Spring 2010

The Demography and Community Characterization of he Federally Endangered Herb, Trillium Persistens: A Study Across Its Range Including A Fire-Dependent Habitat

Cassandra M. Plank

Recommended Citation

Plank, Cassandra M., "The Demography and Community Characterization of he Federally Endangered Herb, Trillium Persistens: A Study Across Its Range Including A Fire-Dependent Habitat" (2010). *Electronic Theses and Dissertations*. 745.
https://digitalcommons.georgiasouthern.edu/etd/745

THE DEMOGRAPHY AND COMMUNITY CHARACTERIZATION OF THE FEDERALLY ENDANGERED HERB, *TRILLIUM PERSISTENS*: A STUDY ACROSS ITS RANGE INCLUDING A FIRE-DEPENDENT HABITAT

by

Cassandra M. Plank

(Under the Direction of Lissa M. Leege)

ABSTRACT

Trillium persistens, a federally endangered perennial herb, exists as fragmented populations in Georgia and South Carolina, U.S.A. I quantified the demography of *T. persistens* across its range and described its associated plant community, including a fire-dependent habitat. To examine the demography of *T. persistens*, one study site was established in each of four population fragments (Battle Creek, Moccasin Creek, Moody Creek, and Panther Creek) located in and around Tallulah Gorge State Park. At Moody Creek, *T. persistens* occurs in the threatened, fire-dependent Table Mountain pine ecosystem, dominated by *Pinus pungens*. To determine whether prescribed fire would impact *T. persistens*, 40 4-m^2 plots containing *T. persistens* were established, with half receiving early-season prescribed fires in February, 2009. All four sites were censused over the 2009 and 2010 growing seasons to characterize *T. persistens* population dynamics and the composition of its associated plant community. I used these Census data to construct stage-based demographic matrix models to project population dynamics within each site over 25 years. Projections were determined using a low estimate and high estimate of fecundity.

More individuals were found across sites in 2010 compared to 2009, but life stage structure did not differ between years. Fruit set was greater in 2010 compared to 2009 across sites. The associated plant community was variable among sites. Species richness differed significantly, ranging between 0.6-1.9, 0.65-1.8, and 4.2-6.35 species per site for herb, shrub, and tree, respectfully. Projections of *T. persistens* population size over 25 years based on the matrix models suggest overall decline of *T. persistens* across its range, with mean λ across sites ranging from 0.84-1.08.

While the burn in 2009 did not show negative effects on *T. persistens*, land managers conducting prescribed burns in the future should use early-season cooler surface fires. *Trillium persistens* numbers should be monitored post-burn to see if they affect population sizes over time. Across sites, land managers should work to protect life stage transitions with the greatest impact on λ according to site, and monitor recruitment of new individuals.

INDEX WORDS: *Trillium persistens*, endangered species, plant community composition, demography, demographic matrix models, Table Mountain pine, *Pinus pungens*, prescribed fire

THE DEMOGRAPHY AND COMMUNITY CHARACTERIZATION OF THE FEDERALLY ENDANGERED HERB, *TRILLIUM PERSISTENS*: A STUDY ACROSS ITS RANGE INCLUDING A FIRE-DEPENDENT HABITAT

by

Cassandra M. Plank

B. S., Purdue University, 2008

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

2010

THE DEMOGRAPHY AND COMMUNITY CHARACTERIZATION OF THE FEDERALLY ENDANGERED HERB, *TRILLIUM PERSISTENS*: A STUDY ACROSS ITS RANGE INCLUDING A FIRE-DEPENDENT HABITAT

by

Cassandra M. Plank

Major Professor: Lissa M. Leege
Committee: Ray Chandler
Risa Cohen

Electronic Version Approved:
December 2010

ACKNOWLEDGMENTS

I would like to first thank my advisor, Lissa Leege, for her support and advice as I conducted my research. I thank my committee members, Ray Chandler and Risa Cohen, for their advice, help with statistics, and guidance on this project. I thank Ed Mondor for assistance with statistics. I appreciate all the hard work of my undergraduate research assistants, Chase Patrick and Aaron Murphy, who assisted me with data collection, provided welcome companionship in the field, and protected me from any snakes that crossed our paths while out in the field. Special gratitude goes to Tom Patrick for sharing his vast knowledge about *Trillium persistens* and showing me study sites. I thank Jim Candler for helping me locate field sites. I thank Nathan Klaus and volunteers from the Georgia Department of Natural Resources and Georgia Fish and Wildlife for their help in conducting the prescribed burns. I thank Georgia Power for allowing me to conduct this research on their land as well as providing me housing while in the field. Special thanks go to Danny Tatum and Tallulah Gorge State Park for allowing me access to their wildlife management area.

Funding for this project was provided by the Georgia Department of Natural Resources, Allen E. Paulson College of Science and Technology Academic Excellence Award, Georgia Southern University College of Graduate Studies Student Professional Development Research Fund, Georgia Botanical Society Marie Mellenger Field Botany Research Grant, Irene Burt Boole Botany Scholarship, and in-kind assistance from Georgia Power.

Last and certainly not least, I thank my family, especially my parents, for emotional support, especially the late-night phone calls. I thank my Georgia Southern student family for all their motivation, help with research, and for providing so much happiness over the past two years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review

Definition of rarity

Plant biodiversity is in rapid decline with nearly 25% of approximately 250,000 vascular plant species at risk of extinction (Schemske et al. 1994, Young et al. 2007). Because of their low abundance, rare species are thought to have the highest risk of extinction (Hartley and Kunin 2003, Lyons et al. 2005). Rarity is defined by the abundance or range of individuals in a species (Fiedler and Jain 1992, Hartley and Kunin 2003, Young et al. 2007) however Rabinowitz et al. (1986) further described rarity based on three factors: geographic distribution, habitat specificity, and the local population size of an organism. These three traits combine to characterize seven forms of rarity. An example of a rare population is the federally-endangered herb, *Trillium persistens*, which has a narrow geographic distribution, broad habitat specificity, and small populations (Duncan et al. 1971). *Trillium persistens* occurs in small, isolated populations in northeastern Georgia and western South Carolina, U.S.A. within a broad range of pine- and hardwood-dominated habitats (Rayner 1984).

In order to help populations of rare species to increase in number, conservationists must identify the factors that affect a population's size, range, and/or habitat (Rabinowitz et al. 1986, Schemske et al. 1994). One of the major shortcomings of conservation management is a lack of life history information on rare organisms (Young et al. 2007). Conservationists must work to understand intrinsic factors limiting the distribution of rare species in order to conserve these organisms (Young et al. 2007). One way to elucidate characteristics of a rare species is to understand the biology of a common congener (Bevill and Louda 1999). For example Young et al (2007) showed the federally endangered legume *Baptesia arachnifera* was less-adapted to heat treatment, a poorer colonizer and more prone to insect herbivory than the common *B. lanceolata*. Their findings illustrate the traits of the rare species may be limiting when compared with the widespread congener. Once the factors affecting the populations of a species are understood, causes of endangerment, conservation strategies, and criteria to determine recovery can be developed (Schemske et al. 1994).

Causes of rarity

Of the 728 federally-listed threatened or endangered organisms in the United States, nearly 50% are plants, with 22% of vascular plants in need of conservation actions (Schemske et al. 1994). Flowering plants are highly represented in the proportion of plants susceptible to extinction (Young et al. 2007). A variety of threats against plants have been cited, including suppression of natural disturbances, habitat destruction and degradation leading to population fragmentation, as well as the introduction of alien species (Hobbs and Huenneke 1992, Bomhard et al. 2005).

For some communities, natural forms of disturbance are required to maximize species richness and preserve biotic diversity (Hobbs and Huenneke 1992). When natural disturbance regimes are disrupted, persistence of disturbance-dependent species may be limited. Fire is a disturbance that is an important component of many ecosystems because it maintains fire-dependent vegetation in these communities (Dumas et al. 2007). Periodic fire regulates fuel accumulation because excess fuel can increase fire severity, potentially leading to wildfires (Varner III et al. 2007). The lack of periodic fire can result in successional replacement with fire-intolerant species. For example, Longleaf pine (*Pinus palustris*), native to the southeastern

United States, has been reduced to 5% of its original range due to disruption of fire regime. Longleaf pine stands are being replaced by deciduous hardwood trees, which has changed species composition across this ecosystem (Outcalt 2008). A review by Wilcove et al. (1998) showed that disruption of fire ecology is one of the top five threats to imperiled plants, affecting 20% of endangered and threatened species in the United States.

