. Miao. 1992. Dispersal can limit local plant distribution. *Conservation Biology* 6(4): 513-519.
- Rabinowitz, D. 1981. Seven forms of rarity. p. 205–217. *In*: Synge, H. (ed.). *The biological aspects of rare plant conservation*. John Wiley & Sons, Chichester.
- Reed, D.H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. *Conservation Biology* 17(1): 230-237.
- Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. p. 3-54. *In*: Rosenthal, G.A. and D.H. Janzen (eds.). *Herbivores: their interaction with secondary metabolites*. Academic Press, New York.

- Rooney, T.P. 1997. Escaping herbivory: refuge effects on the morphology and shoot demography of the clonal forest herb *Maianthemum canadense*. *Journal of the Torrey Botanical Society* 124 (4): 280-285.
- Rooney, T.P. and D.M. Waller. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management* 181: 165-176.
- SAS Institute. 2005. Statview 5.0. Thomson—Brooks/Cole, Belmont, CA, USA.
- Satterthwaite, W.H., K.D. Holl, G.F. Hayes, and A.L. Barber. 2007. Seed banks in plant conservation: Case study of Santa Cruz tarplant restoration. *Biological Conservation* 135: 57–66.
- Schemske, D.G., B.C. Husband, M.H. Ruckelshaus, C. Goodwillie, I.M. Parker, and J.G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75(3): 584-606.
- Schotz, A. 2007. Supplemental surveys for *Clematis morefieldii*, Morefield's Leatherflower, in Alabama. Alabama Natural Heritage Program, Montgomery, Alabama. Unpublished report for the U.S. Fish and Wildlife Service. 3pp. + 3 Appendices.
- Schowalter, T.D., D.C. Lightfoot, and W.G. Whitford. 1999. Diversity of arthropod responses to host-plant water stress in a desert ecosystem in Southern New Mexico. *The American Midland Naturalist* 142(2): 281-290.
- Session, L.A. and D. Kelly. 2001. Heterogeneity in vertebrate and invertebrate herbivory and its consequences for New Zealand mistletoes. *Austral Ecology* 26(6): 571-581.

- Simon, J., M. Bosch, J. Molero, and C. Blanché. 2001. Conservation biology of the Pyrenean larkspur (*Delphinium montanum*): a case of conflict of plant versus animal conservation. *Biological Conservation* 98: 305-314.
- Simpson, M.G. 2006. *Plant Systematics*. Elsevier Academic Press. Burlington, MA.
- Sletvold, N. and J. M. Grindeland. 2008. Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. *Acta Oecologica* 34 (1): 21-25.
- Smith, T.J. 1987. Seed predation in relation to tree dominance and distribution in mangrove forests. *Ecology* 68(2): 266-273.
- Söber, V., T. Teder, and M. Moora. 2009. Contrasting effects of plant population size on florivory and pollination. *Basic and Applied Ecology* 10(8): 737-744.
- Staley, J.T., S.R. Mortimer, G.J. Masters, M.D. Morecroft, V.K. Brown, and M.E. Taylor. 2006. Drought stress differentially affects leaf-mining species. *Ecological Entomology* 31: 460-469.
- Stein, B.A. 2002. *States of the Union: Ranking America's Biodiversity*. NatureServe. Arlington, Virginia. 27 pp.
- Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* 12: 253-279.
- Strauss, S.Y., J.K. Conner, and S.L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: Implications for male and female plant fitness. *The American Naturalist* 147(6): 1098-1107.
- Tanaka, Y. 2000. Extinction of populations by inbreeding depression under stochastic

