AIDING CONSERVATION OF THE FEDERALLY THREATENED *SCUTELLARIA*

MONTANA (LAMIACEAE, LARGE-FLOWERED SKULLCAP) THROUGH

ABUNDANCE MONITORING AND TRANSPLANTATION STUDIES

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ABSTRACT

Scutellaria montana Chapm. (large-flowered skullcap) is a federally threatened species endemic to Georgia and Tennessee. This thesis presents conservation work on this species through abundance monitoring at a single site in Catoosa County, Georgia, and transplantation experiments at two sites located in Hamilton County, Tennessee, and Catoosa County, Georgia. Plants naturally occurring in the fixed plots surveyed over seven years had an overall stable growth status, though relatively low floral induction and seedlings. Transplantation revealed influences of abiotic and biotic factors. One transplantation site was fenced to prevent human trampling and apparently discouraged vertebrate grazing, resulting in dramatic flower number increases. The other transplantation site without fencing had mixed results with prescribed burning and canopy thinning treatments. Evidence suggested that grazing pressures increased with the treatments, so the control plot was apparently best suited for transplantation when herbivory exclosures are absent. Controlling herbivory to maximize transplantation success is recommended.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A, Net photosynthetic rate

ANOVA, Analysis of Variance

B, Prescribed burning only

C, Control

E, Transpiration rate

ESIP, Enterprise South Industrial Park

g_s, Stomatal conductance

LMA, Leaf mass per unit area

PCA, Principal Component Analysis

PPFD, Photosynthetic photon flux densities

SE, Standard error of the mean

T, Canopy thinning only

T + B, Combined canopy thinning and prescribed burning

TNARNG, Tennessee Army National Guard

USFWS, United States Fish and Wildlife Service

VTS, Volunteer Training Site

CHAPTER 1

INTRODUCTION

Scutellaria L. is a subcosmopolitan genus in Lamiaceae with approximately 360 known

species of largely non-aromatic and predominately perennial herbs and shrubs (Paton 1990;

Kadereit 2004). Bentham and Hooker (1965) originally placed subtribe Scutellariae under

Lamiaceae's tribe Stachydeae. Erdtman (1945, as cited in Erdtman 1952) reorganized

Lamiaceae into two subfamilies, Lamioideae and Nepetoideae, but Cantino and Sanders (1986)

later suggested that Lamioideae was not monophyletic. More recently, researchers split

Lamiaceae into many subfamilies, including Lamioideae, which contained tribe Scutellarieae,

based on DNA evidence (Cantino et al. 1997; Wagstaff and Olmstead 1997). Monophyletic

subtribes Scutellariinae, including genera *Scutellaria* and *Tinnea*, and Holmskioldiinae,

including one Asian species (Cantino 1992), were placed under Scutellarieae (Cantino et al.

1997; Wagstaff and Olmstead 1997). Later, Kadereit (2004) regrouped Lamiaceae to include

subfamily Scutellarioidea with five genera: *Scutellaria*, *Tinnea*, *Holmskioldia*, *Renschia*, and

Wenchengia.

The infrageneric structure of *Scutellaria* has been reorganized many times as well. Epling

(1942) split *Scutellaria* into 17 sections, including *Annulatae* that contained *Scutellaria montana*

Chapm. (large-flowered skullcap). Section *Annulatae* contained 13 species of North American

Scutellaria, mostly occurring in the southeastern part of the United States (Collins 1976). Paton

(1990) split *Scutellaria* into two subgenera, *Scutellaria* and *Apeltanthus*, based on differences in inflorescence morphology. Subgenus *Scutellaria* has one-sided or rarely spiral inflorescences usually with opposite flowers subtended by leaves or leaf-like bracts and was further subdivided into five sections due to differences mostly in the flowers and nutlets. He considered section *Scutellaria* to be paraphyletic and again split this section into 34 species-groups. Species-groups comprised of herbaceous plants with distributions that include the eastern United States were *S. galericulata,* which is a wetland or waterside species with rhizomes, *S. incana,* which has terminal inflorescences with annulate corollas and subtended by reduced leaf-like bracts, *S. lateriflora*, which has axillary inflorescences, S. *ovata*, which is similar to the *S. incana* species-group except with exannulate corollas, and *S. parvula*, which has corollas less than 10 mm and subtended by cauline-like leaves. Paton (1990) placed all of *Annulatae* within the *S. incana* species-group along with *S. bushii* Britton, *S. incana* Spreng., and *S. integrifolia* L., but he did not specifically address the classification of exannulate *S. montana* within *Annulatae*. Kadereit (2004) recognized Paton's splitting of *Scutellaria* but did not elaborate. *Scutellaria* is considered one of the largest genera in Lamiaceae (Epling 1942; Cronquist 1981; Kadereit 2004), and Estill and Cruzan (2001) included *Scutellaria* in their top ten endemic genera of the southeastern United States.

Chapman (1878) was the first to describe *S. montana* from a specimen collected in Georgia, likely from Floyd County (Epling 1942; Collins 1976). He described it as a tomentose pubescent perennial having simple stems with opposite, serrated leaves that were acute apically and basally and having large blue corollas. Epling (1942) added glandular-tipped retrorse trichomes on the stem, evenly hirsute on both sides of the crenate-serrate-margined leaves, and described that corollas of *S. montana* were exannulate. Building on Epling's work, Collins

3

(1976) found that *Annulatae* species have stipitate-glandular trichomes on the second internode from base of the inflorescence and do not produce rhizomes, and he cautioned against using basal leaves for identification. He also described anthesis occurring in late spring or early summer and that flowering could occur in the first year of growth, contrary to Cruzan's (2001) observation that seedlings required a few years to mature. In general, *Scutellaria* species have decussate or helical flower arrangements, decussate phyllotaxis, and schizocarp fruits (Kadereit 2004). Forty-five species of *Scutellaria* occur in the United States with 22 of these occurring in the southeastern region, and nine of these species in particular have native geographical ranges that coincide with that of *S. montana*, which occurs only in southeast Tennessee and northwest Georgia (USDA 2011). *Scutellaria pseudoserrata* Epling is a sympatric species that has the closest morphological and phenological resemblance to *S. montana* (Collins 1976; USFWS 2002; USDA 2011; personal observation). Unlike *S. montana*, however, *S. pseudoserrata* has punctate glandular leaf surfaces with short, eglandular trichomes, especially located at leaf veins and margins, and corollas with an annulus at the bend (Collins 1976; USFWS 2002).

Research interest in *S. montana* has been motivated largely by its status as federally threatened, rare, and locally endemic species. Conservation efforts have tended to focus on rarity due to the assumption that organisms with small population sizes are most vulnerable to extinction at least partly from stochastic processes (Schemske et al. 1994; Flather et al. 1998; Matthies et al. 2004). Though there is little evidence to show that endemics are necessarily stenotopic, rare plants may be limited in range due to some unique combination of environmental constraints from climate, geology, or interactions with other organisms (Kruckeberg and Rabinowitz 1985). If species habitats are sharply delimited from other habitats along with poor dispersal mechanisms and more aggregated distributions, colonization of new areas and the

4

rescue effect between populations would be improbable (Quinn et al. 1994). Collins et al. (2001) cited land clearing and forest fragmentation as reasons for the rarity of *S. montana*, and seeds of *S. montana* are apparently clitochorous, limiting long-distance dispersal (Cruzan 2001). However, asking how a species becomes rare is less important than asking about the consequences of this rarity, particularly in terms of its population status (Schemske et al. 1994; Watson et al. 1994). If populations are declining or only stable due to individuals with long lives, it is recommended to look at underpinning mechanisms in life histories that may be contributing to this status (Schemske et al. 1994). Each stage of life history acts as a series of filters, in which only some individuals pass, so evaluating germination, recruitment, adult survival, pollination, and seed set is essential for at-risk species (Dixson and Cook 1989). Given their importance, discussion of these life history stage filters is included within the following chapters. I have made a conscious attempt to assemble a comprehensive collection of our current knowledge on *S. montana*, sourcing as much literature from previous observations and research as possible. Additionally, this thesis examines aspects of the conservation of *S. montana* through studying the abundance trends at a single site and transplantation of the species at two separate sites.

In the following chapter, I report on the findings of two years of abundance monitoring of *S. montana* at a military site in Catoosa County, Georgia. Starting in 2009, Drs. Shaw and Boyd (University of Tennessee at Chattanooga) were contracted to continue conducting these surveys using established protocols for documenting the number of plants, stage classes, and morphological characteristics within previously established long-term monitoring plots. Habitat species richness was recorded as well. These data and historical census data were compiled and reported yearly. Our objectives were to describe the general abundance trends of *S. montana* within the monitoring plots at this site and to evaluate any negative impacts on these plants.

A study conducted in Hamilton County, Tennessee, on the transplantation of 49 *S. montana* into a protected area is reported in the third chapter, and another transplantation study of 100 *S. montana* at the military site in Catoosa County, Georgia, is presented in the fourth chapter. While preserving natural habitat is an obvious priority, habitat destruction is sometimes an unavoidable impact of anthropogenic activities. Studying transplantation of *S. montana* can help conservation efforts when plant rescue is necessary. Morphological and developmental characteristics were used to evaluate plant responses to transplantation. The objectives of these studies, specifically, were to minimize the impacts of land-clearing disturbances on *S. montana* occurring in condemned habitat through the action of transplantation and to quantify the transplantation success of *S. montana* at both sites. An additional objective in the fourth chapter's transplantation study was to quantify the effects of pre-transplantation canopy thinning and prescribed burning on *S. montana* transplants at the military site. The purpose of these treatments was to improve our understanding of *S. montana* responses to light availability and ground disturbance from fire with the intention of making recommendations concerning macro-site selection for any future transplantation.

In the final chapter, I assess the combined results from the studies presented in this thesis in terms of their contribution to the species recovery plan. Future research directions needed to fully assess the conservation status of *S. montana* are presented as well. In conclusion, I reiterate the salient points of this thesis and future research needed to be performed before the species should be considered for delisting.

CHAPTER 2

SHORT-TERM TRENDS IN ABUNDANCE OF *SCUTELLARIA MONTANA* CHAPM.

(LAMIACEAE, LARGE-FLOWERED SKULLCAP) AT A MILITARY SITE IN

CATOOSA COUNTY, GEORGIA

Introduction

Scutellaria montana Chapm. (Lamiaceae, large-flowered skullcap) is an herbaceous perennial species that is endemic to the Ridge and Valley and Cumberland Plateau physiographic provinces in Tennessee and Georgia. *S. montana* was first listed in 1986 as federally endangered under the Endangered Species Act (USFWS 1986) and later reclassified as federally threatened

7

with 48 known populations in 11 counties (USFWS 2002). Since that time, *S. montana* has been found in nine counties in Georgia and four counties in Tennessee (USFWS 2011) and suspected to occur in Alabama (USFWS 1996, 2002). A five-year review required by the Endangered Species Act to evaluate the appropriateness of the species' status was initiated (USFWS 2007), but has not been finalized at the time of this writing (personal communication from G. Call, USFWS, 19 May 2011, unreferenced). For the species to be delisted, the recovery plan stated that 15 managed and protected *S. montana* populations distributed throughout its range must be maintained and sustainable for ten years (USFWS 1996). A viable population is generically defined as one having more than 100 individuals separated by a distance of at least 0.8 km from another occurrence (USFWS 2002). Additionally, this species is afforded state protection in both Georgia and Tennessee (GDNR 2008; TDEC 2008).

 Scutellaria montana occurs in low densities with a clumped spatial distributional pattern (Cruzan 2001). Its associated habitat has been described as mid-to-late successional forest with a relatively open and predominately oak-hickory or mixed-oak canopy that has a deciduous shrub layer with an evergreen *Vaccinium* spp. component and a moderately dense herbaceous layer with vine and grass components occurring in shallow, rocky soils (USFWS 2002; Mulhouse et al. 2008). Since a population of *S. montana* was known to occur in a nearby county park (Elsie A. Holmes Nature Park), the United States Fish and Wildlife Service (USFWS) requested that the Tennessee Army National Guard search for the species at its 659-ha Volunteer Training Site (VTS), located 6.4 km east of Ringgold, Georgia, in Catoosa County (SAIC 2002; Figure 2.1). This initial coarse scale site survey in 2002 yielded a finding of more than 1,500 plants in 60 occurrences that were subsequently grouped into 26 management areas for the purpose of minimizing human disturbance by prohibiting vehicular traffic and limiting foot traffic during

periods of flowering and fruiting (SAIC 2002, 2006). Army regulations require that military installations with significant natural resources draft resource management plans that consider endangered species, written in conjunction with the USFWS and state wildlife agencies, to ensure sustainable use of military lands while maintaining the installations' missions (Melton et al. 2004; Army 2007). The species recovery plan for *S. montana* also recommends that new populations be annually monitored for at least five years (USFWS 1996). Given these directives, 46 fixed monitoring plots were selected non-randomly in high density occurrences of *S. montana* within each of the 26 management groups across the spread of the facility in 2004 for annual surveys conducted by formerly contracted investigators to monitor abundance trends and increase awareness of any negative impacts on the species (SAIC 2006).