While natural disturbance may be necessary for the regeneration of some species, other forms of disturbance may negatively affect species. The number one threat to biodiversity is habitat destruction and degradation with reported that 81% of listed endangered and threatened plant species in the United States were threatened by human activities (Cook and Dixon 1987, Wilcove et al. 1998b). Disturbances can lead to habitat destruction that can cause fragmentation which changes in ecosystem function and structure by increasing the spatial isolation between populations of a species (Fiedler and Jain 1992, Wilcove et al. 1998b, Tomimatsu and Ohara 2002, Ohara et al. 2006). Fragmentation disrupts mutualistic interactions between plants and animals, increasing the chance these populations will become rare (Wilcove et al. 1998a, Tomimatsu and Ohara 2002, Knight 2003, Aguilar et al. 2008). Small population fragments may also experience microclimatic edge effects because seedlings recruitment can be limited around forest edges (Jules and Rathcke 1999, Tomimatsu and Ohara 2004). Increased edge habitat and greater isolation from neighboring populations increases the chance that populations within habitat fragments will become extinct (Jules 1998).

Fragmentation may have a greater effect on some plant species, depending on their life history traits. For example, fragmented populations of plants with breeding systems dependent on pollinators often experience lower reproductive success because of insufficient transfer of pollen by pollinators (Pino et al. 2007). Pollen transfer may be reduced as fragmented populations fall outside of a pollinator’s foraging range (Kearns et al. 1998). Lower reproductive success can result in reduced seedling recruitment and ultimately population decline (Tomimatsu and Ohara 2010). Establishment and expansion of populations are dependent upon spread of new individuals, therefore plant species that exclusively proliferate through sexual reproduction may have more limitations on population growth than species that reproduce asexually (Menges 1990). Therefore, the type of breeding system can have important ecological effects on population dynamics (Bierzychudek 1982).

Invasive species are ranked second as a threat to regional biota, affecting 57% of rare plants (Thomson, 2005; Wilcove et al. 1998). Invasive species are thought to compete with natives for resources, potentially driving rare individuals to extinction (Bomhard et al. 2005, Thomson 2005). According to Lyons and Schwartz (2001), the loss of rare species in a community is often assumed to open niches, and if dominant species cannot fill these open niches, invasive species may be able exploit the available resource or space. Therefore, the increase in invasive species coupled with the loss of native species can further reduce the diversity of the plant community. Invasive species establishment can be promoted by habitat disturbance, therefore land managers restoring natural disturbances must monitor for potential invasive establishment (Hobbs and Huenneke 1992).

Causes of rarity may also be multifactorial (Fiedler and Jain 1992). For example Thompson (2005) examined the effect of two factors, invasive vines and herbivory, on another endangered trillium, *Trillium reliquum*. He showed that invasive vines reduced recruitment of new *T. reliquum* seedling and herbivory reduced the successful production of seeds in *T. reliquum* (Thompson 2005). Therefore, one cause of rarity, such as encroaching non-native invasives combined with other causes of rarity, such as habitat fragmentation, may hasten the decline of a species (Jules and Rathcke 1999, Gonzales and Hamrick 2005).

Consequences of rarity

Because plants provide valuable contributions to their ecosystem, it is important to understand the consequences of their disappearance (Lyons and Schwartz 2001). While few studies actually quantify the contribution of rare species to ecosystem function, some studies suggest that less-common and rare species make significant ecosystem contributions (Lyons et al. 2005). In a study examining N uptake rates in alpine meadows among rare, subdominant, and dominant species, rare species retained more N, demonstrating that the loss of these species could reduce nutrient retention in a community (Theodose et al. 1996). High-diversity communities are more likely to recover from environmental stochasticity and disturbance than low-diversity communities, which further supports the idea that a community with higher biodiversity of less-common species can contribute to ecosystem maintenance (Chapin III et al. 1998, Chapin III et al. 2000). Species traits may regulate abiotic components such as nutrient cycling and retention in a habitat, therefore losing a species may change an ecosystem's ability to mediate energy and resources(Chapin III et al. 2000, Lyons et al. 2005).

Fragmented populations of rare plants are vulnerable to extinction due to environmental and demographic stochasticity and reduced genetic variability caused by inbreeding depression and genetic drift (Aguilar et al. 2008, Zietsman et al. 2008). While extinction is a natural process, human activity that increases spatial isolation of species drives organisms to extinction at an unnatural rate (Chapin III et al. 2000). Human activities can lead to increased spatial isolation and decreased population sizes, leading to changes in ecological and genetic properties of plant populations (Tomimatsu and Ohara 2002).

Demography and rare plants

Demographic traits such as survivorship, life stage transitions, and fecundity describe the intrinsic characteristics of individuals in a population (Menges 1990, Caswell 2009). Birth and death rates in a population determine population size, therefore quantifying mortality and fertility can predict a population's structure through time (Bierzychudek 1982). By studying the demography of a rare plant, factors restricting their abundance and distribution can be determined (Schemske et al. 1994).

One way to analyze demographic data is to construct projection matrix models, which are also known as transition matrix models. Projection matrix models analyze how individuals in a population move through life stages over time (Bierzychudek 1999, Caswell 2009). Projection matrix models are useful when studying rare species because they provide a measure of the health of the population and assess whether a population is growing, stable, or shrinking (Bierzychudek 1982, Schemske et al. 1994, Horvitz and Schemske 1995). Life stage-based projection matrices can determine which stage is the most sensitive to perturbations (Lefkovitch 1965). For example, a study by Crowder et al. (1994) on the endangered loggerhead sea turtle (*Caretta caretta*), determined that the survival of the large juvenile stage affected λ, or the long-term intrinsic growth rate of the population, more so than other life stages, which helped conservationists determine that particular life stage should be the focus of protection. Therefore, stage-based models can predict future trajectories in the population so that appropriate management methods can be developed and applied (Menges 1986a, Bierzychudek 1999).

Community structure associated with rare plants

The diversity of forest structure depends on the interaction of different plant species (Mirkin et al. 2010). Interactions such as symbiosis, trophic interactions, and competition for resources, as opposed to the general presence or absence of a species, determines characteristics of the ecosystem by modifying abundances or traits of species (Chapin III et al. 2000). Therefore, studying the forest community that interacts with a rare plant can elucidate whether this intrinsic interaction is affecting the abundance of the rare species (Wang et al. 2010).

Few studies actually quantify the effect of removal of a rare species on an ecosystem, but most support the idea that rare or less-common species contribute to ecosystem function with no studies reporting negative results to their presence (Lyons et al. 2005). For example, a case study about the removal of the rare *Trinia glauca* shows that the removal of a rare plant can have community level consequences when the food web is disrupted (Carvalheiro et al. 2008). When food webs between pollinators and plants are disrupted, secondary endangerment and/or extinction of pollinators can ensue (Dunne et al. 2002). This illustrates that maintaining diversity in an ecosystem through conserving a less-abundant organism is important.

***Trillium persistens*, a federally endangered herb**

The federally endangered perennial herb *Trillium persistens* is endemic to a mountainous region in northern Georgia and western South Carolina, most notably in four fragmented populations in and around Tallulah Gorge State Park within the Tugaloo watershed (Rayner 1984). The maximum distance between these four populations is 8.53 km (Rayner 1984). These populations may have been reduced by damming due to rising water levels as Yonah Lake formed. Though samples of *T. persistens* were first collected in 1937, *T. persistens* was determined to be a new species by Duncan et al. in 1971 (Duncan et al. 1971, Rayner 1984). *Trillium persistens* was officially listed as a federally endangered species on April 26, 1978 due to its restricted distribution and potential threats from development and silvicultural practices (Rayner 1984; U.S. Fish and Wildlife Service 1978).