- environments. *Population Ecology* 42(1): 55-62.
- Tennessee Department of Environment and Conservation (TDEC). 2010. Public Notice M2010-11. Nashville, TN. Available:
http://www.state.tn.us/environment/wpc/ppo/mining/pn_10_15_tinsleyasphalt.pdf.
- Timmerman-Erskine, M. and R.S. Boyd. 1999. Reproductive biology of the endangered plant *Clematis socialis* (Ranunculaceae). *Journal of the Torrey Botanical Society* 126(2): 107-116.
- U.S. Census Bureau. 2009. American Community Survey (ACS).
<www.huntsvillealabamausa.com/new_exp/community_data/demographics/summary.html>. (Accessed: September 23, 2009).
- U.S. Census Bureau. 2010. U.S. Census Bureau delivers Tennessee's 2010 census population totals, including first look at race and hispanic origin data for legislative redistricting. <<http://2010.census.gov/news/releases/operations/cb11-cn93.html>>. (Accessed: May 10, 2011).
- U.S. Census Bureau. 2011. Population Distribution and Change: 2000 to 2010. United States Census 2010. <www.census.gov/prod/cen2010/briefs/c2010br-01.pdf>. (Accessed: December 12, 2013).
- U.S. Fish and Wildlife Service (USFWS). 1992. Endangered and threatened wildlife and plants; determination of *Clematis morefieldii* (Morefield's leather flower) to be an endangered species. *Federal Register* 57(98): 21562-34420.
- U.S. Fish and Wildlife Service (USFWS). 1994. Recovery plan for Morefield's leather flower (*Clematis morefieldii*). Atlanta, Georgia. 15 pp.
- U.S. Fish and Wildlife Service (USFWS). 2010. Morefield's leather flower (*Clematis*

- morefieldii*) 5-Year Review: Summary and Evaluation. U.S. Fish and Wildlife Service Southeast Region Mississippi Ecological Services Field Office Jackson, Mississippi. 12 pp.
- Walck, J.L., J.M. Baskin, and C.C. Baskin. 1999. Effects of competition from introduced plants on establishment, survival, growth and reproduction of the rare plant *Solidago shortii* (Asteraceae). *Biological Conservation* 88(2): 213-219.
- Wall, M.A., M. Timmerman-Erskine, and R.S. Boyd. 2003. Conservation impact of climatic variability on pollination of the federally endangered plant, *Clematis socialis* (Ranunculaceae). *Southeastern Naturalist* 2(1): 11-24.
- Waller, D.M. and W.S. Alverson. 1997. The white-tailed deer: a keystone herbivore. *Wildlife Society Bulletin* 25(2): 217-226
- Weakley, A.S. 2012. Flora of the Southern and Mid-Atlantic states, Working draft of November 2012. North Carolina Botanical garden. <<http://www.herbarium.unc.edu/flora.htm>> Last updated 10/2/2012. (Accessed: October 6, 2013).
- Weber, S.F. 1990. The population structure of *Cotinus obovatus* Raf., an ecologically narrow, endemic tree of cedar woodlands of the southern Cumberland Plateau. MA thesis. The University of Alabama in Huntsville.
- Weber, S.F. 1991. Status Report on *Clematis morefieldii*. Unpublished report to U.S. Fish and Wildlife Service, Jackson, Mississippi. 6 pp. + appendix.
- Weber, S.F. 1994. Status Report on *Clematis morefieldii*. Unpublished report to U.S. Fish and Wildlife Service, Jackson, Mississippi. 17 pp. + appendix.

- Weller, S.G. 1994. The relationship of rarity to plant reproductive biology. p. 90-117. *In*:
Bowles, M.L. and C.J. Whelan (eds.). Restoration of endangered species: conceptual
issues, planning, and implementation. Cambridge University Press, Cambridge, UK.
- Wenny, D.G. 2000. Seed dispersal, seed predation, and seedling recruitment of a neotropical
montane tree. *Ecological Monographs* 70(2): 331-351.
- White, T.C.R. 1969. An index to measure weather-induced stress of trees associated with
outbreaks of Psyllids in Australia. *Ecology* 50(5): 905-909.
- White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of
nitrogen in stressed food plants. *Oecologia* 63(1): 90-105.
- White, J. and J.L. Harper. 1970. Correlated changes in plant size and number in plant
populations. *Journal of Ecology* 58(2): 467-485.
- Wiser, S.K., Peet, R.K., and P.S. White. 1998. Prediction of rare-plant occurrence: A southern
Appalachian example. *Ecological Applications* 8(4): 909-920.