Population monitoring is a key activity valued in documenting species' status and identifying threats to species. Typically, such monitoring involves collecting count data and analyzing these for abundance trends. Increasingly, monitoring also is being used to evaluate species' management approaches and learn about species' life histories (Philippi et al. 2001; Marsh and Trenham 2008). Within the VTS, monitoring involved collecting abundance data, allowing for the evaluation of temporal trends and providing some insight into potential demographic mechanisms (Menges and Gordon 1996). Starting in 2009, we began conducting the annual monitoring of *S. montana* at the VTS, using established protocols of previous workers with some additional quantitative variables measured. The primary objective of this study was to evaluate the status of sampled *S. montana* in the existing monitoring plots at the VTS. Our 2009 and 2010 reports add to the growing body of information about the abundance of *S. montana* at this location and assist with the evaluation of site impacts on *S. montana* from a natural resources management perspective.
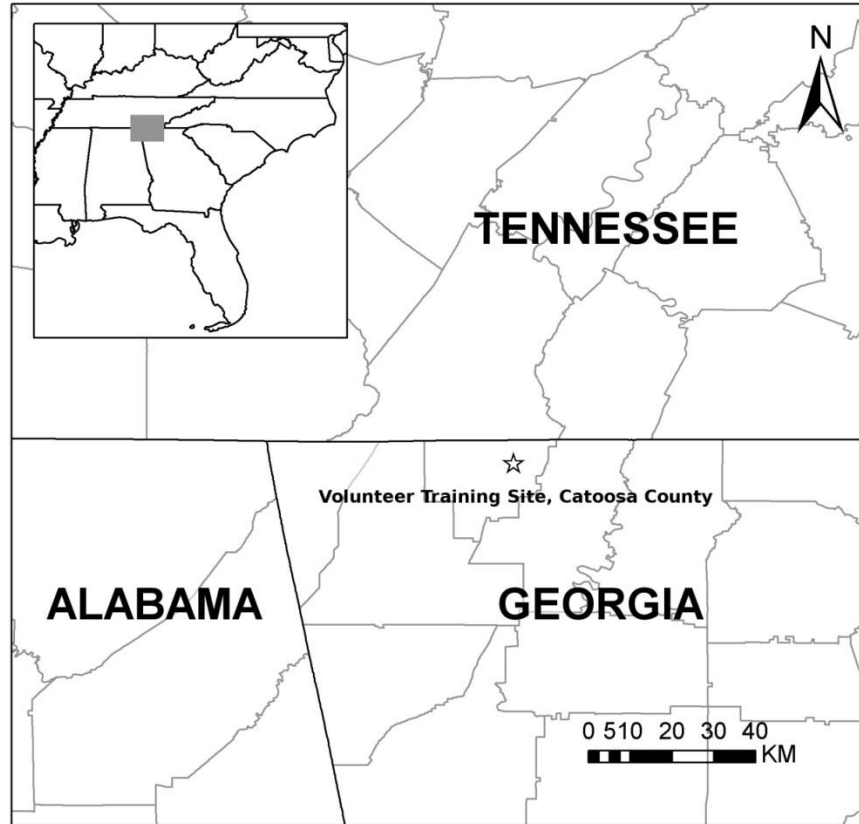
Figure 2.1   Location of a *Scutellaria montana* population at the Tennessee Army National
Guard Volunteer Training Site in Catoosa County, Georgia.

Material and Methods

The 46 established plots, which had been previously selected non-randomly within the 26

management areas of known *S. montana* habitat, were surveyed during the blooming period on

ten days between 25 May and 16 June 2009 and five days between 10 May 2010 and 14 May

2010. Since one plot was removed in 2010 due to the plants having been transplanted to another

part of the facility earlier that year, the results are reported for the remaining 45, 20-m-diameter

plots for 2010 and the comparison between 2009 and 2010. At least four surveyors examined

each plot, walking side-by-side to flag all individuals of *S. montana*. The 2004 monitoring

protocol approved by the USFWS and the Georgia Department of Natural Resources was

followed (SAIC 2006), and observations were recorded for the identification of plant species within the plots, morphological growth and development characteristics, and any damage. To track growth, we counted the number of stems (one plant was defined as stems within 5 to 7.5 cm of each other) and the number of leaves, and measured the height of the tallest stem of each individual plant. Developmental stage classes were defined as flowering adults, which were noted with the presence of any fruit or flowers; vegetative adults, which were plants taller than 10 cm and not flowering or shorter than 10 cm with obviously missing aboveground biomass; juveniles, which were intact plants less than 10-cm tall and not flowering; and seedlings, which were plants less than 3-cm tall consisting of only two leaves. Stem damage was noted if stems were found broken or missing apical biomass, such as from vertebrate grazing. Leaf damage was noted if leaves were found with irregular patterns of missing biomass from leaf tissue or with fungal colonization or blighting.

Using IBM SPSS Statistics 19 (SPSS, Inc., Somers, New York, 2010; unreferenced), the annual overall means and ranges for quantitative data and the proportional composition for qualitative data were analyzed. A paired-samples t-test was used to compare variables from 2009 and 2010. Historical data from the permanent plots were consistently recorded for total plant counts and number of flowering adult stage plants from 2004 to 2008 (SAIC 2006; SpecPro 2008; Holt and Stanborn 2009). Since these data did not meet the sphericity assumption, the historical data were compared to our data using a Greenhouse-Geisser-corrected, one-way, repeated-measures analysis of variance and, where significant ($P \leq 0.05$), a Bonferroni-corrected, paired-samples t-test was used (Field 1998, 2009).

Results and Discussion

Plot Habitat

Shaw et al. (2010) found a total of 147 associated species and lesser taxa distributed in 112 genera and 66 families in the 46 plots surveyed in 2009. Asteraceae was the largest family represented with nine species and lesser taxa in nine genera followed by Rosaceae with eight species from six genera, and two rare species, *Castanea dentata* (Marsh.) Borkh. (American chestnut) and *Panax quinquefolius* L. (American ginseng), were observed in a few plots. They found seven introduced taxa in multiple plots, making up 5% of the richness of associate species with *Lonicera japonica* Thunb. (Japanese honeysuckle) as the most frequently found introduced species in 43% of the plots. The species most commonly found by percentage of total plots ($n = 46$) were *Carya* Nutt. species (hickories), *Vitis rotundifolia* Michx. (muscadine)*,* and *Hexastylis arifolia* (Michx.) Small (little brown jug) at 89%, *Cornus florida* L. (flowering dogwood) at 87%, *Toxicodendron radicans* (L.) Kuntze (poison ivy) and *Quercus alba* L. (white oak) at 83%, and *Nyssa sylvatica* Marsh. (blackgum) at 80%. Additionally, 24% of the species (35 species) observed were located in only one plot, and 15% (21 species) occurred in 50% of the plots or more.

While vegetation cover estimates were not performed, these findings agreed with the common assertion that *S. montana* often occurs in hickory-oak or mixed-oak forests (Cruzan 2001; Mulhouse et al. 2008, USFWS 2002), but dominant midstory and understory vegetation seemed to vary from site to site. The frequency of *V. rotundifolia* and *T. radicans* suggested an agreement with previous findings that *S. montana* habitat has a vine component (Mulhouse et al. 2008). It is unknown if this vine component relationship is competitive, facilitative, or neither. While competition for light availability has been speculated as a threat to *S. montana*, especially

from invasive species such as the shrub *Ligustrum japonicum* Thunb. (Japanese privet) and the vine *L. japonica* (Nix et al. 1993; USFWS 2002), one study found that increasing herbaceous vegetation cover within a 0.5-m radius of sampled *S. montana* had strong positive relationships with both leaf number and flower number and that no relationship existed between percent canopy openness and number of leaves (Hopkins 1999). This suggests a mechanism in *S. montana* for dealing with interspecific shading is to produce more leaves, which may increase its reception of external signals that promote floral induction (Taiz and Zeiger 2006). Hopkins (1999) further suggested that interspecific competition may have a positive effect on fitness by increasing flowering, at least in the short term. Perhaps this slight advantage may only be seen in non-drought years when soil water content and nutrients are not limiting to growth.

2009 Inventory

In the 2009 survey of *S. montana* in 46 monitoring plots, we found 1,282 total individuals with 12.0% flowering adults, 40.7% vegetative adults, 36.6% juveniles, and 10.8% seedlings. Mean density of sampled plots occurred at 0.089 plant/m^2, and, overall, the plots had ranges of 4 to 93 total plants. Across all plots, plants exhibited ranges of 1 to 7 stems per plant, 1 to 46 leaves per plant, and 2-cm to 53-cm height per plant. Of all sampled plants, 7% had evidence of stem damage, suggesting vertebrate herbivory, and 50% showed signs of leaf damage, suggesting arthropod or fungal predation.

2010 Inventory

In the 2010 survey of *S. montana* in 45 monitoring plots, we found 1,346 total individuals with 28% flowering adults, 40% vegetative adults, 30% juveniles, and 2% seedlings. Mean density of sampled plots occurred at 0.095 plant/m^2, and, overall, the plots had ranges of 1 to 150

total plants. Across all plots, plants exhibited ranges of 1 to 6 stems per plant, 1 to 61 leaves per plant, and 3-cm to 88-cm height per plant. Of all plants, 8% had evidence of stem damage and 53% showed signs of leaf damage.

Comparison of 2009 and 2010

No statistical differences were found between 2009 and 2010 for some variables (Table 2.1), including the means per plot for total plants ($P = 0.341$; $t_{44} = -0.963$), vegetative adult stage plants ($P = 0.581$; $t_{44} = -0.556$), juvenile stage plants ($P = 0.232$; $t_{44} = 1.213$), stem number ($P = 0.096$; $t_{44} = -1.701$), and stem or leaf damage (all $P > 0.262$). However, other variables showed significant differences between the 2009 and 2010 censuses (Table 2.1). The mean per plot for seedlings ($P < 0.001$; $t_{44} = 4.916$) were significantly reduced by 77% in 2010, while significant increases in 2010 were evidenced in the means per plot by 15% for stem height ($P = 0.016$; $t_{44} = -2.499$), by 29% for leaf number ($P < 0.001$; $t_{44} = -4.565$), and by 167% for flowering adults ($P < 0.001$; $t_{44} = -4.037$).

While the total number of plants was similar between 2009 and 2010, the composition of stage classes did change for flowering adults and seedlings that suggested maturation of sampled plants. Though without following tagged individuals over several years, it is unknown whether these are the same individuals developing into the more mature stage classes. The total number of adult plants had strong positive relationships with the variables quantifying growth, including number of leaves ($n = 45$; $P < 0.001$; $r^2 = 0.96$) and stem height ($n = 45$; $P < 0.001$; $r^2 = 0.80$), possibly explaining these increases. A drought period extended through 2007 and 2008 (NDMC [date unknown]), so a dormant seed bank may have collected during this time that led to an increase in seedlings when the first non-drought season arrived in 2009, presenting optimal conditions for seed germination. While it is unknown how long *S. montana* seeds may remain

14

viable in the seed bank, it is thought that Lamiaceae, including *S. montana*, seeds have shallow physiological dormancy that may require stratification (Cruzan 2001; Finch-Savage and Leubner-Metzger 2006). The increased number of flowering adults in 2010 could have been a timing delay effect resulting from physiological stress during the extended drought (Inghe & Tamm 1988), and while seedlings decreased in 2010, the increase in flowering plants could have a positive influence on seedling recruitment in the near future. It has been suggested that seedlings may not flower for the first few years (Cruzan 2001), so the maturation of the assumed recruits seen in this population may increase flowering in the coming years.

Table 2.1   Mean ($\pm$ 1 SE) values of variables of *Scutellaria montana* from 45 plots in 2009 and 2010 at the Tennessee Army National Guard Volunteer Training Site, Catoosa County, Georgia. The asterisk (*) denotes that the variables were significantly different (all $P < 0.016$).

	Year	
Variable	2009	2010
Total plants	28 $\pm$ 3.3	30 $\pm$ 4.5
Flowering adults *	3 $\pm$ 0.5	8 $\pm$ 1.3
Vegetative adults	11 $\pm$ 1.5	12 $\pm$ 2
Juveniles	10 $\pm$ 1.4	9 $\pm$ 1.5
Seedlings *	3 $\pm$ 0.6	0.7 $\pm$ 0.2
Stem height (cm) *	13 $\pm$ 0.5	15 $\pm$ 0.9
Number of stems	1 $\pm$ 0.04	1 $\pm$ 0.06
Number of leaves *	7 $\pm$ 0.4	9 $\pm$ 0.5
Stem damage	2 $\pm$ 0.5	2 $\pm$ 0.7
Leaf damage	14 $\pm$ 1.8	16 $\pm$ 2.2

Our 2009 and 2010 results had similar growth patterns to the *S. montana* population

Hopkins (1999) studied over a three-year time period in the Tennessee River Gorge. She

classified plants into two groups, inflorescence oscillators and non-oscillators. The majority of

her sampled plants were non-oscillators with declining size and flowering. This was attributed to

either chronic resource deficiency or genotypic variation, in which these plants could not achieve

the oscillating flowering pattern. Plants with inflorescence oscillation were apparently able to

sequester resources and alternate between having larger and smaller sizes and inflorescences.