Trillium persistens is generally found in mixed pine-hemlock-hardwood forests and contributes to the herbaceous layer of its community (Duncan et al. 1971). In Tallulah Gorge some fragmented populations also overlap with the threatened Table Mountain pine ecosystem, dominated by *Pinus pungens*. The Table Mountain pine ecosystem requires fire for establishment and maintenance though fire in Tallulah Gorge State Park has been restricted by human activity and has not been replaced by regular prescribed burns. No pine recruitment has occurred in any of the Georgia Table Mountain pine stands in the past 40-50 years (Brose and Waldrop 2006), but one prescribed burn in March 2007 was conducted in Tallulah Gorge within the range of *T. persistens* in an attempt to facilitate ecosystem recovery. Other prescribed burns within the park outside of the range of *T. persistens* have been conducted since that time. While reintroducing fire may help restore the Table Mountain pine ecosystem, it is questionable whether the endangered *T. persistens* located in the area will withstand the burn. Fire, necessary for the establishment and maintenance of the Table Mountain Pine ecosystem, could be a threat to *T. persistens* and may lead to a potential management conflict between the conservation of the rare plant and the threatened ecosystem.

Objectives

The objectives of this study were to 1) determine if an early-season prescribed burn affected *T. persistens* and *P. pungens* population dynamics 2) investigate germination patterns of

T. persistens and *P. pungens* under heat shock conditions and 3) examine population dynamics of *T. persistens* with demographic matrix models.

CHAPTER II

The Effect of Fire on *Trillium persistens* Demography

Introduction

Trillium persistens is a perennial herb that occupies an area with an 8 km radius along the Georgia-South Carolina border (Duncan et al. 1971, Rayner 1984). The species has only four known populations and was placed on the endangered species list in 1987 (Rayner 1984). It is located in fragmented populations in and around Tallulah Gorge State Park where it overlaps with the threatened Table Mountain pine ecosystem, dominated by Table Mountain pine (*Pinus pungens*). This ecosystem is fire-dependent (Zobel 1969), however burns in Tallulah Gorge State Park have been restricted by human activity and have not been replaced by regular prescribed burns. While reintroducing fire may help restore this endangered ecosystem, it questionable whether the *T. persistens* located in the area will withstand the burn, which may lead to a potential management conflict between protecting the rare plant and the threatened ecosystem.

Fire has historically played a role in shaping habitats by influencing the evolution of plant species within forests and grasslands (Barbour et al. 1999, Knapp et al. 2007). Plants within fire-evolved ecosystems often require fire to complete their life cycles (Keeley and Bond 1997). Fire-dependent species have life history strategies that include either allocating resources to resprouting following fire or by ensuring they have seeds accumulated in the seed bank (Clarke and Dorgi 2008). Dormancy of seeds in the seed bank increases the chance that seeds will germinate at a favorable time (Clarke and Dorgi 2008). Seeds in fire-prone habitats are often refractory seeds that require fire to germinate, and are cued to germinate after scarification by fire. Exposure to high heat, or heat shock, breaks the seed coat and allows for moisture to penetrate the seeds (Williams et al. 2003). Seeds have developed other germination cues over time, including response to combustion products such as smoke, elevated nutrient levels in the soil, and/or altered light intensity after fire (Thomas et al. 2003).

Factors that promote periodic fire are the presence of fuel, favorable weather conditions to allow for dry fuel, and a source of ignition (Barbour et al. 1999). Knapp et al. (2007) found that seasonality influences post-fire vegetation recovery, with communities receiving growing-season (spring through summer) and late-season (late summer and early fall) burns showing greater decline in species richness when compared with early-season burns. They also suggest that fire intensity varies more with fuel load than seasonality (Knapp et. al 2007). Fuel is a primary determinant of the intensity of fire. A more intense fire yields greater heat and rate of spread (Barbour et al. 1999). Intensity of fires in forests is classified into three major groups: ground, surface, and crown. Ground fires burn and smolder below the surface of the soil, and for habitats where fire has become infrequent, this type of fire can be destructive because it damages roots, tubers, and rhizomes (Barbour et al. 1999). Surface fires burn only along the soil surface, and are cooler, fast-moving fires that consume organic matter on the forest floor, open pine cones and scorch pine needles and bark (Aber and Melillo 1991, Barbour et al. 1999). Crown fires are above-ground burns and feature high flames that affect mature trees, and therefore are only seen in forests with tall trees and shrubs (Barbour et al. 1999).

Biotic and abiotic components of habitats

Fires can affect both the biotic and abiotic components of habitats. Biotically, fire affects forests by maintaining or increasing diversity in these habitats (Dumas et al. 2007). Because forest floor vegetation and herbaceous and woody shrubs are removed post-fire, diversity increases as fire-dependent plants and tree seedlings are able to emerge and compete. Abiotically, fire changes resource availability in a habitat (Brown and Smith 2000). Fire affects resource availability of the soil structure through ash deposits that contain macronutrients and through the loss of gaseous carbon and nitrogen from surface organic layers (Aber and Melillo 1991). Studies have indicated that ash can increase soil pH from 2 to 3 units and increase the negative surface charge of soil and, consequently, the cation exchange capacity (Molina et al. 2007). Fire itself is an abiotic factor, which can have the greatest effect on forests at the seedbank level (Tozer 1998). Biotic and abiotic effects combine when loss of layers of vegetation in forests after fire cause increases in light levels on the forest floor as well as transforming detritus into ash that ultimately alters nutrient and carbon flux in the habitat (Dumas et al. 2007).

History of fire suppression

In many areas of the United States, fire has been suppressed in ecosystems historically maintained by fire (Varner III et al. 2007). In the southeastern United States, fires from sources such as lightning and humans began to be suppressed in the 1920s as forested land was developed into plantation forests and croplands (Gilliam and Platt 1999, Mulligan and Kirkman 2002, Mulligan et al. 2002). Fire suppression along with logging and urbanization have fragmented and reduced vegetation in habitats dependent on fire, causing some of these habitats to become endangered (Heuberger and Putz 2003). Besides a reduction of fire-dependent habitat, fire suppression can cause increases in understory and overstory density, changes in species composition, and an increase in the buildup of leaf litter, which acts as fuel that alters the severity of fire (Varner III et al. 2007). Therefore, fire suppression affects both the range and the composition of vegetation within fire-prone habitats. The Table Mountain pine ecosystem has experienced range restriction as a consequence of fire suppression (Brose and Waldrop 2006).

Prescribed fire is used by land managers to maintain fire-dependent habitats and restore ecosystem function and structure (Knapp et al. 2007, Vermeire and Rinella 2009). Prescribed fires remove excess vegetation and reduce fuel load as well as restore cues for regeneration on which fire-dependent habitats rely (Knapp et al. 2007). Prescribed fires also clear herbaceous and woody shrubs, some of which may have grown due to lack of periodic fire and may be competing with native vegetation (Heuberger and Putz 2003, Garten Jr. 2006).

Prescribed burns must provide necessary life cycle cues for fire-dependent species, otherwise they will not be effective (Mulligan et al. 2002). Also, while some taxa have responded positively to prescribed fire management, it may be difficult to predict overall whether these methods will be effective for all habitats (Bradstock et al. 2006). Another problem occurs when managing the focal population interferes with other species in the community (Maina and Howe 2000). In situations such as these, proper management of one species may negatively affect non-target species. For example, in habitats where the ranges of fire-dependent and fire independent natives overlap, the addition of prescribed fire may effectively manage the fire-dependent species but may be incompatible with managing species independent of fire (Vitt et al. 2009).

Demography of rare plants

One way to evaluate the effect of fire on *Trillium persistens* is by studying its demographic traits (Gonzales and Hamrick 2005, Thomson 2005, Heckle and Leege 2006). Demographic traits include vital rates such as annual survivorship, population growth, life stage transitions, and fecundity (Menges 1990). Documentation of these traits can be used to project changes in population structure over time (Bierzychudek 1982). Demographic analyses can further estimate a population's growth rate and project future population dynamics, quantify age or stage distribution and reproductive efforts, and determine the contribution of stage or age class to the overall population growth rate (McPeek and Kalisz 1993).