Further, Hopkins (1999) found that plants were pollination limited, and that successful fruit set,

which was generally low, had a strong positive correlation with number of flowers. Therefore, it

was proposed that inflorescence oscillation was a fitness strategy in which larger inflorescences

would be produced in an effort to attract greater numbers of pollinators, resulting in an increased

fruit set.

It is unknown whether *S. montana* has sensitivity to leaf damage, such as from

invertebrate predation, early in the growing season that could contribute to low flowering

numbers (Inghe & Tamm 1988). In general, plants are thought to grow and reproduce better in

the absence of herbivory (Hawkes & Sullivan 2001). While impacts and perpetrators of stem

damage on *S. montana* are unknown at the VTS, herbivory could potentially have negative

consequences for *S. montana* growth, reproduction, and survivability (Rooney & Waller 2001).

Recently, research has begun at the VTS to help elucidate impacts of vertebrate herbivores on

S. montana there.

Historical Data Comparison

There were significant differences in the means of total plants per plot since 2004 ($P <$

0.001; $F_{2.513} = 37.862$; Figure 2.2). The 2006 sampling year was significantly greater than all

16

other years, and 2007 and 2008 were each significantly lower than all other years. Though the number of total plants oscillated over the seven years of sampling (Figure 2.2), the last two years (2009, 2010) were similar to the first two sample years (2004, 2005), suggesting an apparent overall stability in the monitoring plots of *S. montana* at the VTS.

Previous researchers suggested that the significant decline in the population in 2007 and 2008 was the result of drought conditions in the area during that time (SpecPro 2008; Holt and Stanborn 2009). From January to August of 2007, Georgia experienced its second driest period on record in 100 years (Fuchs 2008). While abnormally dry periods in Catoosa County, Georgia, have been common since 2004, actual drought conditions began in February 2007, starting with moderate status (Category D1), and escalated into an exceptional drought status (Category D4) in August 2007 (NDMC [date unknown]). Category D4 is the highest intensity of drought and is considered to have a 2% chance of occurrence in any given year (NDMC 2005). Category D4 conditions lasted in Catoosa County until March 2008, but the county did not entirely exit lower levels of drought conditions until January 2009. Abnormally dry conditions eased in April 2009, and normal conditions persisted from that point through our monitoring and data collection in May 2010 (NDMC [date unknown]).

While it is plausible that drought conditions increased the decline seen in the historical data set, a high sampling error also may have contributed to the 262% increase in total plant number between 2008 and 2009, especially when 676 fully developed adult plants were counted in 2009 compared to a total of 354 plants in 2008 (Shaw et al. 2010). The growth of *S. montana* individuals has not been well studied, and its response to stressful conditions, such as drought, is largely unknown. Prolonged dormancy by not emerging from belowground in the spring thereby preventing water loss through the absence of aerial tissues throughout drought years is another

possibility; however, this is unlikely as this stage is generally noted in geophytic species, especially in Orchidaceae (see Lesica and Steele 1994; Waite and Farrell 1998; Shefferson et al. 2001; Volaire 2002; Gremer 2010).
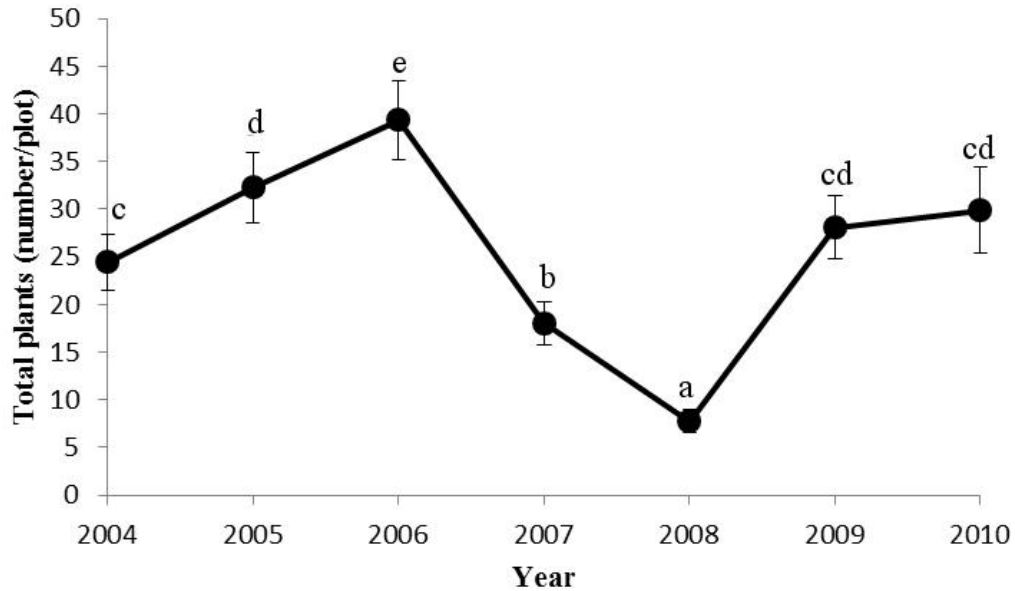
Figure 2.2   Mean (± 1 SE) total number of *Scutellaria montana* per monitoring plot from 2004-2010 at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia. Means shown below the same letters are not significantly different at the P ≤ 0.05 level.

The means of number of flowering adult stage plants since 2004 were significantly different ($P < 0.001$; $F_{2.757} = 20.101$; Figure 2.3). The 2006 number of flowering plants was significantly greater than all other years, while counts in 2004, 2005, and 2007 were all similar. Additionally, flowering plant number in 2007 was also similar to 2008 and 2010, and 2008 was similar to the lowest mean in 2009. One apparent factor that may have contributed to the significantly greater number of flowering plants in 2006 was that in nearby Chattanooga, Tennessee, the average monthly temperature (18º C) and total precipitation (15 cm) in April 2006 was elevated compared to other census years (Weather Underground 2011). These

favorable conditions in 2006 may have accelerated adult phase inductions and increased floral

evocation in plants (Taiz and Zeiger 2006). Drought may have induced a prolonged

physiological stress on plants preventing floral stimulus, and only showing some recovery of this

trend in 2010.
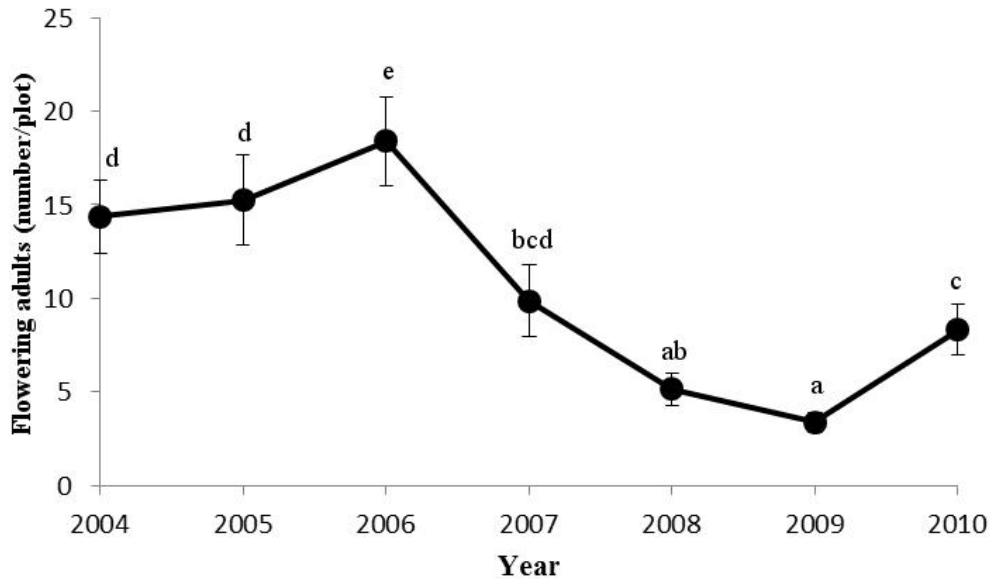
Figure 2.3   Mean (± 1 SE) flowering adults of *Scutellaria montana* per monitoring plot from
2004-2010 at the Tennessee Army National Guard Volunteer Training Site in
Catoosa County, Georgia. Means shown below the same letters are not
significantly different at the $P \leq 0.05$ level.

Since the *S. montana* abundance in the monitoring plots at the VTS is apparently stable

overall, we see no reason to recommend management changes at this time. However, we found

some concerning patterns, including the drastic reduction of total plants between 2007 and 2008

and the reduced flowering, in the temporal trends at the VTS that may be related to annual

meteorological events, so annual monitoring should continue. Clearly, the life history strategies

of *S. montana* are perplexing and have been of interest to researchers given their implications to

fitness and long-term population viability of this threatened species. However, the type of

19

demographic data collection needed to elucidate answers to these questions by annually

following marked individuals (Menges and Gordon 1996) has apparently not been investigated.

The variables we added to the data set in field collection provided additional information, but

tracking at least a small random, sample of individuals over several years would produce a

clearer picture of the life history and population dynamics of *S. montana*, especially that of the

life stage filters of survivability, reproduction, and recruitment (Dixson and Cook 1989).

Considering the relatively low flowering capacity and recruitment of *S. montana* in the

monitoring plots at this study site, this type of research could help determine if the stability in the

abundance trend in our findings was due to steady replacement recruiting or merely to a

suspected long-lived nature of individuals in *S. montana* (personal communication from J.

Brown, Tennessee River Gorge Land Trust, 2 June 2011, unreferenced). In the latter case, a

decline in abundance may be inevitable without management intervention as older individuals

complete their lives (Schemske et al. 1994).

Another area of which we lack understanding is the poor fruit and seed set of *Scutellaria*

species (Collins 1976) and *S. montana* in particular (Kemp 1987; Nix et al. 1993; Hopkins 1999;

Cruzan 2001; USFWS 2002). Although poor pollination of gynoecia has been cited as a possible

cause (Nix et al. 1993; Hopkins 1999; Cruzan 2001), even pollinated pistils of other *Scutellaria*

species had poor fruit set (Collins 1976), and Paton (1990) mentioned that sterile pollen was

sometimes found in *Scutellaria* species. Considering the impact of poor seed set on recruitment

potential, this is an area that could use more research. Additionally, relatively low recruitment

should instigate study into factors that facilitate and hinder seedling survival in *S. montana*. To

ensure the full recovery of *S. montana* and make informed decisions on the legal status of this

species in the future, it is important that we understand if the apparent stability of this threatened species is due to adequate recruitment rather than to long-lived individuals with poor recruitment within the populations.

CHAPTER 3

RELOCATION SUCCESS OF FEDERALLY THREATENED *SCUTELLARIA MONTANA*

(LAMIACEAE, LARGE-FLOWERED SKULLCAP) FROM A PROPOSED

HIGHWAY CORRIDOR

Introduction

  Scutellaria montana Chapm. (large-flowered skullcap) is an herbaceous perennial endemic to nine counties in northern Georgia and four in southeastern Tennessee (USFWS 2011). It is found in the Ridge and Valley physiographic province and the eastern escarpment of the Cumberland Plateau. First described by Chapman (1878) from a location that

was likely in Floyd County, Georgia, *S. montana* was first listed in 1986 as federally endangered

under the Endangered Species Act (USFWS 1986), but it was reclassified as federally threatened

in 2002 (USFWS 2002). At the time of reclassification, 48 populations of *S. montana* were

documented with a viable population defined as having over 100 individuals and separated by a

distance of 0.8 km from another occurrence (USFWS 2002). Additionally, this species is

considered endangered at the state level by both Georgia (GDNR 2008) and Tennessee (TDEC

2008). As suggested by its protection statuses, *S. montana* is usually found at low density, rarely

exceeding more than a few plants per square meter (Cruzan 2001). Generally, *S montana* occurs

in mid- to late-successional forests, often positioned near streams. Forests containing this species

are primarily mixed-oak or oak-hickory dominated with a strong understory grass component

and some occurrences of native pine and blueberry (Mulhouse et al. 2008). Soils are generally

shallow, loose, rocky, well-drained, and slightly acidic (Bridges 1984, as cited in USFWS 2002;

personal observation).

The Enterprise South Industrial Park (ESIP) houses several populations of this rare

species in Chattanooga, Tennessee (Figure 3.1). Specifically, more than 200 *S. montana*

individuals occur in seven defined occurrences across the site (personal communication from D.

Estes, Austin Peay State University, 2008, unreferenced; personal communication from L.