Environmental conditions influence plant population demography by affecting plant growth, reproduction, and survivorship (Torang et al. 2010). Factors within forest communities that affect demographic parameters include herbivory and environmental stochasticity (Vitt et al. 2009). Herbivory as well as stochastic events that remove leaves reduce a plant's ability to manufacture and store carbohydrate reserves because leaves are a major photosynthetic organ in a plant (Lubbers and Lechowicz 1989). Without the necessary store of carbohydrates, an individual is less likely to transition to the next life stage and may even regress to a smaller size or life stage (Knight 2003). Nutrient stores contribute to the formation of fruit, so fruit size may be affected as well. Random changes in environmental conditions, or environmental stochasticity, can influence the establishment of new individuals as well as the survivorship of current individuals in a population. An example of a stochastic environmental event that affects forest communities is forest fires.

Moody Creek: a habitat for *Trillium persistens* and Table Mountain pine

At Tallulah Gorge State Park, which lies along the county line between Habersham and Rabun counties in northeastern Georgia, U.S.A., (Figure 2.1) *T. persistens* overlaps with the Table Mountain pine ecosystem at one fragmented site, Moody Creek

(34°42'29.83"N 83°22'16.69"W). Prescribed fire has been proposed to manage the Table Mountain pine, with one burn having been conducted in 2007.

Moderate-intensity fire like a surface fire has been suggested to be enough to regenerate *P. pungens*, the dominant fire-dependent tree species in the Table Mountain pine ecosystem (Brose and Waldrop 2006). Early-season burns tend to be less severe than late-season burns because fuels tend to be moist and less combustible (Knapp et al. 2007). Therefore, combining a moderate-intensity surface fire with early seasonality might not affect the growth of *T. persistens*, which would be dormant underground as rhizomes and seeds during that time (Knapp et al. 2007).

Seeds in the seedbank of a burned area experience a brief elevation in soil temperature, or heat shock, which is necessary for germination of some seeds in fire-dependent habitats (Williams et al. 2003). *Pinus pungens* cones are serotinous, however *T. persistens* fruits are not serotinous, meaning these seeds are released at maturation and not in response to fire. Therefore, application of heat shock could yield different germination rates between the two species.

In this study I examined the demographic traits of *Trillium persistens* as well as how return of fire to the habitat might affect *T. persistens.* The objective of this study was to examine the demography of *T. persistens* in a fire-dependent habitat. I hypothesized that fire would negatively affect the population dynamics of *T. persistens* because it not been documented to be a fire-dependent species. I predicted that the *T. persistens* reproductive success and density, and leaf litter depth would decrease in burned plots and that unburned and burned plots would have differing soil nutrient content. I also hypothesized that *P. pungens* seeds would have greater germination and viability when exposed to heat shock compared to *T. persistens* because *P. pungens* is a fire-dependent species.

METHODS

Study sites

I established a study site at Moody Creek, a wildlife management area in Tallulah Gorge State Park. At Moody Creek, the Table Mountain pine ecosystem and *T. persistens* co-occur. One prescribed burn was conducted in 2007 at this location, but the response of *T. persistens* was not studied and no burning occurred again until this study. This site was used to document the demography of *T. persistens* over two consecutive growing seasons and to study the effects of prescribed burning on the *T. persistens* population.

Trillium persistens **biology**

Trillium persistens produces a single flower with narrow white petals and uniformly-colored leaves. Upon aging, the white petals turn pink to purple (Duncan et al. 1971).The life history of *T. persistens* has not been formally studied, however this species exhibits the same life stages seen in other trilliums (Figure 2.2), which include seedling (single oblong-lanceolate cotyledon), juvenile (single ovate leaf), subadult (three leaves) and reproductive (three leaves and a flower) (Heckle and Leege 2006, Ohara et al. 2006). *Trillium persistens* flowers from early March to mid April and produces fruit from June to mid July. Unlike other trilliums that die back in the summer, these plants persist through September before dying back to become dormant underground as a rhizome during winter (personal observation; Duncan et al. 1971). Seeds of *T. persistens*, like other trilliums, have a lipid-rich, fleshy appendage called an elaiosome, which is attractive to ants as a food source (Ohara and Higashi 1987). Ants collect the seeds and transport them to their nests where the elaiosome is removed and eaten and the seeds are carried outside the nest (Rayner 1984) where they later germinate. Ants can disperse trillium seeds from 0.6 m to 3.3 m (Ohara and Higashi 1987). Little is known about the pollination biology of *T. persistens*, but potential pollinators include honeybees and other small bees, ants, butterflies, midges and wasps (Rayner 1984).

Table Mountain pine biology

Pinus pungens Lambert (Table Mountain pine) is an endemic conifer of the Appalachian Mountain region of eastern North America and is distributed from southern Pennsylvania to northern Georgia (Williams and Johnson 1990, Brose and Waldrop 2006). *Pinus pungens* is shade-intolerant, bears serotinous cones and is limited by the absence of fire, competition with shade-tolerant hardwoods, low seed rain and poor seedbed conditions (Zobel 1969, Williams and Johnson 1990). The Table Mountain pine ecosystem is comprised of stands of *P. pungens* on steep ridges in the southern Appalachians as well as other hardwood trees (Williams and Johnson 1990).

Experimental design

In September 2008, I flagged *T. persistens* individuals located in areas within the Table Mountain pine stands at Moody. Then in February 2009, prior to trillium emergence in the spring, I established 40 2x2 m plots containing the flagged *T. persistens*. I randomly divided the forty plots into the categories of burned (20) and control (unburned) (20). Burns in the burned plots were conducted with the assistance of the Department of Natural Resources. To prepare the burn plots, the burn crew used leaf blowers to add an extra 0.5 m perimeter buffer to control for moisture leachate as an edge effect from the non-burned exterior of the plot and prevent unwanted fire spread (Figure 2.3). If a large piece of wood crossed through the plot and into the buffer region, it was cut with a chainsaw at the edge of the 2x2 m plot and removed from the buffer in order to maintain the natural state inside the plots. The burns were conducted February 6, 2009. Fires were ignited with a cigarette lighter unless the fire would not start easily. Under those conditions we used a drip torch.

Following the burn and the emergence of *T. persistens* in Spring 2009, I censused each plot and recorded life stages. I also marked each individual with a numbered metal tag. I recensused the plots in July 2009 for fruit set to determines reproductive success. If fruits were present, I measured the diameter, or the widest point between two carpel ridges. I censused life stage again in April 2010. I also recorded reproductive success again in June 2010. Individuals that did not have fruit were noted with some plants being unpollinated (a dried flower was present), experienced herbivory (the flower-end was missing), or the fruit had already fallen from the plant.

To facilitate the study of both *P. pungens* and *T. persistens* at Moody Creek plots, I planted *P. pungens* seeds into each of the *T. persistens* plots in February 2009 post-burn. In order for the seeds to be planted in the same location in each plot, I used a PVC pipe grid covered with bird netting to ensure the seeds were planted with even distribution. I placed the grid over each plot and planted the seeds within marked coordinates. The average *P. pungens* cone contains 50 seeds with approximately 81% viability (Burns and Honkala 1990), therefore planting 24 seeds would elicit the effect of having approximately half a cone release seeds in the plot. After planting, I tagged each seed with a colored cocktail fork and monitored for germination throughout the course of the study. I purchased the *P. pungens* seeds from Sheffield's Seed Company, New York.

Demography and the effect of fire on *Trillium persistens*

I compared the density of *T. persistens* within plots for 2009 and 2010 to determine if density differed between years by treatment. I calculated density by dividing the number of individuals per plot by the plot area, which was 4 m^2. I used a t-test to compare density per plot between the burned treatment and control at Moody Creek in 2009. The 2009 data were square root transformed because they did not meet the assumption of normality. To compare density per plot between the burned and control treatments in 2010 I used a non-parametric Mann-Whitney U-test because these data did not meet the assumptions of normality and equal variance. All statistical analyses were conducted with JMP 8.0. (2010).

I examined stage structure and reproductive success of *T. persistens* between the burned and control plots at Moody Creek. To determine if stage structure varied by treatment, I used a chi-square analysis for both 2009 and 2010. To examine reproductive success, I determined percent fruit set by dividing the number of fruit per site by the number of flowers per treatment and multiplying by 100. I compared fruit set between the burned and control plots at Moody Creek with a chi-square analysis for 2009 and 2010.

Abiotic and biotic measurement

In February 2009, I used a trowel to take soil samples from the upper right-hand corner facing upslope of each plot to determine if there was a difference in soil nutrient content and pH after fire. Soil was taken from an area of approximately 5x5 cm to a depth of approximately 15 cm. In the field, samples were placed in re-closable polyethylene bags in the field, and then transferred to paper bags provided by the University of Georgia Laboratory for Environmental Analysis, where the samples were analyzed. I combined samples from two neighboring plots of the same treatment to meet the required volume. Total nitrate, total carbon, P, K, Ca, Mg, Zn, Mn, pH, and cation exchange capacity (CEC) were measured for each sample. To determine total C and N, 1 gram of combustion catalyst was added to weighed soil samples to ensure complete combustion before being incinerated in a furnace at 1350˚C. Combustion gasses were collected in a 4.5 L ballast tank before being allowed to flow into the detectors of the Leco 2000 analyzer. Infra-red absorption measurement was used for total C and thermal conductivity detection was used for total N. pH was determined with an H-sensitive glass electrode calibrated against solutions of known hydrogen ions. The ICP-MS, a mass-selective detector determined P, K, Ca, Mg, Zn, and Mn content in the soil samples through a semi-quantitative element scan called "TotalQuant". CEC was determined by displacing absorbed cations with a neutral salt ($BaCl_2$) and measuring the displaced cations using atomic absorption. Results reported in lbs/acre were divided by 2 to convert values to ppm (Miller et al. 2010). Nutrient content was compared between the control and pre-burn plot data using t-tests. Finally, pre-burn and post-burn data were compared to determine if nutrient content differed between pre- and post- burn to see if there was a significant difference between the nutrient content before and after the burn. Ca was the only micronutrient analyzed with a repeated measure ANOVA; I analyzed all other micronutrients with Wilcoxon Signed Ranks tests because they did not meet the assumptions of normality and equal variance.

I measured litter depth within each pre-burn, post-burn, and control plot. A ruler was inserted through the profile of the leaf litter until it came into contact with the mineral soil surface. Litter depth in pre-burn and control plots were compared because they had not been in contact with fire. A non-parametric Mann-Whitney U-test was used for the analysis because these data did not meet the assumptions of equal variance and normality. Finally, litter depth in pre-burn and post-burn was analyzed with a non-parametric Wilcoxon Signed Ranks test to determine if litter depth differed between the pre-burn and post-burn.

Heat shock experiment

I conducted a heat shock germination experiment (Young et al. 2007) to determine the response of seeds to heat stress. I harvested *Trillium persistens* seeds in July 2009 from a site not used in these analyses and allowed the seeds to dry at room temperature for one month before being placed in the refrigerator. Five replicates of 10 seeds (250 seeds total) were subjected to one of five heat treatments in a laboratory drying oven: no heat control, 70˚C, 85˚C, 100˚C, and 115˚C, with each heat treatment lasting 4 minutes. The duration of heat exposure and range of temperatures were based on previous studies of prescribed burns (Heyward 1938, Tozer 1998, Iverson and Hutchinson 2002, Sullivan et al. 2003). I placed a thermometer in the drying oven to ensure the temperature was maintained among replicates. The same heat shock experiment was applied to *P. pungens* seeds to determine whether there was a similarity with *T. persistens* between the temperatures they can withstand and still germinate, which would be beneficial knowledge for land managers. I acquired *T. persistens* seeds from the field at another population fragment (Panther Creek), and *P. pungens* seeds were acquired from Sheffield's Seed Company, New York.

After exposure to the heat treatments, I placed them on two sheets of filter paper within polystyrene Petri dishes. They were imbibed with enough deionized water to moisten the paper, exposed to 12:12 hr light:dark cycle for a period of three weeks. *Pinus pungens* germinate under a wide variety of light conditions (Barden 1979, Burns and Honkala 1990, Pavek 1993). Light was provided by 52 W soft white light bulbs. I watered and monitored the seeds daily and recorded germination. After 3 weeks, I tested the seeds that did not germinate for viability with a 1% triphenyl tetrazolium chloride (TTZ) test (Grabe 1970). TTZ reacts with hydrogen in the respiration process of living tissue, where dehydrogenase enzymes reduce the TTZ to formazan, a reddish, water-soluble compound (Patil and Dadlani 2009). Non-germinated seeds were placed in the solution for 24 hours, and then each seed was split in half with a razor blade to see if the interior was red, indicating viability. To test if germination was affected by heat shock treatment, I used a model I one-way ANOVA. The number germinated and the number viable were added to find total viability. I used the non-parametric Scheirer-Ray-Hare test to test for differences in total viability between *T. persistens* and *P. pungens* seeds and among heat treatments. To test if viability differed by treatment for each species, I used a model I one-way ANOVA. Significant data were further analyzed with the Bonferroni method (Sokal and Rohlf 1995).

RESULTS

Demography and the effects of fire on *Trillium persistens*

In 2009, I censused 67 *T. persistens* in the control plots and 116 in the burned treatment plots. In 2010, there were 75 individuals in the control and 100 in the burned treatment. Though the number of *T. persistens* increased in the control treatment and decreased in the burned treatment between 2009 and 2010, density did not differ significantly for both the burn and control treatments for both years (Figure 2.4). In the burned plots, density ranged from 0-4.5 and 0-2.75 *T. persistens*/m^2 in 2009 and 2010, respectively. In the control plots, density ranged from

0-4 *T. persistens*/m^2 for 2009 and 2010. Because density did not differ between treatments, fire did not have a negative effect for either years. Any effect of fire in 2009 did not have a lasting effect in 2010.

The prescribed burn at Moody Creek did not affect stage structure of *T. persistens* in 2009 (Figure 2.5 a). Additionally, fire from 2009 did not affect *T. persistens* in the 2010 growing season (Figure 2.5 b). In the burned treatment, the percent of seedlings and juveniles ranged from 51 in 2009 to 41 in 2010. In the control plots, they ranged from 35 in 2009 to 54 in 2010. The percent of subadults in the burned treatment ranged from 44 in 2009 to 45 in 2010. In the control, percent of subadults ranged from approximately 38 in 2009 to 40 in 2010. Few seedlings were found in burned treatment and control plots in both years. Besides seedlings, the reproductive life stage was the least represented for both the burned and control plots for both years.

In 2009, fruit set was approximately 50% greater in the control compared to the burned treatment (Figure 2.6), suggesting a negative effect of fire on fruit set. Fruit set did not differ between treatments in 2010, however, suggesting that fire effects were short-lived. A low number of reproductive *T. persistens* were present within both treatments for both years. In 2009, nearly half of the 14 reproductive individuals in the burned treatment produced fruit, versus all 4 in the control plots. Approximately 1/4 of the 21 reproductive individuals set fruit in the burned plots in 2010, versus only 1 out of 8 in the control plots.

Germination of *P. pungens* seeds within the burned and control plots was monitored throughout the course of the study. While *Pinus strobus* (white pine) seedlings were observed, no germination was seen in the out-planted *P. pungens* seeds within the plots. Similarly, few *T.*

persistens seedlings were seen for both 2009 and 2010. For 2009, no seedlings were observed for either treatment, however in 2010, 4 *T. persistens* seedlings were observed in control plots and 3 in the burned plots.

Abiotic and biotic measurement analyses

Soil micronutrients did not differ between pre-burn and control plots or, surprisingly, between pre- and post-burn (Table 2.1, 2.2). Litter depth did not differ significantly between the mean pre-burn and mean control samples (Figure 2.7 a). However, as expected, litter depth decreased by approximately 75% post burn (Figure 2.7 b).

Heat shock experiment

After heat shock, *T. persistens* seeds did not germinate and therefore could not be analyzed to determine if treatment affected germination. *Pinus pungens* seed germination was significantly affected by the heat shock treatments, however (Figure 2.8). Germination occurred at the highest proportion in the lowest heat treatment (70˚C) and germination was lowest at the hottest heat treatment (115˚C). After pair-wise comparisons of treatments, the 115˚C and control treatments were found to differ from the 70˚C treatment.