Green, Hamilton County Parks and Recreation, unreferenced). Four of these occurrences were

located in close proximity to an approximately 250-m-wide corridor designated to become a new

four-lane divided highway and its associated right-of-way as part of the Volunteer Ordnance

Connector project described to us by the Tennessee Department of Transportation. Each of these

groups was estimated initially to contain two to three individuals (Estes 2008) for a total of

approximately 11 plants. Two other occurrences of *S. montana* in the industrial park are located

in areas that were scheduled to be fenced during summer 2009 with deed restrictions having no encumbrance established for the two fenced areas. One of these occurrences has been estimated to contain more than 140 individuals spread over approximately 1.2 ha, while the other occurrence has been estimated to contain more than 50 individuals spread over approximately 0.08 ha. The remaining occurrence of *S. montana* is located in the adjacent protected Enterprise South Nature Park and has been estimated to contain about eight individuals spread over approximately 1.4 ha. Prior to our transplantation activities, this group contained several individuals caged for protection from large herbivores. For additional protection, a fence was scheduled to be built around this area during summer 2009.
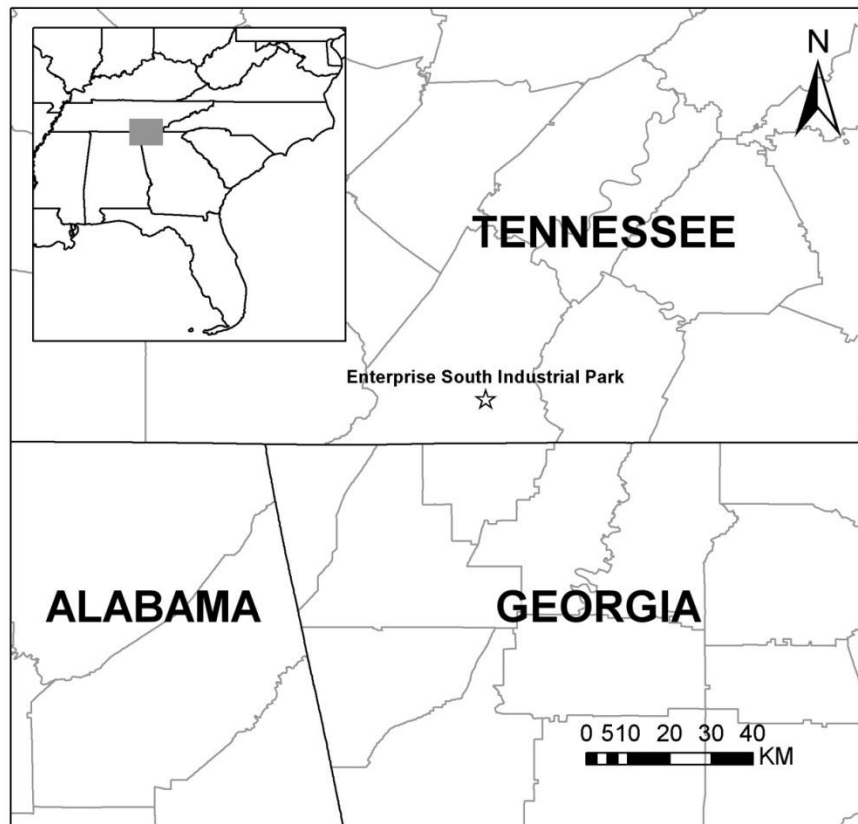
Figure 3.1   Location of *Scutellaria montana* at the Enterprise South Industrial Park in Chattanooga, Tennessee.

To minimize the effects of the proposed Volunteer Ordnance Connector project on *S. montana* populations growing in the ESIP and to promote their recovery from any associated negative impacts, the United States Fish and Wildlife Service recommended that all individuals located within occurrences in close proximity to the planned Volunteer Ordinance Connector project be transplanted among nearby, protected occurring populations prior to the onset of road construction activities and subsequently monitored.

Material and Methods

We surveyed four occurrence groups of *S. montana* directly threatened by planned construction activities at the ESIP in early June 2009, near the end of the skullcap flowering season, with assistance provided by students trained in *S. montana* identification. As a group, we walked north-south transects (with spacing ≤ 2 m between each person) through the entire area of these occurrences. All observed *S. montana* individuals in these occurrences were counted and evaluated for basic morphological characteristics and health. Because *S. montana* is cespitose, all stems within 10 cm of each other were considered to be parts of an individual plant. We measured the height, and number of stems, leaves, and flowers for each individual found to provide baseline data on the overall morphology of these individuals. Adult plants were defined as those individuals greater than 10-cm tall, while seedlings were defined as those less than 3-cm tall; juvenile individuals were considered those of intermediate height between these measures. Damage by herbivores and/or fungal disease also was noted when present to evaluate the general health of each individual found. These measurements were made to allow us to reassess the short- and long-term general health of relocated individuals pre- and post-transplantation.

The day after our evaluations of plant morphology and health were made, we removed all *S. montana* individuals from the four occurrences surveyed for transplantation into the location

of the protected *S. montana* occurrence located in the nearby Enterprise South Nature Park. We chose this site for transplantation because it is located in close spatial proximity to the threatened occurrences, which we assumed would help to minimize differences in soil type, resource availability, and microclimate, and it was planned to be protected by the construction of a new fence during summer 2009. Given the shallow soils characteristic of the site and to minimize root disturbance, an approximately 10-cm-diameter, 15-cm-deep cylinder of soil was carefully dug around each *S. montana* individual. Each individual (and its surrounding soil) was transferred quickly into a large nursery container for transport by air-conditioned vehicle to the relocation site during the same day. There, four linear transects were established along east-west gradients at least 1-m apart. Each transect included both shaded areas beneath the overstory canopy and fully sunlit areas in a clearing to encompass a full gradient of light availability at the site and to allow for potential future evaluation of the effects of light availability on *S. montana*, which has been described as potentially important to its growth and survival (Nix et al. 1993). Holes were dug approximately 1-m apart along each linear transect to accommodate the transplants and their soil. Transplants were placed randomly in these holes, covered with soil and then leaf litter, and immediately and thoroughly watered. To help to minimize transplant shock, we watered all transplanted individuals every day for three days immediately following transplantation and then once weekly through August 2009 during weeks in which it did not rain.

One month after the threatened *S. montana* individuals were moved to the their new location, an approximately 1.25-m-tall chain-link fence was constructed around a 15.25-m^2 area that included all of the transplants. This fence was intended to deter people from the site, since it is visible from a nearby road and also located within close proximity of both hiking and biking trails. Subsequent monitoring of the transplanted individuals was conducted approximately three

weeks (early July 2009) and one year (late-May 2010) post-transplantation using methods identical to those used to assess their general morphology prior to their relocation to this area. A paired t-test was used to compare the mean height, and numbers of stems, leaves and flowers of *S. montana* individuals in 2009 and 2010 (SPSS for Windows, Rel. 7.5.1, 1996, SPSS, Inc., Chicago, Illinois, USA, unreferenced).

Results and Discussion

Pre-transplantation Inventory

In total, 49 *S. montana* individuals were identified within the four occurrences in close proximately to the Volunteer Ordnance Connector project during June 2009. This number was far greater than the 11 plants that we anticipated finding based on previous reports (Estes 2008; personal communication with L. Norris, City of Chattanooga Division of Public Works, unreferenced). We suggest that this discrepancy stemmed from the different methods used during the 2008 and 2009 surveys. The 2008 survey involved one researcher using flowers to 'spot' plants from a distance (personal communication with D. Estes, Austin Peay State University, unreferenced). In contrast, the 2009 group observed nearly every square meter of ground in the study area and found many non-flowering individuals.

Of the 49 individuals that we identified, about 43% were adults, 55% were juvenile, and one individual was a seedling suggesting that there was a seed bank in this area. The majority of the individuals (about 75%) were single stemmed, while eight individuals were double stemmed, three were triple stemmed, and one plant was quadruple stemmed. Individual plant heights ranged from 3 cm to 38 cm, with an average plant height of 11.6 cm. At the time of our evaluation, only four plants were flowering or fruiting. Grazing by large herbivores (e.g., rabbit,

deer) was apparent on approximately 29% of plant individuals. About 49% of transplanted individuals had evidence of insect herbivory or fungal damage.

Preliminary Post-transplantation Evaluation

Of the 49 *S. montana* individuals that had been relocated, one was missing, five were desiccated, and five showed indications of experiencing water stress but did not appear to be past the permanent wilting point. The remaining 38 plants appeared to be in excellent health at approximately three weeks after transplantation.

Post-transplantation Inventory

Overall, we consider the transplant work to have been an overwhelming success. Approximately, one year after the 49 *S. montana* individuals were relocated, only one plant had died. This individual had exhibited water stress beginning shortly after transplantation which persisted throughout the remaining of the 2009 growing season. However, the other nine plants that also showed signs of water stress during the 2009 growing season appeared healthy in 2010. In addition to the extremely low amount of mortality observed from 2009 to 2010, there was a very noticeable and quantifiable increase in general plant size and health (Table 3.1). Specifically, the mean plant height increased about 190% ($P < 0.001$; $t_{47} = -13.219$), the number of stems per plant increased about 45% ($P = 0.001$; $t_{47} = -3.493$), and the average number of leaves per plant increased about 130% ($P < 0.001$; $t_{47} = -7.103$). Flowering also increased dramatically from 2009 to 2010. Prior to transplantation, only four plants were flowering—one plant had 30 flowers, and the other plants had three, two, and one flowers, respectively. However, in 2010, approximately 90% of the transplanted individuals were flowering, producing 713 flowers total. Most notably, the mean number of flowers per individual was 21-times greater

in 2010 than in 2009 ($P < 0.001$; $t_{47} = -6.779$; Table 3.1). Like most species in the mint family (Lamiaceae), *S. montana* typically produces four seeds per fruit. As a result, the total seed production of the 49 transplanted individuals could have increased from approximately 144 seeds in 2009 to 2852 seeds in 2010. However, it should be recognized that such numbers are dependent on assumptions that no seeds are lost due to growth disruption or predation. Additionally, the viability of seeds of *S. montana* is unknown and probably low given its small total population size.

Table 3.1   Mean ± 1 SE values of morphological variables of *Scutellaria montana* individuals pre- (spring 2009) and post- (spring 2010) transplantation in the Enterprise South Industrial Park, Chattanooga, Tennessee.

	Year	
Variable	2009	2010
Stem height (cm)	11.7 ± 0.9	33.9 ± 1.8
Number of stems	1.4 ± 0.1	2.0 ± 0.2
Number of leaves	8.0 ± 0.6	18.4 ± 1.7
Number of flowers	0.7 ± 0.6	14.9 ± 2.6

Although our post-transplantation evaluation evidenced that our efforts were highly successful, we suggest that the increase in overall development and health of these plants was less likely due to their being transplanted and more likely due to their protection from large herbivores by a chain-link exclosure. It was very evident to us upon making our 2010 post-transplantation evaluations that the chain-link fence constructed around the relocated individuals was preventing or dramatically deterring grazing by large herbivores on both *S. montana* and associate species at the site such as *Toxicodendron radicans* (poison ivy) and *Parthenocissus*

virginicus (Virginia creeper) given their observably greater percent ground cover and overall height. In 2009, about 29% of transplanted *S. montana* individuals were noticeably grazed at the time of transplantation, as indicated by their stems being severed. The year following fencing of the transplants, only one plant exhibited evidence of being grazed. This is an important factor to consider in evaluating and determining the cause of the improved health of the transplanted individuals because grazing typically removes flowers and could cause enough stress via leaf loss in individual plants that they may not flower again in that same year or perhaps the next year. In contrast to herbivory by large mammals, the relative amount of insect/fungal damage was comparable for the transplants in 2009 and 2010 (about 49% of plants in 2009 and 62% of plants in 2010).

Future Directions

Investigating the success of transplantation of *S. montana* along various biotic and abiotic gradients could provide information useful for selecting sites that could maximize the success of its transplantation in future instances in which transplantation is recommended to minimize the negative impacts of human activities. For example, high densities of deer can impact the survival, growth and reproduction of spring-flowering herbaceous plants (Frankland and Nelson 2003). Although deer herbivory had been observed at several sites where *S. montana* occurs (USFWS 2002), to date, the susceptibility of *S. montana* to herbivory by deer or other large animals has not been quantified or studied scientifically. Depending on its extent and selectivity, deer grazing in the relocation site could impact the success of transplantation of *S. montana* individuals and the health of their new population through the potential conflicting impacts of direct biomass removal or the removal of biomass from competing plant species. While we have observed and reported anecdotally the positive impacts of protecting transplants from large

herbivores in this report, potential future investigations that involve quantitative comparisons intact *S. montana* individuals and/or transplants in areas that are open versus exclosed could help to better elucidate the impacts of large herbivore grazing on its success in a more quantifiable way.

The presence and extent of invasive plants species within *S. montana* relocation sites also could impact the success of its transplantation, due to competition for shared resources or apparent competition via the increased attraction of shared herbivores. The findings of previous research have suggested that *S. montana* does not compete well with other herbaceous species within the deciduous forest understory communities with which it is associated (Bridges 1984, as cited in USFWS 2002), yet it is unknown if such competitive effects would change if that community were to contain more or less invasive species. Future comparisons of *S. montana* individuals transplanted into areas with and without associated invasive species could help to investigate their potential influence on transplantation success.