Heat treatment affected the total viability of *T. persistens* and *P. pungens* seeds (H = 9.49, DF = 4, P < 0.0001). However, species did not differ, nor was there an interaction between treatments and species. Total viability differed significantly for *T. persistens* and for *P. pungens* (Figure 2.9). The mean percent viability for *T. persistens* was 68.4% versus 78% for *P. pungens*. The TTZ test showed that of the 78 *P. pungens* seeds that did not germinate, 29.5% of the seeds were viable. For both species, the lowest viability occurred at 115˚C, with *T. persistens* also having a reduced viability at 100˚C. *Trillium persistens* seeds were more sensitive to heat than *P. pungens*. Viability in the remaining three treatments (70˚C, 85˚C, and no heat control) was statistically similar for both species. For the *T. persistens* seeds, control, 70˚C, and 85˚C viabilities were nearly three times greater than the 110 and 115˚C treatments.

DISCUSSION

In this study, three major results occurred in response to fire and heat shock, which include reduced *T. persistens* fruit set in the burned treatment, the lack of tolerance of *T. persistens* seeds to temperatures higher than 85˚C, and the significant reduction of leaf litter by fire. The percent fruit set in burned plots was reduced in 2009, which was an immediate effect fire. Therefore, the hypothesis that *T. persistens* in the burned treatment would have lower fruit set was supported. Fruit set time has been estimated for this species to be between June and July (Rayner 1984), however by the beginning of July 2009, most of the fruits had fallen away from the plants. Because many of the fruits in 2009 were gone by the July census, I censused for reproductive success approximately two weeks earlier in 2010. One difference between 2009 and 2010 is the number of plants missing by the time of census. In 2009 amongst the burned plots, there was one reproductive with a dried flower, indicating it had not been pollinated, two plants had herbivory of the fruit where the fruit was removed due to herbivory, and two plants were missing from plots. In 2010, two plants in the burned treatment and one in the control had fruits that experienced herbivory. The major difference between 2009 and 2010 is that 12 plants were missing from the plots. Because there were instances of herbivory on other fruits, one explanation for the absence of plants is deer browsing that occurred earlier in the season. Many trilliums are known to experience deer browsing, including *T. cuneatum, T. maculatum,* and *T. reliquum*, all of which occur in Georgia (Thompson 2007). These trilliums experienced a reduced number of individuals in the reproductive stage as well as decreased fruiting success (Thompson 2007), possibly because deer have been shown to prefer flowering plants over non-flowering plants (Augustine and Frelich 1998, Rooney and Gross 2003).

The heat shock experiment showed that *T. persistens* seeds were sensitive to any temperature over 85˚C. The lack of *T. persistens* germination, despite viability, could have occurred because these seeds require a longer germination period than three weeks. Previous research shows that other trillium species require double dormancy (Hanzawa and Kalisz 1993, Rooney and Gross 2003). In the first year, the species develops a primary root, or radicle, then in the second growing season, the seedling expands and becomes photosynthetic (Hanzawa and Kalisz 1993). The experiment was ended at three weeks, however, because mold was beginning to grow on the surface of the filter paper and seed coats, which could have potentially damaged the seeds if they were left for any longer.

The lethal effect of fire on seeds is dependent on the magnitude of heat along with the interaction of other variables, such as the insulation of soil and tissues from the surrounding environment (Vermeire and Rinella 2009). For surface fires, only about 5% of the heat released by fire affects soil temperature (Boerner 2000). Nonetheless, because this heat shock experiment was done in the absence of soil, future studies could expose seeds to both soil and heat to determine if and to what degree soil acts as a buffer, especially at higher temperatures, where seed bank mortality can range from 55 to 80% (Esque et al. 2010).

The temperature range used in the heat shock falls within the range observed at real prescribed fires, which vary from 84 to 195˚C at the soil surface (Glasgow and Matlack 2007). Temperatures ranging from 70 to 100˚C simulate the temperature of soil five centimeters below the soil surface during fire passage (Bossuyt and Honnay 2008), so this heat shock simulated the exposure of seeds to heat in the seed bank. Temperature decreases as soil depth increases, with soil depths of approximately 20 to 30 cm experiencing temperatures ranging from 35 to 55 ˚C during fire (Sackett et al. 1992).

Brose and Waldrop (2006) suggest a moderate intensity fire is enough for *P. pungens* to regenerate. From the heat shock test, *P. pungens* seeds were highly viable in all treatments except 115˚C, whereas *T. persistens* seeds were only viable up to 85˚C (Figure 2.16). Past 85˚C, viability of *T. persistens* dropped by about 50%. While *P. pungens* seeds may survive a hotter fire, the same fire could be lethal for *T. persistens*. Both *P. pungens* and *T. persistens* were had lower viability at 115˚C, with *T. persistens* having reduced viability at 100˚C as well. This suggests that if less-intense burns are used, then the health of the seed bank may be better preserved and both *T. persistens* and *P. pungens* may not be negatively affected.

Finally, leaf litter was significantly reduced within the burned plots compared to the control plots. Leaf litter plays a role in herbaceous communities (Facelli and Pickett 1991). When leaf litter is removed it no longer serves as a barrier to seed germination and establishment and light is able to reach the humus and mineral layer of soil (Hutchinson 2006). Leaf litter serves as fuel for fires, and was greater between pre-burn and control compared to post-burn, which was expected. Because leaf litter volume affects fire severity, fire-dependent habitats that do not have frequent fire can accumulate excess litter, causing fires when they do occur to be more severe (Murphy et al. 2006). Early season burns, like the one conducted in this study, consume less fuel and therefore decrease fire intensity and severity and can have less effect on herbaceous species (Knapp et al. 2007).

I expected that fire would negatively affect the stage structure of *T. persistens*, especially in year two of this study (2010) because the previous year's growing season affects the outcome of the next year. The results did not support the hypothesis because fire did not negatively affect the stage structure of *T. persistens* within the burned treatment for either growing season. One reason why *T. persistens* may not have been affected by fire is that, though this species does not have classic fire adaptations, it lives in a part of the country where it has had to deal with fire and fire-adapted species (Appendix C).

Dormancy is a trait that occurs amongst other trillium, such as *T. reliquum*, and other herbaceous perennials (Hanzawa and Kalisz 1993, Gonzales and Hamrick 2005, Heckle and Leege 2006, Shefferson 2009, Leege et al. 2010). While more individuals were added to the population in 2010 due to re-emergence from a dormant stage, other individuals present in 2009 died or went dormant and could not be censused in 2010. This study documents that dormancy did occur in 2009, because individuals larger than seedlings that were not in the census in 2009 were present for the 2010 census. Though there was emergence from dormancy in 2010, it was not enough to affect density. I predicted that density would decrease in plots that received the burned treatment, however the burned and control plots both exhibited no difference between years. This suggests that density was not affected by the fire.

The purpose of the prescribed burns was to provide information as to whether managing Table Mountain pine with fire would negatively affect *T. persistens* growth. Out-planting the *P. pungens* seeds was a method of comparing *P. pungens* germination between the burned treatment and controls. Because no planted seeds germinated, this would suggest fire did not affect the germination of *P. pungens,* however, it is highly probable that spring rains washed many of those seeds away. The rains created large washes in ravines throughout the range of the study (personal observation). Therefore, this outcome may not be a lack of fire effectiveness; rather, that the seeds may not have been present to germinate.

The prescribed fires used in this study differ from many other studies in that they were small plot burns. Few studies apply the method of using small plots for prescribed burns (Glasgow and Matlack 2007, Esque et al. 2010), with most studies focusing on the effects of prescribed burns on larger ranges (Heuberger and Putz 2003, Sullivan et al. 2003, Kobziar et al. 2006). Because there is little information differentiating between small plots fires and large fires, it is difficult to determine whether these results would be similar had the whole range been burned in one large treatment area. However, Glasgow and Matlack (2007) showed that even in their 2x2 m plots, fire effects were evident through the response in the understory within temperate deciduous forests in the eastern United States. They found cooler burning fires reduced leaf litter and increased germination of disturbance-adapted species from the seed bank.

It was expected that nutrient content would differ between the pre- and post-burn samples due to the effect of fire, however there was no difference for any of the measured nutrients. Nutrients that were anticipated to decrease post-burn, in accordance with previous studies, included total carbon, total nitrogen, and K (Murphy et al. 2006). Because this was a lower-temperature surface fire, carbon and nitrogen were expected to decrease because they volatilize at low burning temperatures (Neary et al. 1999). Other elements such as P, Mg, and Ca require higher burning temperatures, so little effect was anticipated for these nutrients. Soil pH was expected to increase between the pre- and post-burned treatments, and while it appeared to increase, it was not significant (Neary et al. 1999). The recovery plan for *T. persistens* by Rayner (1979) indicated that soil samples had not been analyzed from the Tallulah Gorge area, so the analyses in this study represent the first to provide information about the soil profile for that area.

For refractory seeds, or seeds that require heat for germination, mineral content of the soil and ash after a fire can act to fertilize seeds newly released post-fire (Barbour et al. 1999). While the mineral content may not affect the non-refractory *T. persistens* seeds, it may be necessary for *P. pungens* seeds. *Pinus pungens* seeds normally germinate post-fire because cones are serotinous (Zobel 1969), but it is unknown whether the seeds are refractory. The results from the heat shock experiment suggest *P. pungens* seeds are not refractory because more seeds germinated at a lower than higher temperatures. Barden (1979) observed about 40% of *P. pungens* cones open on their own while the rest appear to be permanently serotinous. Seeds from these cones could be opened with exposure to a 100˚C heat treatment no longer than four minutes (Barden 1979). While fire is needed to open a proportion of pine cones so seeds can be released, perhaps the seeds are exploiting soil nutrient changes post-fire after soil has returned to temperatures closer to pre-burn. If a surface fire cannot generate necessary micronutrients, then surface fire may not be ideal for *P. pungens*.

Management implications

This study provides documentation of the population structure of *T. persistens* at Moody Creek. This study also examined the effect of fire both in the field and the effect of fire on seeds within the lab. At Moody Creek, the 2009 burn did not negatively affect *T. persistens* population growth for 2009 and 2010. The heat shock experiment showed approximately a 50% reduction in viability at higher temperatures.

The fire for this study was conducted on February, 2008 before *T. persistens* were above ground. Combining the results of the field study and the heat shock experiment shows that *T. persistens* were not negatively affected except for the heat shock treatments above 85˚C. Future prescribed fires to manage *P. pungens* conducted at Moody under the same conditions of this study may have the same results. If prescribed fire is used in the future, land managers conducting burns should only use lower-temperature surface fires and follow burns with a census of *T. persistens* to determine any affect on stage structure, fruit set, density, and recruitment.

Table 2.1. Micronutrient content comparison between pre-burned and control plots. T-tests were used to analyze pre-burn and control.

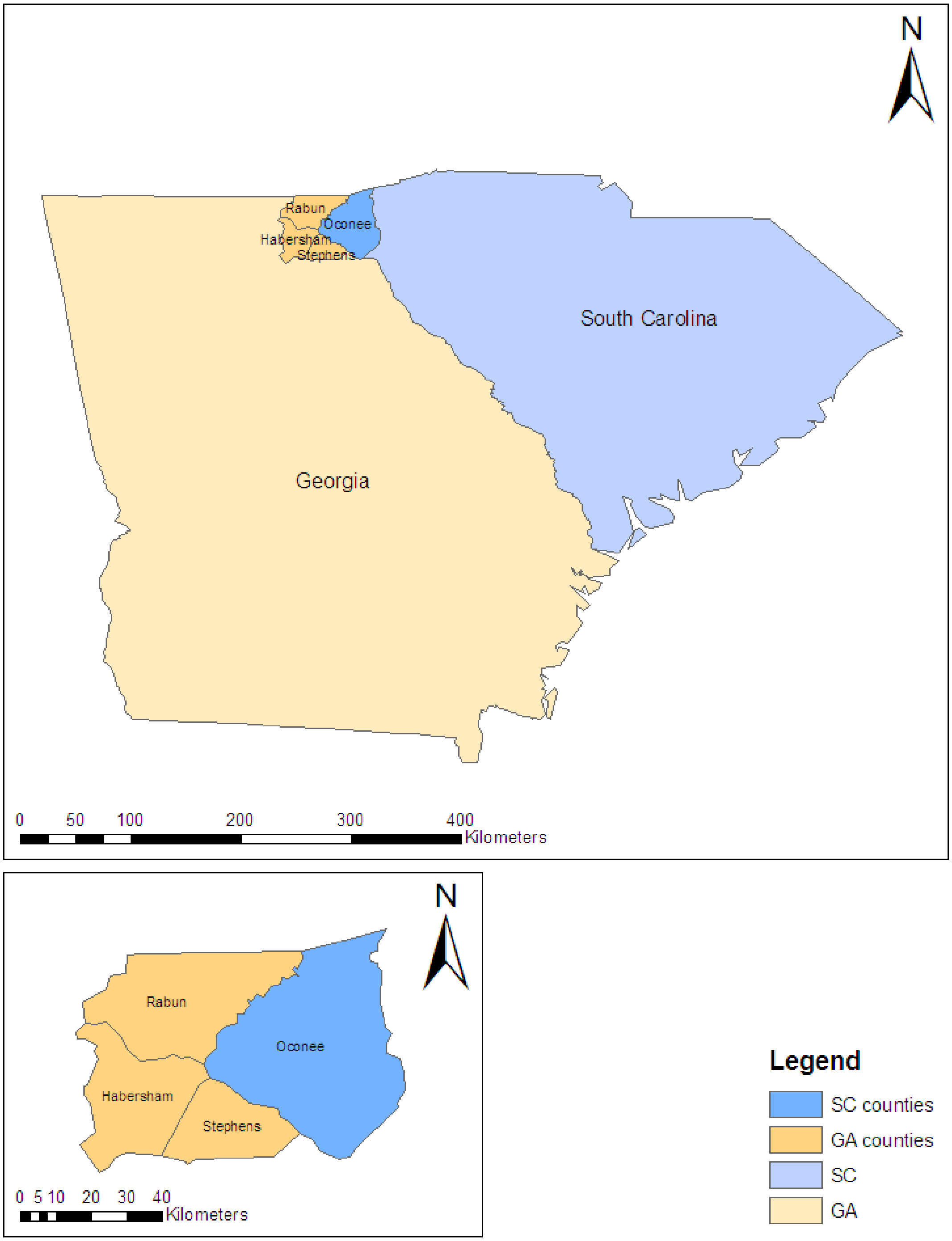
	Control (ppm)		***Pre (ppm)***		
Variable	***Range***	***Mean (±SE)***	***Range***	***Mean (±SE)***	***t***
Ca (ppm)	165.65-1021.00	819.900 (±285.04)	356.75-2010.00	470.630 (±132.11)	0.994
K (ppm)	79.15-166.2	112.080 (±16.07)	73.45-172.75	104.080 (±14.08)	0.337
Mg (ppm)	50.25-110.95	105.280 (±28.97)	50.55-229.15	72.305 (±8.16)	0.980
Mn (ppm)	61.60-85.40	89.060 (±10.65)	52.3-121.65	78.267 (±22.89)	0.382
P (ppm)	7.50-26.29	17.023 (±2.73)	12.68-27.20	13.603 (±2.02)	0.900
Zn (ppm)	1.94-4.76	4.624 (±0.85)	2.82-8.22	3.304 (±0.32)	1.300
CEC	4.56-13.92	8.089 (±0.42)	5.20-7.88	6.571 (±0.87)	1.406
pH	4.72-5.54	4.942 (±0.10)	4.77-5.48	5.114 (±0.11)	1.009
Total C%	5.40-28.3	9.400 (±1.14)	4.91-12.69	8.090 (±1.07)	0.749
Total N%	0.26-1.14	0.497 (±0.05)	0.24-0.59	0.394 (±0.06)	1.193

- $*$ $P > 0.05$, DF = 8

Table 2.2. Micronutrient content comparison between pre- and post-burn. A repeated measure ANOVA was used for Ca, with Wilcoxon Signed Ranks used for the others.