In addition, investigating the success of *S. montana* transplantation along natural light gradients could provide information useful to selecting sites that could maximize its success. During our post-transplantation evaluation at the ESIP, we noted that *S. montana* individuals located toward the center of the fenced area of their relocation, where light availability was observably greatest due to a significant canopy gap, seemed generally larger and more advanced in their development than individuals located toward the periphery of the site, which was closer to tree cover. We also noted in the field, the possibility that *S. montana* grows in environments with substantial light availability provided by canopy gaps during our annual monitoring of *S. montana* occurrences at the Tennessee Army National Guard Volunteer Training Site in nearby Catoosa County, Georgia. Currently, we are conducting a transplantation study there that

includes evaluating the influence of light availability on transplantation success, which we hope will better elucidate such effects in a quantitative way.

Ultimately, the successful relocation of *S. montana* individuals threatened by human activities will be influenced by both the establishment of sound transplantation techniques and the selection of sites for relocation that will maximize the success of transplanted individuals and support population growth. Collectively, the activities we have completed to date along with these future research directions could help to maximize the effectiveness of management strategies for dealing with the protection of this threatened species in locations where anthropogenic disturbance is planned.

CHAPTER 4

RESPONSES OF TRANSPLANTED *SCUTELLARIA MONTANA* CHAPM. (LAMIACEAE,

LARGE-FLOWERED SKULLCAP) TO CANOPY THINNING AND BURNING

Introduction

Moving plants from one location to another is a tool that can be used to rescue plants from areas with planned habitat modification or destruction (Fahselt 2007). Issues that could impede successful transplantation have been well documented and include transplantation site selection, poorly understood life histories, disturbance and stress to plants, stochastic loss of genetic diversity leading to inbreeding depression or hybridization at the transplantation site leading to outbreeding depression, high economic costs, poor survivability, and few long-term

monitoring protocols (Allen 1994; Walters et al. 1994; Montalvo and Ellstrand 2000; Fahselt 2007). Some of these impacts can be minimized through methodology; however, poor conceptual planning for success goals and long-term monitoring—all identified as causes of transplantation failure—have led to some discouragement of this practice (Berg 1996).

Reservations concerning transplantation as a form of conservation mitigation have been voiced (Allen 1994; Falk et al. 1996; Fahselt 2007; Wendelberger et al. 2008). Transplantation of at-risk species is especially troubling to critics, since the persistence of such species is often dependent on undisturbed, intact habitat, and transplantation could allow habitat destruction to become more acceptable (Falk and Olwell 1992; Fahselt 2007). In general, habitat preservation is essential to conservation efforts, because habitat destruction due to human activities is considered the primary threat to biological diversity (Primack 2006). Since habitat destruction has not yet been abated as a practice, and when the alternative is plant loss in a condemned area, transplantation is an option for the rescue of at-risk plants (Falk and Olwell 1992; Fahselt 2007; Wendelberger et al. 2008). For mitigation to be successful, projects must move beyond the traditional view of success, in which transplants are expected to survive for only a few years, into an ecological view of success where transplants become a viable, self-maintaining population reflecting natural communities (Pavlik et al. 1993; Primack 1996; Jusaitis 2005; Fahselt 2007). The latter perspective requires long-term monitoring of survival, reproduction, seedling establishment to recruitment, and population viability estimations in comparison to natural reference populations (Primack 1996; Menges 2008). Since transplantation mitigation can be costly and characterized by low success, small scale transplantation experiments can guide the feasibility of such action and help avoid some of its common pitfalls (Jusaitis 2005).

This transplantation study was prompted by the unavoidable land clearing at a Tennessee Army National Guard (TNARNG) military training facility, Volunteer Training Site (VTS), in Catoosa County, Georgia, that would drastically disturb the existing habitat of federally-threatened *Scutellaria montana* Chapm. (large-flowered skullcap). The individuals that were transplanted were largely adult stage plants, which have often been found to have higher initial survivability than establishment with seeds or seedlings (Drayton and Primack 2000; Wendelberger et al. 2008). Initial survival, growth, and fecundity of transplants are all early fitness indicators that are thought to be site dependent and influenced by ecological pressures, such as herbivory and competition (van Andel 1998; Jusaitis 2005; Menges 2008). Since transplants in this study were sourced from a local population, many problems associated with genetic incompatibilities at the transplant receiving site that could reduce fitness due to local adaptations, including any ecotypic variation and genetic differentiation between populations, should be minimized (Walters et al. 1994; Montalvo and Ellstrand 2000). It is unknown whether ecotypes of *S. montana* occur; however, previous genetic work revealed that at least two distinct populations exist, a northwesterly Tennessee River region and a southwesterly Oostanaula River region divided by geographical ridge feature called Taylor Ridge (Cruzan 2001). Our study site, VTS, is within the Tennessee River region.

The preferred habitat of *S. montana* has been a subject of scientific interest and contradictions. Its canopy is generally described as relatively open, mid-to-late successional, and predominately oak-hickory or mixed-oak (Cruzan 2001; Mulhouse et al. 2008, USFWS 2002). Faulkner (unpublished work, as cited in USFWS 2002) found that *S. montana* survived after logging and prescribed burning activities, but speculated that recruitment was not likely in disturbance zones for unspecified reasons. However, it has also been suggested that canopy

disturbances resulting in greater light availability are beneficial to the species (Nix et al. 1993; personal communication from Sutter, as cited in USFWS 2002), but that soil disturbances could negatively impact *S. montana* due to competition pressures (Nix et al. 1993). Fail and Sommers (1993) suggested that fire suppression activities may be a factor in the rarity of *S. montana*, yet as late as 2005, the United States Forest Service classified *S. montana* as adversely affected by fire (Owen and Brown 2005). Mulhouse et al. (2008) reasoned that since *S. montana* habitat seemed to have a strong grass component that its habitat would tend to also have relatively high light availability. However, Hopkins (1999) evaluated canopy openness at the location of *S. montana* individuals sampled in the Tennessee River Gorge with hemispherical photography and found no correlation between percent canopy openness and variables including leaf number and flower number.

The VTS accommodates a tank firing range, making prescribed burning necessary to reduce fuel loads to prevent and control resultant forest fires. Since some existing *S. montana* habitat falls within this area, it is relevant to evaluate transplantation responses to prescribed burning in an investigation into habitat suitability for transplants. With conflicting observations regarding the influence of light availability where *S. montana* occurs, further investigation was warranted. Here we studied the responses of *S. montana* transplants to separate and interactive canopy thinning and low-intensity prescribed burning pre-transplantation treatments with the intention of making recommendations for land managers concerning site selection for any future transplantation. Previous attempts at transplantation of *S. montana* have had mixed results (USFWS 1996; USFS 2002, 2005; Snyder and Lecher 2010), yet high initial survivability was found after our transplantation of *S. montana* at a site in Chattanooga, Tennessee, in 2009 (see Chapter 3). While continued monitoring of transplants will be needed to understand long-term

trends, the purposes of this study were to determine if transplantation as a form of conservation mitigation is feasible in instances where plant rescue is a necessary and unavoidable impact, and investigate how site selection could influence transplantation success. By quantifying early indicators of fitness, including initial survivability, growth and development characteristics, and underlying mechanisms with physiological characteristics, our objectives were to evaluate the overall success of transplantation of *S. montana* and how to maximize that success through site selection by measuring the response of transplants to canopy thinning and prescribed burning treatments.

Material and Methods

Study Site

The VTS is a 659-ha military facility that provides habitat for more than 1,500 individuals of *S. montana* (SAIC 2002; Figure 4.1). A security directive requires vegetation clearing of an approximate 7.6-m (25-ft) interior buffer around the fenced property line, which would result in a drastic habitat change from forest to an open area without a canopy. Even if individuals of this understory species along the VTS boundary survive the disturbance caused by clearing equipment, *S. montana* has been predicted to respond poorly to an open habitat (Ash 1983, as cited in Johnson 1991). To mitigate potentially negative impacts of this disturbance, the TNARNG is required to replace three times the number of affected plants with *S. montana* grown from local seeds (personal communication from L. Lecher, State of Tennessee Military Department, 8 March 2011, unreferenced). Conservation efforts on military lands must be able to maintain the training objectives; therefore, additional transplantation efforts may be necessary in the future (Melton et al. 2004; Army 2007).

Figure 4.1   Map of the study area depicting portions of Tennessee, Alabama, and Georgia with counties outlined in light gray. The inset depicts Catoosa County featuring the Tennessee Army National Guard Volunteer Training Site (VTS) in dark gray. The dark gray counties surrounding Catoosa County have records of *Scutellaria montana* occurrences.

Plot Treatments

The site for transplantation was chosen because it is located within one of the existing management groups of known *S. montana* habitat at the VTS and had no plans for future development. Within the site, four 40-m^2 plots were situated on a gentle slope of an east-facing aspect approximately 25-m below a gravel road and several meters upland to a wet weather conveyance. A low-grade prescribed burning treatment was applied to two of the plots in March 2011, and only the leaf litter was consumed with this treatment. In one of the burned and one of the unburned plots, woody stems less than 15-cm diameter at breast height were cleared, and

38

larger woody stems were girdled. The resultant plot treatments were a control (C), canopy thinned only (T), burned only (B), and combined canopy thinned and burned (T + B). The burned area was spaced approximately 100 m from the unburned area, and the plots within each area were approximately 5-m apart. According to previous methods, hemispherical photographs (Rich 1990; Zhang et al. 2005) were taken with a digital camera (Canon EOS Rebel, Canon U.S.A., Inc., Lake Success, New York, unreferenced) mounted on a tripod from the center of each plot in August 2010 to determine the percent canopy openness. Photographs were analyzed with Gap Light Analyzer 2.0 (Simon Fraser University, Burnaby, British Columbia, Canada, 1999, http://www.ecostudies.org/gla/, unreferenced). Percent canopy openness was 10.4% in plot C, 20.8% in plot T, 13.5% in plot B, and 18.7% in plot T + B. Soil samples collected in July 2010 at a depth of approximately 10 cm from the plot centers were homogenized and sent to the Soil, Plant, and Water Laboratory at the University of Georgia in Athens, Georgia, in April 2010 for soil nutrient analysis. Compared to plot C, potassium (K) was 67% greater in plot B and 75% greater in plot T + B; nitrate (NO_3^-) was 313% greater in plot T + B; ammonium (NH_4^+) was 26% greater in plot B (Table 4.1).

<center>Transplantation</center>

Scutellaria montana individuals occurring in the buffer zone along the VTS boundary were originally flagged for potential transplantation in June 2009, and 100 of these individuals were assigned randomly in April 2010 to one of the transplantation plots and to a specific position within that plot. As a result, each plot received 25 transplants. Transplants were sourced from three clusters on the western perimeter and one cluster on the eastern perimeter of the VTS. Nine days before transplantation began, holes were dug in the transplantation plots, spaced 1-m apart along north-south transects. Transplantation occurred on three days between 29 April and 3

May 2010. When plants were dug out of the ground, we attempted to minimize root disturbance by collecting an approximately 30-cm-diameter-by-15-cm-deep cylinder of intact soil around each transplant. However, this collection size was not always possible due to shallow and/or rocky soils. Once removed, each individual was transported in a large nursery container by vehicle to the transplantation location the same day. Each individual was placed in its assigned hole and covered with soil and any available leaf litter. Since transplanting disturbs the soil and root contact, water stress can result in dry soils before sufficient establishment takes place (Jusaitis 2005; Taiz and Zeiger 2006). To prevent this, all transplants were watered daily through the first week after transplantation and then on a weekly basis, if it did not rain until the end of July 2010.

Table 4.1   Soil nutrients ($\mu g\ g^{-1}$) in four *Scutellaria montana* transplantation plots, control (C), canopy thinning only (T), prescribed burning only (B), and combined canopy thinning and prescribed burning (T + B), at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia.

	Soil nutrients ($\mu g\ g^{-1}$)			
Plots	NO_3^-	NH_4^+	P	K
C	0.8	8.8	10.5	68.8
T	1.7	9.8	8.9	76.5
B	1.7	11.1	11.8	115.0
T + B	3.3	9.7	9.5	120.5

Measurements

Surveys to monitor the transplants were conducted throughout the 2010 growing season. Specifically, inventories were conducted on 7 May 2010 (for baseline metrics four days after the

last plants were transplanted), 27 May 2010 (for number of flowers per plant when most individuals should have undergone floral induction), 20 May 2011 (for growth about a year after transplantation), and 15 July 2011 (for transplant survival only). Survivability was expressed as transplants with existing aboveground biomass as a percentage of the initial total number of transplants. For growth characteristics, we determined stem number, stem height, leaf number, and any damage for each transplant. Stems within 7.5 cm of each other were considered one plant, and the height of the tallest stem of an individual was measured for stem height. Damage was noted as leaf damage, such as irregular patterns of biomass missing from leaf tissues from invertebrate herbivory, or as stem damage, which was largely assumed to result from vertebrate grazing, especially when apical biomass was found missing. We strongly suspected deer to be herbivores of *S. montana*, since hoof impressions were occasionally observed in the plots. Additionally, stems were occasionally found bent or broken after heavy rains, or possibly from vertebrate trampling. While fecundity was not measured, we quantified the reproductive potential of each individual transplant by counting flower numbers and determining developmental stage classes. These stage classes were defined as juvenile for intact vegetative stems less than 10-cm tall, vegetative adult for plants with stems greater than 10-cm tall, or flowering adult for flowering or fruiting plants.

To elucidate the physiological mechanisms underlying our morphological observations, we also measured instantaneous leaf-level gas exchange with a LI-6400XT portable gas exchange analyzer (LI-COR, Lincoln, Nebraska, unreferenced). Net photosynthetic rates and other physiological variables give a snapshot of how a plant is performing in its environmental conditions and has been correlated with long-term plant success (McAllister et al. 1998). We noticed if more than two days had passed since plants were watered or received rain that they

were mostly respiring, so all plants were watered before gas exchange was measured on 21 July 2011 (12 weeks after transplantation). While ambient light was used for gas exchange measurements, the leaf chamber conditions were controlled for a CO_2 concentration at 400 µmol CO_2 mol^{-1} with constant air flow rate at 500 µmol s^{-1} and the block temperature at 29º C. Variables were reported for net photosynthetic rate (A; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}). Additionally, small subsamples of leaf biomass were collected on 21 June 2010 and dried to calculate the leaf mass per unit area (LMA; g m^{-2}).

Statistical Analysis

All statistical analyses were performed with IBM SPSS Statistics 19 (SPSS, Inc., 2010, Somer, NY, unreferenced). Means were reported for all measured variables. Principal Component Analysis (PCA) with an orthogonal rotation was used test relationships between variables. A factorial ANOVA was used to evaluate plant responses to plot treatments with fixed main effects of canopy thinning and burning and their interaction effect.

Results

Overall Transplantation Success

Following the first day of transplanting, two transplants were found missing. Four plants senesced during the first growing season: two from what appeared to be mechanical basal damage and two for unknown reasons. Two of these regenerated new shoots within a few weeks, and the other two did not regenerate until the next year. As a result, the transplantation survival was 98% as of August 2010. During the May 2011 inventory, 12 plants were not found;

however, due to resprouting of individuals with missing aboveground biomass, only nine plants were still not found in July 2011, thus resulting in 91% survivability.

In the first week after transplantation, plants were mostly single stemmed but ranged from 1 to 3 stems, the stem height ranged from 2.5 cm to 45 cm, and leaf number ranged from 2 to 16 leaves (Table 4.2). Leaf damage was observed on 58% of the transplants, and 14% exhibited stem damage. Stage classes were 27% juvenile, 51% vegetative adult, and 22% flowering adult. A total of 50 flowers were counted, ranging from 1 to 8 flowers per individual.

About a year after transplantation, the stem number ranged from 1 to 5 stems, the stem height ranged from 1.0 to 54.5 cm, and the leaf number ranged from 0 to 78 leaves (Table 4.2). Leaf damage was observed on 67% of the plants, and 57% exhibited stem damage. Stage classes were 5.7% juvenile, 56.8% vegetative adult, and 37.5% flowering adult. A total of 293 flowers were counted, ranging from 1 to 27 flowers per individual.

Table 4.2   Morphological variable means ± 1 SE of *Scutellaria montana* transplants across plots at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia, were all significantly different at the $P \leq 0.05$ level.

	Survey date	
Variable	May 2010 ($n = 98$)	May 2011 ($n = 88$)
Stem height (cm)	12.0 ± 8.1	20.0 ± 1.1
Number of stems	1.1 ± 0.38	1.7 ± 0.10
Number of leaves	6.8 ± 2.7	21.2 ± 1.6
Number of flowers	0.51 ± 1.5	3.3 ± 0.6

A PCA of morphological variables (flower number, stem height, stem number, and leaf number) resulted in finding two components that explained 87.2% of the variation in the morphology found in the transplants (Table 4.3). Stem number had a strong positive relationship to leaf number, and flower number had a strong positive relationship to stem height (Figure 4.2).

Table 4.3   A Varimax-rotated matrix of a Principle Component Analysis showing the variable loading per component, total eigenvalue, and cumulative percentage of variance explained in variables measured in May 2011 from *Scutellaria montana* at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia.

	Component	
Morphological variables	1	2
Flower number	0.903	
Stem height	0.880	
Stem number		0.973
Leaf number	0.468	0.837
Total eigenvalue	1.81	1.68
Cumulative variance explained (%)	45.3	42.0
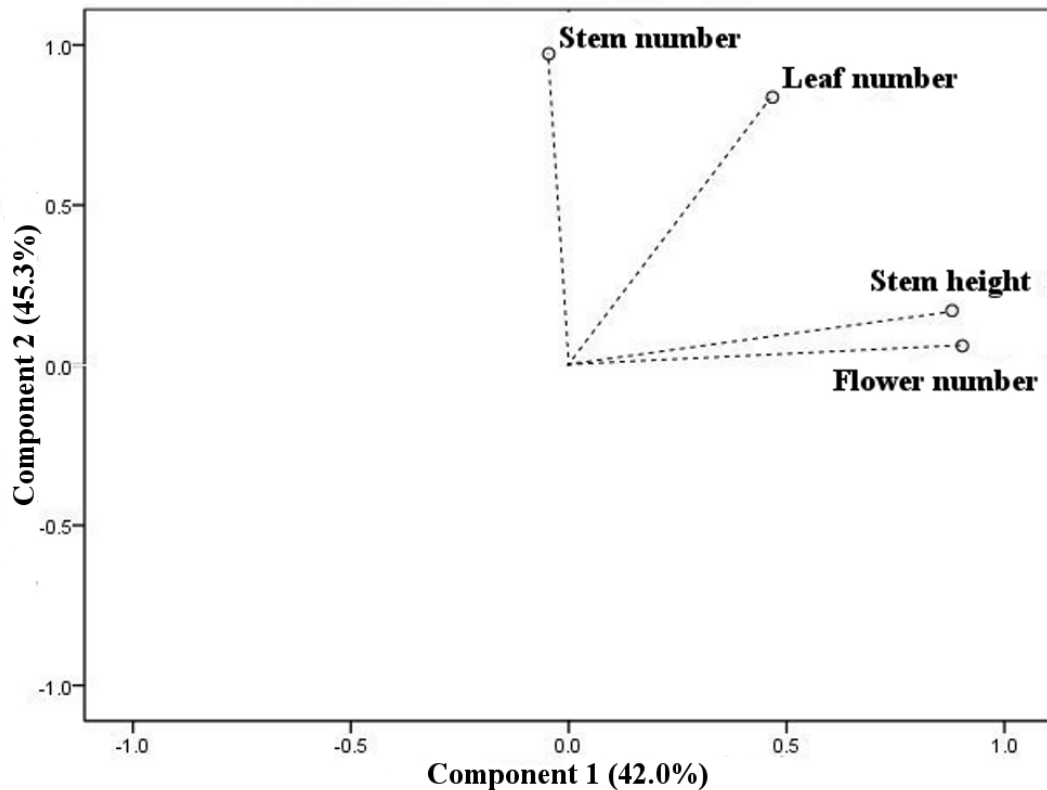
Figure 4.2   Principal component analysis projection of flower number, stem height, leaf number, and stem number measured in May 2011 of transplanted *Scutellaria montana* at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia. Percentages of the two components represent total variance explained, and projections in the same direction show positive correlations, while opposite directions would have represented negative correlations.

<div align="center">Responses to Plot Treatments</div>

In July 2011, the survivability per plot ($n = 25$) was 96% in plot C, 84% in plot T, 100% in plot B, and 84% in plot T + B. While in May 2010 there were no significant differences in any of the measured morphological variables between the transplantation plots (all $P > 0.109$), several response variables in May 2011 showed significant differences in both the fixed main effects and their interaction effect. For the fixed main effect of canopy thinning, significant increases were found in stem damage ($P = 0.001$; $F_{3,87} = 10.838$; Figure 4.3A), vegetative adults

($P = 0.025$; $F_{3,87} = 5.185$; Figure 4.3B), LMA ($P < 0.001$; $F_{3,76} = 27.526$; Figure 4.3D), E ($P = 0.004$; $F_{3,60} = 8.919$; Figure 4.3E), and g_s ($P = 0.011$; $F_{3,60} = 6.824$; Figure 4.3F) and significant decreases were found in flowering adults ($P = 0.009$; $F_{3,87} = 7.075$; Figure 4.3C) and stem height ($P = 0.009$; $F_{3,87} = 7.107$; Figure 4.3G). No significant differences were found in stem number ($P = 0.074$; $F_{3,87} = 3.265$), leaf number ($P = 0.926$; $F_{3,87} = 0.009$), leaf damage ($P = 0.123$; $F_{3,87} = 2.428$), juvenile stage class ($P = 0.595$; $F_{3,87} = 0.284$), flower number ($P = 0.083$; $F_{3,87} = 3.074$), or A ($P = 0.229$; $F_{3,60} = 1.481$) between thinned and unthinned plots. For the fixed main effect of prescribed burning, significant increases were found in stem damage ($P = 0.030$; $F_{3,87} = 4.845$; Figure 4.4A), vegetative adults ($P = 0.009$; $F_{3,87} = 7.242$; Figure 4.4B), LMA ($P < 0.001$; $F_{3,76} = 55.223$; Figure 4.4D), E ($P = 0.007$; $F_{3,60} = 7.710$; Figure 4.4E), and A ($P = 0.045$; $F_{3,60} = 4.191$; Figure 4.4F) and significant decreases were found in flowering adults ($P = 0.003$; $F_{3,87} = 9.036$; Figure 4.4C) and leaf damage ($P < 0.001$; $F_{3,87} = 14.322$; Figure 4.4G). No significant differences were found in stem number ($P = 0.819$; $F_{3,87} = 0.053$), leaf number ($P = 0.277$; $F_{3,87} = 1.199$), stem height ($P = 0.254$; $F_{3,87} = 1.319$), juvenile stage class ($P = 0.714$; $F_{3,87} = 0.135$), flower number ($P = 0.099$; $F_{3,87} = 2.785$), or g_s ($P = 0.078$; $F_{3,60} = 3.213$) between the burned and unburned plots. Significant interaction effects were found in stem height ($P = 0.004$; $F_{3,87} = 8.844$; Figure 4.5A) and leaf number ($P = 0.015$; $F_{3,87} = 6.151$; Figure 4.5B). Additionally, the percentage of stem damage by individual plot was 27% in plot C, 65% in plot T, 54% in plot B, and 82% in plot T + B.
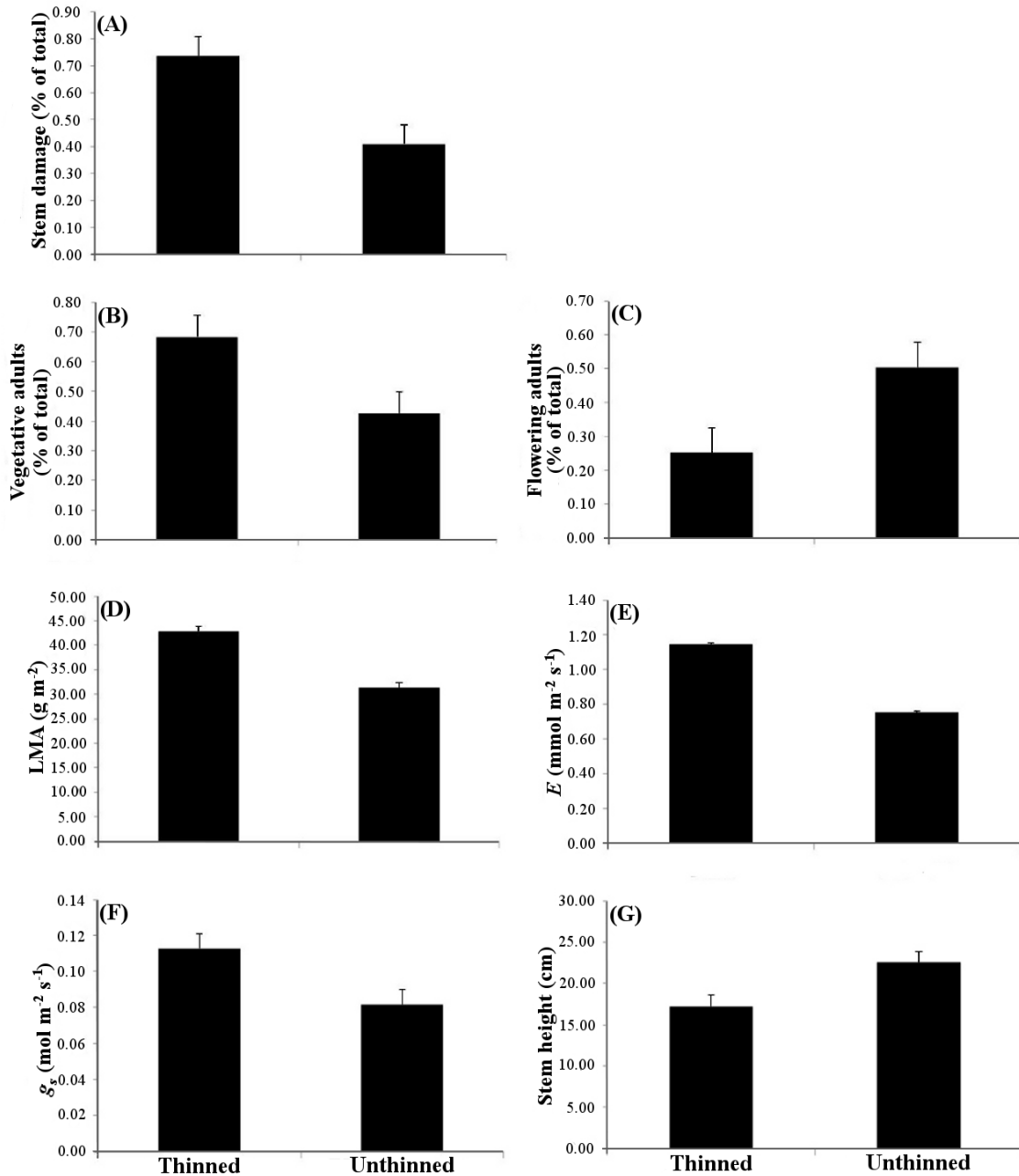
Figure 4.3   The canopy thinning treatment as a fixed main effect. Means (± 1 SE) with significant differences at the P ≤ 0.05 level are shown for transplanted *Scutellaria montana* individuals at the Tennessee Army National Guard Volunteer Training Site in Catoosa, Georgia, including stem damage (a), vegetative adults (b), flowering adults (c), leaf mass per unit area (LMA; d), transpiration rate (E; e), stomatal conductance (g_s; f), and stem height (g). Samples were collected for LMA in June 2010, gas exchange in July 2010, and the other variables in May 2011.
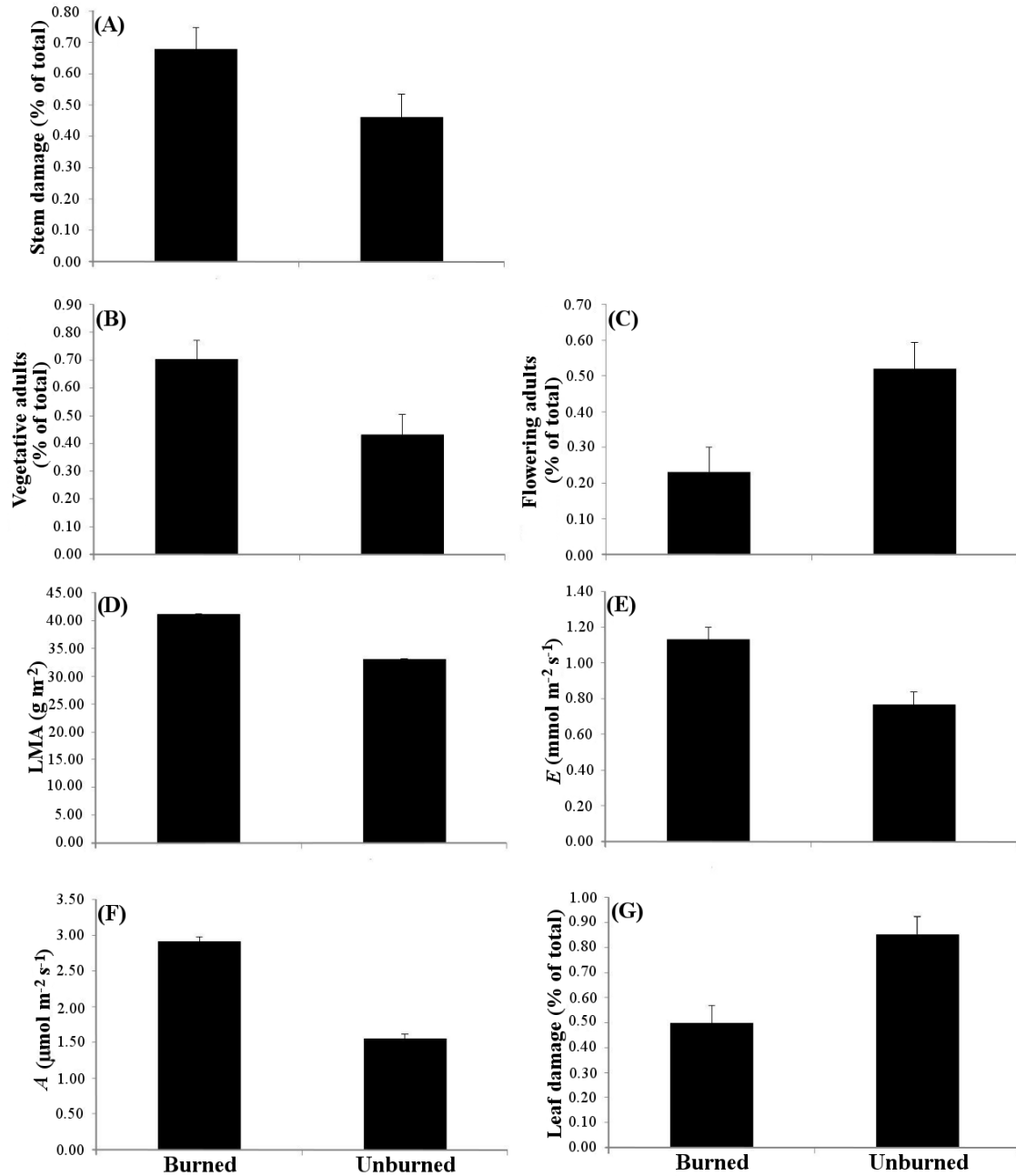
Figure 4.4   The prescribed burning treatment as a fixed main effect. Means ($\pm$ 1 SE) with significant differences at the $P \leq 0.05$ level are shown for transplanted *Scutellaria montana* individuals at the Tennessee Army National Guard Volunteer Training Site in Catoosa, Georgia, including stem damage (a), vegetative adults (b), flowering adults (c), leaf mass per unit area (LMA; d), transpiration rate (*E*; e), net photosynthesis rate (*A*; f), and leaf damage (g). Samples were collected for LMA in June 2010, gas exchange in July 2010, and the other variables in May 2011.
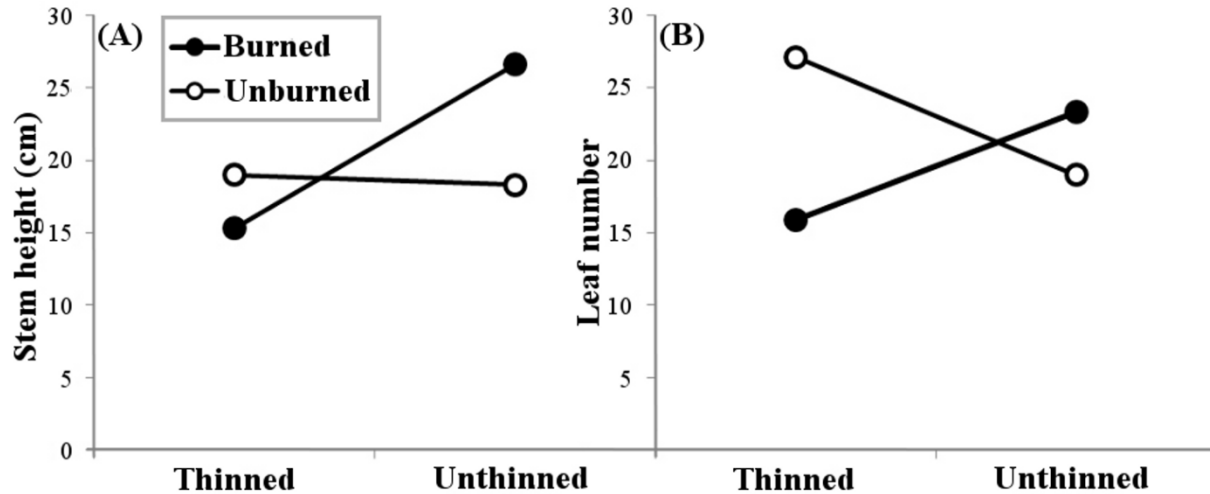
Figure 4.5   The significant interaction effect at the P ≤ 0.05 level of canopy thinning and prescribed burning for stem height (a) and leaf number (b) of transplanted *Scutellaria montana* individuals in May 2011 at the Tennessee Army National Guard Volunteer Training Site in Catoosa County, Georgia.

Discussion

Scutellaria montana individuals in this study were overall transplanted successfully in terms of early fitness indicators (van Andel 1998; Jusaitis 2005; Menges 2008), as they exhibited high survivability, continued to mature in the second year with increases in both growth and development, and produced more flowers suggesting an increased reproductive potential. However, because seedlings were not found in the second year, we cannot yet consider the transplants to be self-maintaining. Since the transplantation of rare plant species has a reputation of less than ideal long-term results in viability due to inherent features, such as low seed set and recruitment (Fahselt 2007), it will be important to continue monitoring tagged individuals for survival and search for seedlings that have established in the vicinity of our transplant plots (Primack 1996; Menges 2008). Seedling survival has been identified as one of the largest barriers to self-maintenance in transplanted populations, especially in at-risk species (Jusaitis 2005; Lofflin and Kephart 2005), and recruitment is often conditional on seed availability and

favorable environmental conditions (Eriksson and Ehrlen 1992). Previous researchers have observed *S. montana* to be characterized by low flowering, fruit set, and seed set in natural conditions (Kemp 1987; Nix et al. 1993; Hopkins 1999; Cruzan 2001; USFWS 2002). However, Hopkins (1999) found a positive relationship between flower number and fruit set, so the increased flower number found in our 2011 transplant survey suggested both an overall suitability of site selection and perhaps future increased seed availability. Yet, seedling establishment in the future could be negatively impacted by summer drought (Manzaneda et al. 2005), as well as herbivory and competing vegetation (Jusaitis 2005).

In an effort to maximize transplantation success, we investigated site selection and found differences in the fixed main effects of canopy thinning and prescribed burning treatments. While plots with canopy openness of greater than 18% had a lower percent survival, the plots with canopy openness of less than 14% had very high survivability. Both treatments as fixed main effects showed increased stem damage, LMA, and gas exchange, and increases were also found in plot B for stem height and plot T for leaf number. Additionally, both treatments had reduced development of flowering adults, suggesting a reduced reproductive effort. Apparently, no treatment had the best success, as these results do not clearly demonstrate that either of the plot treatments met criteria for successful transplantation.

The increased stem damage found in our canopy thinning and prescribed burning treatments compared to plot C suggests that our plot treatments increased grazing interest by attracting herbivores. We further suggest a strong influence of suspected vertebrate grazing on transplant responses that skewed our analysis of the plot treatments as fixed main effects. Stem damage was often evidenced in the form of missing apical portions of biomass, which in general would decrease stem height and may have increased leaf number after grazing by increasing

stem number or lateral branching after losing apical dominance in these individuals (Taiz and Zeiger 2006). Since *S. montana* has a terminal inflorescence, any evidence of flowers would likely have been missing after grazing at the time of data collection, which could have reduced our counts of flower number and flowering adult stage class. Hopkins (1999) reported a positive relationship with leaf number and flower number in *S. montana*, but the PCA model in this study suggested that stem height was a better indicator of flower number in the transplants than leaf number, giving further support that individuals increase leaf number and perhaps lost reproductive organs after grazing.

The 3.6-fold increase in overall stem damage one year after transplanting was an unexpected result. Naturally occurring *S. montana* individuals sampled across the VTS in May 2010 (n = 1346) exhibited 8% stem damage (see Chapter 2), and individuals within the two permanent monitoring plots closest in proximity to the transplantation plots exhibited 12% (n = 150) and 19% (n = 26) stem damage (Shaw et al. 2010). The similarity of stem damage in plot C (27%) to those monitoring plots gives further evidence that prescribed burning and canopy thinning treatments may have attracted herbivores. When this study was implemented, we did not yet have the first year results from our previous *S. montana* transplantation study within an occurrence in nearby Chattanooga, Tennessee, in which stem damage was reduced by 93% and flower number increased by almost 20-fold after fencing the transplantation plot (see Chapter 3). Given these combined findings, we strongly recommend that herbivore exclosures should be used to both maximize transplantation success and better elucidate results from any future utilization of canopy thinning and prescribed burning treatments.

Not all of the response variables examined in the fixed main effect of our treatments were unfavorable to transplants, as all treatment plots were characterized by significantly increased

means of LMA, *A* or g_s, and *E*. Additionally, the treatment plots had at least some increased light availability due to more canopy openness compared to plot C. Changes in LMA can result from changes in either lamina depth or tissue density or both in response to variety of environmental conditions (Witkowski and Lamont 1991); however, increased photosynthetic capacity has a stronger association with increasing thickness (Ellsworth and Reich 1992; Niinemets 1999). Leaf acclimation, including increased photosynthesis, leaf thickness, and palisade mesophyll cells, is sensitive to increased light availability in the form of total irradiance (Chabot et al. 1979), suggesting that the increases in LMA and *A* or g_s in our results may have resulted from the acclimation of *S. montana* to increased light availability. While we did not measure foliar nitrogen (N), leaves grown in high irradiance typically are associated with increased foliar N content per unit leaf area, which in many species strongly correlates with increased photosynthesis capacity (Evans 1989; Reich et al. 1997; Niinemets 1999; Frak et al. 2001). Increased foliar N content per unit leaf area, as a response to increased light availability, is thought to result from N partitioning with greater allocation towards soluble proteins, such as RuBP carboxylase enzyme in the Calvin cycle (Evans 1989).

Increased soil nutrient availability, especially N and potassium from the combustion of leaf litter in plots B and T + B may have occurred (Knoepp and Swank 1993; Wan et al. 2001; Certini 2005; Huang et al. 2007). Positive correlations between foliar N content and gas exchange have previously been evidenced in plant responses to prescribed burning treatments, and *E*, in particular, was increased at all times of the day (Reich et al. 1990). While *E* can be increased with increased light availability and leaf temperature, solute loading, especially with the potassium cation (K^+), into the xylem lowers the xylem resistance and increases flow rate and *E*, which can positively influence photosynthetic rates and growth (Zwieniecki et al. 2001). K^+ is

also a major osmolyte employed to stimulate guard cell turgor to open stomata (Taiz and Zeiger 2006), so the increased availability of K^+ may have enhanced g_s in plots B and plot T + B as well. Additionally, increased E has been associated with increased distribution of cytokinins per unit leaf area (a plant growth hormone), which has also been associated with increased leaf growth, photosynthetic capacity, and foliar N content (Pons et al. 2001). Furthermore, if transplants in the treatment plots were able to reduce their shoot C-to-N ratio with increased foliar N and perhaps in effect become more palatable to vertebrate predators (Davidson 1993), this could help to explain the greater intensity of stem damage found within our treatment plots.

Even though our prescribed burning treatment as a fixed main effect resulted in increased stem damage and decreased number of flowering adults, it is interesting that plot B transplants exhibited both increased stem height and 100% survival. Shoot regeneration suggests at least some fire tolerance, as long as the belowground parts are able to survive (Lambers et al. 2008). Since Native Americans are thought to have greatly influenced the Ridge and Valley physiographic providence with fire before Europeans settlers arrived (Delcourt and Delcourt 1998), *S. montana* should be further researched for fire adaptability, including the effects on seed germination, recruitment, and interspecific competition of surrounding post-fire vegetation.

In conclusion, our overall *S. montana* transplantation results evidenced initial success with high survivability, continued maturity, and increased reproductive effort. However, recruitment has not yet been evidenced, so self-maintenance of these transplants cannot yet be determined. For this reason, monitoring of these transplanted individuals and search for new seedlings should be continued until the viability of the transplants can be determined. Our individual plots produced less clear results in terms of selecting the most suitable site(s) for transplantation. At face value, plot C (no treatments) best met the criteria for initial success with

high survivability and continued maturity with increased flowering adults; however, we suggest our plot treatment results were skewed due to the influence of herbivore grazing. We recommend that selected sites for any future transplantation should not include canopy thinning or prescribed burning treatments without planning for the control of vertebrate herbivory, and, to maximize overall transplantation success, we strongly recommend that herbivore exclosures should be utilized for all *S. montana* transplants.

CHAPTER 5

SYNTHESIS AND CONCLUSIONS

The work presented in this thesis was concerned with the conservation of a federally

threatened and locally endemic plant species, *Scutellaria montana* Chapm. (Lamiaceae, large-

flowered skullcap). The first study presented attempted to analyze trends in sampled plants

occurring naturally in one population of *S. montana*, and subsequently presented studies

quantified transplantation results in two different populations for plant rescue and mitigation.

Within these chapters, I tried to describe areas that were still perplexing and confounding to

recovery efforts for delisting this species, and I synthesized them here in terms of the species

recovery plan (USFWS 1996).

At the time of listing *S. montana* as an endangered species under the Endangered Species

Act of 1973, there were ten known populations of this species, and all were at risk of extinction

due to habitat destruction (USFWS 1986). When *S. montana* was reclassified as a threatened

species in 2002, there were 48 known populations, and many were on protected lands (USFWS

2002). The recovery plan calls for the protection of at least 15 viable populations of *S. montana*

to be maintained for ten years (USFWS 1996). While the first goal of the recovery plan has been

successful in locating previously unknown populations, limited data exist to determine the self-

maintaining nature of these populations. Analysis of the abundance trend of sampled *S. montana*

over a seven-year period at the Tennessee Army National Guard's Volunteer Training Site

(VTS) in Catoosa County, Georgia, suggests a stability of individuals in the monitoring plots, but

55

our sampled plants had relatively low flowering and seedlings (see Chapter 2), as previous researchers at different sites have found as well (Kemp 1987; Nix et al. 1993; Hopkins 1999; Cruzan 2001; USFWS 2002). *Scutellaria montana* is suspected to have individuals with long lives, based on circumstantial evidence that individuals still exist at positions that were flagged at least 13 years prior (personal communication from J. Brown, Tennessee River Gorge Land Trust, 2 June 2011, unreferenced). This leads to the concern that any stability found in monitoring abundance trends could be due more to the persistence of adult individuals rather than to replacement recruiting, so there could be a risk of declining populations in the future as aging individuals complete their lives (Schmeske et al. 1994). Additionally considering the indeterminate growth of herbaceous perennial plants, research into the life history of *S. montana* needs to include small demographic studies in which randomly sampled individuals are tracked for multiple years across its range in Tennessee and Georgia. The recovery plan recommends this type of research (USFWS 1996), and these data would allow researchers to build life history tables and model population viability to predict survival or extinction probabilities (Menges and Gordon 1996), giving authorities more informative results to make decisions on the status of the species' recovery.

The combined results from transplantation studies suggest that vertebrate grazing may be a factor negatively affecting the long-term fitness of *S. montana*. The fenced individuals studied at the Enterprise South Industrial Park (ESIP) in Hamilton County, Tennessee, had results similar to studies with herbivory exclusion (Augustine and Frelich 1998; Kettenring et al. 2009). The ESIP transplants exhibited tremendous increases in floral induction concurrent with a reduction in stem damage from 29% in the first year to 2% a year later after fencing (see Chapter 3). Since previous studies have concluded that *S. montana* is likely pollination limited with low

seed set (Nix et al. 1993; Hopkins 1999; Cruzan 2001), these may still be issues for the ESIP transplants. However, Hopkins (1999) found a positive relationship between flower number and fruit set in this species, suggesting that an increased reproductive effort may lead to increased natality in the future. While the VTS transplants also exhibited an increase in flower number in the second year following transplantation without fencing, suggesting continued maturity and suitability of site selection, these gains were not nearly as impressive as in the ESIP transplants. Further, stem damage in the VTS transplants increased from 14% in the first year to 57% in the second year, and the plot treatments of canopy thinning and prescribed burning may have especially contributed to increased grazing interest by attracting herbivores (see Chapter 4). Even though second-year flowering increased in the VTS transplants, early season grazing, which removes apical biomass that could include terminal inflorescences, may have reduced evidence of reproductive effort. Due to these transplantation results, we strongly recommend herbivore exclosures to maximize success for any future transplantation projects. Research is ongoing to better quantify the effects of vertebrates on the natural occurrences of *S. montana* at the VTS that will be able to determine if herbivore populations should be controlled as well.

When thinking about protecting *S. montana* from potentially harmful impacts from vertebrates, it should be considered that herbivores, such as deer, could be an important long-distance seed disperser of *S. montana* by grazing after seeds have set (Boulanger et al. 2011). Although there is no available evidence to suggest that *S. montana* seeds could survive vertebrate digestive systems, anecdotal evidence suggests that there is a strong representation of *S. montana* in edge habitat, which is prime deer habitat (Alverson et al. 1988), and *S. montana* individuals are often found nearby trails and road cuts (personal observation). Another aspect of herbivore exclusion that should be explored is whether grazing has any positive impact on preventing

57

crowding and reducing competition pressures, if any exist, on *S. montana*. While Hopkins (1999) found a positive relationship between leaf number, flower number, and vegetation percent cover within 1 m of *S. montana* individuals, seed germination and seedling survival were not observed in relationship to surrounding vegetation, so it is unknown if herbivore exclusion or crowding would have any influence on recruitment.

Long-term success of transplantation requires in part that the transplants are able to self-maintain their numbers through recruitment. For this to be determined, an extended period of future monitoring is required. Though reproductive effort appears to have increased at both transplantation sites, no seedlings or recruits have been observed. Since flowering adults without stem damage in natural occurrences seem to be often one-stemmed (personal observation) and *S. montana* has an apparently limited seed dispersal mechanism (Cruzan 2001), I speculate on the possibility that multiple stems per transplant found at the protected ESIP transplantation site may in some cases actually be offspring. Yet, in general, lack of seedling survival is a noted barrier to self-maintenance in rare species transplantation (Jusaitis 2005; Lofflin and Kephart 2005), and seedling establishment can be threatened by drought, herbivory, and crowding from surrounding vegetation (Jusaitis 2005; Manzaneda et al. 2005). Considering the unusual pattern in the abundance trend at the VTS that perhaps resulted from severe drought (see Chapter 2) and that annual precipitation variability has increased throughout the past 30 years with greater intensity in both rainfall and summer drought events in the southeastern United States (Wang et al. 2010), drought tolerance and water stress response is another area that should be investigated in regards to seedling and adult survival.

The recovery plan calls for research into the effects of disturbance on *S. montana*, including canopy thinning and prescribed burning (USFWS 1996), and this has been examined in

this thesis and previously with mixed results. Hopkins (1999) found no influence of canopy openness, which was quantified with hemispherical photographs taken at the location of each sampled individual, on measured growth and development variables in natural occurrences of *S. montana* in the Tennessee River Gorge, in Hamilton County, Tennessee; however, in the VTS transplantation study, differences in survivability, flowering, and herbivory were significantly less favorable with the canopy thinning treatment as a fixed main effect (see Chapter 4). These results suggest that canopy openings at 18% or more apparently put *S. montana* individuals at greater risk of early season stem damage, which could negatively impact reproductive effort.

While the prescribed burning treatment as a fixed main effect also had less favorable results than no burning for flowering and herbivory, the burn only plot yielded an increased stem height, which was positively associated with increased flower number, and 100% survivability (see Chapter 4). The burned only plot transplants seemed to benefit from the increased soil nutrients released when the leaf litter was combusted. In general, this geographical area was prehistorically burned by Native Americans (Delcourt and Delcourt 1998), and as a resprouter, fire impacts on *S. montana* should be further explored. While initial results have not evidenced recruitment in any of the VTS transplantation plots, low-grade prescribed burning could potentially be beneficial by increasing light and nutrient availability for seedlings when leaf litter is combusted (Glasgow and Matlack 2007), if these seedlings can avoid desiccation. Furthermore, if embryos can survive prescribed burning, scarification of the pericarp from fire treatment could be beneficial to germination (Baskin and Baskin 1997), especially since Collins (1976) found that the seeds of other *Scutellara* species germinated much earlier when he mechanically removed the pericarp.

The survival status of *S. montana* is not as dire as presumed when it was first listed, but there are still several relevant research questions that should be addressed before this species should be considered for a recovery status and delisted. These research areas include determining factors that facilitate and hinder seed germination and seedling establishment, finding the recruitment status within natural populations with detailed study of *S. montana* demographics, and determining if drought could be a future threat to the species' natural populations. Vertebrate herbivory may be a larger threat to the fitness of *S. montana* than first surmised (USFWS 2002), and this research is ongoing at the VTS. We found evidence suggesting that herbivores were attracted to our canopy thinning and prescribed burning treatments in one transplantation study and that success of transplantation was likely increased when herbivores were seemingly excluded in the other transplantation study, so we strongly recommend that herbivore exclosures should be utilized in any future transplantation efforts. Without herbivory exclusion, we found that the control (no treatment) habitat with canopy openness at 10.4% had the best overall success in terms of survivability and reproductive effort. Given that some positive results were found with prescribed burning treatments and that *S. montana* is a resprouter, this is an area that should draw further attention. In conclusion, we found that the monitoring plots at the VTS had a stable abundance trend over seven years, and *S. montana* is amenable to transplantation, though self-maintenance is unclear and requires continued monitoring. Overall, these studies have enhanced our understanding of biotic and abiotic effects on this species.

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VITA

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