	Pre (ppm)		***Post (ppm)***			
Variable	***Range***	***Mean (±SE)***	***Range***	***Mean (±SE)***	***F***	***z***
Ca (ppm)	356.75-2010.00	819.900 (±285.04)	369.15-801.5	581.33 (±65.53)	0.4774	-
K (ppm)	73.45-172.75	112.080 (±16.07)	81.25-156.4	116.29 (±13.43)	-	1.5
Mg (ppm)	50.55-229.15	105.280 (±28.97)	47.08-101.95	78.52 (±10.67)	-	2.5
Mn (ppm)	52.3-121.65	89.060 (±10.65)	28.66-173.5	71.13 (±4.48)	-	-3.5
P (ppm)	12.68-27.20	17.023 (±2.73)	8.77-21.96	14.33 (±2.91)	-	-1.5
Zn (ppm)	2.82-8.22	4.624 (±0.85)	2.39-4.25	3.55 (±0.45)	-	-0.5
CEC	5.20-7.88	8.089 (±0.42)	5.08-10.80	7.28 (±1.51)	-	-2.5
pH	4.77-5.48	4.942 (±0.10)	4.50-5.23	5.14 (±0.12)	-	6.5
Total C%	4.91-12.69	9.400 (±1.14)	4.50-12.84	10.99 (±3.90)	-	-1.0
Total N%	0.24-0.59	0.497 (±0.05)	0.30-0.71	0.50 (±0.15)	-	-2.5

- ∗ $P > 0.05$, DF = 4



s

Figure 2.1. Counties of Georgia and South Carolina containing *Trillium persistens*.

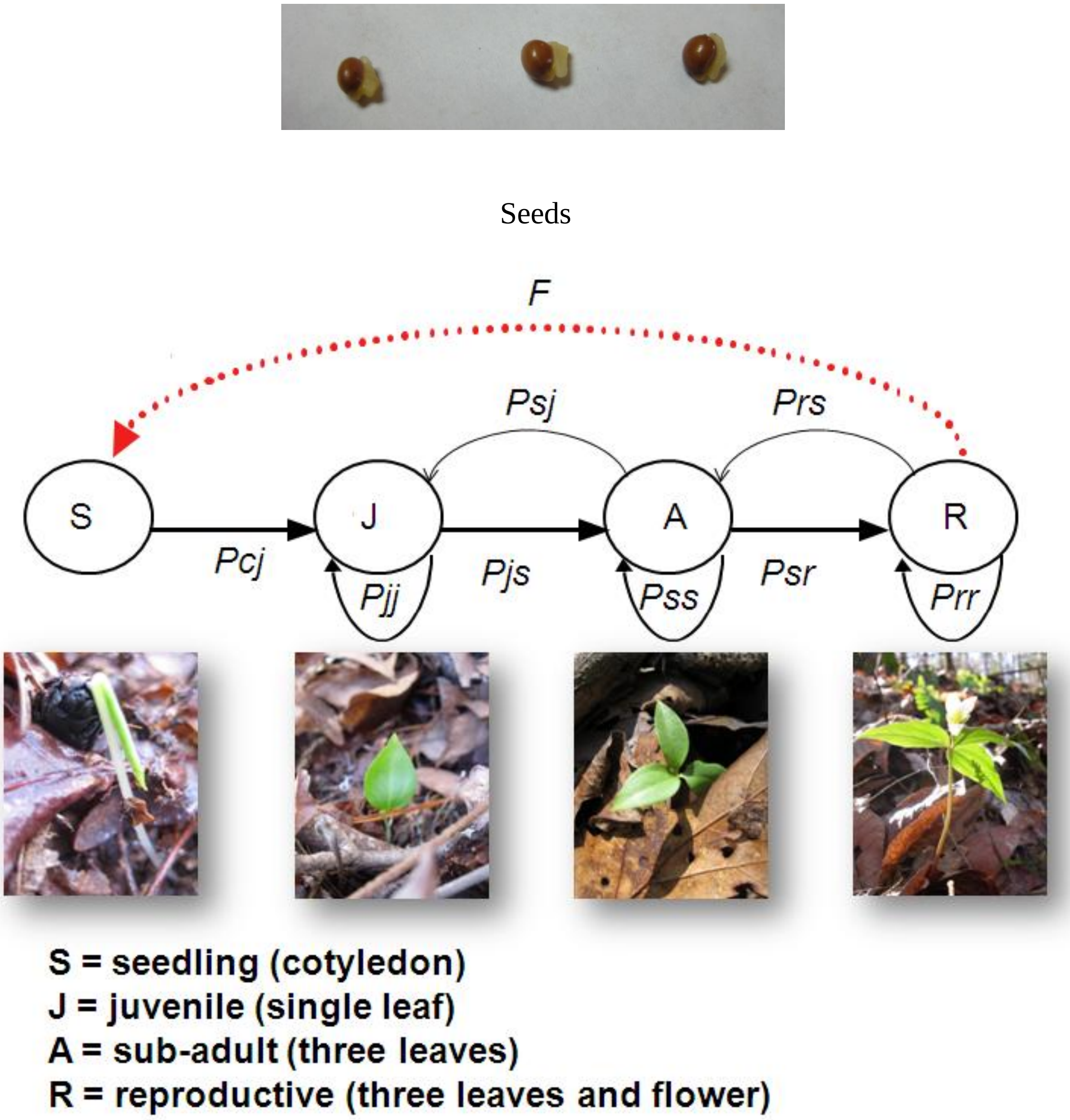


Figure 2.2. Stage-based life cycle diagram of *Trillium persistens*. *Pij* indicated the probability of an individual in stage *i* in year one transitioning to stage *j* in year two.

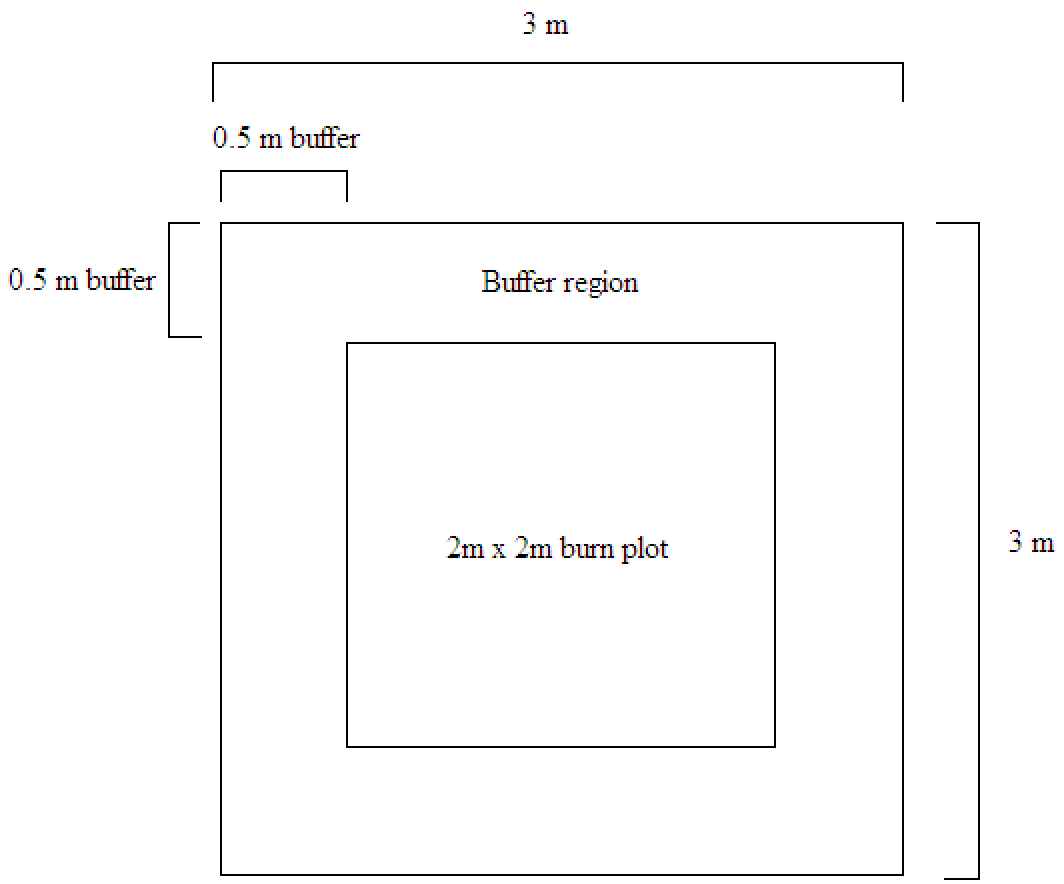


Figure 2.3. Diagram of 4 m^2 burn plot with 0.5 m buffer perimeter.

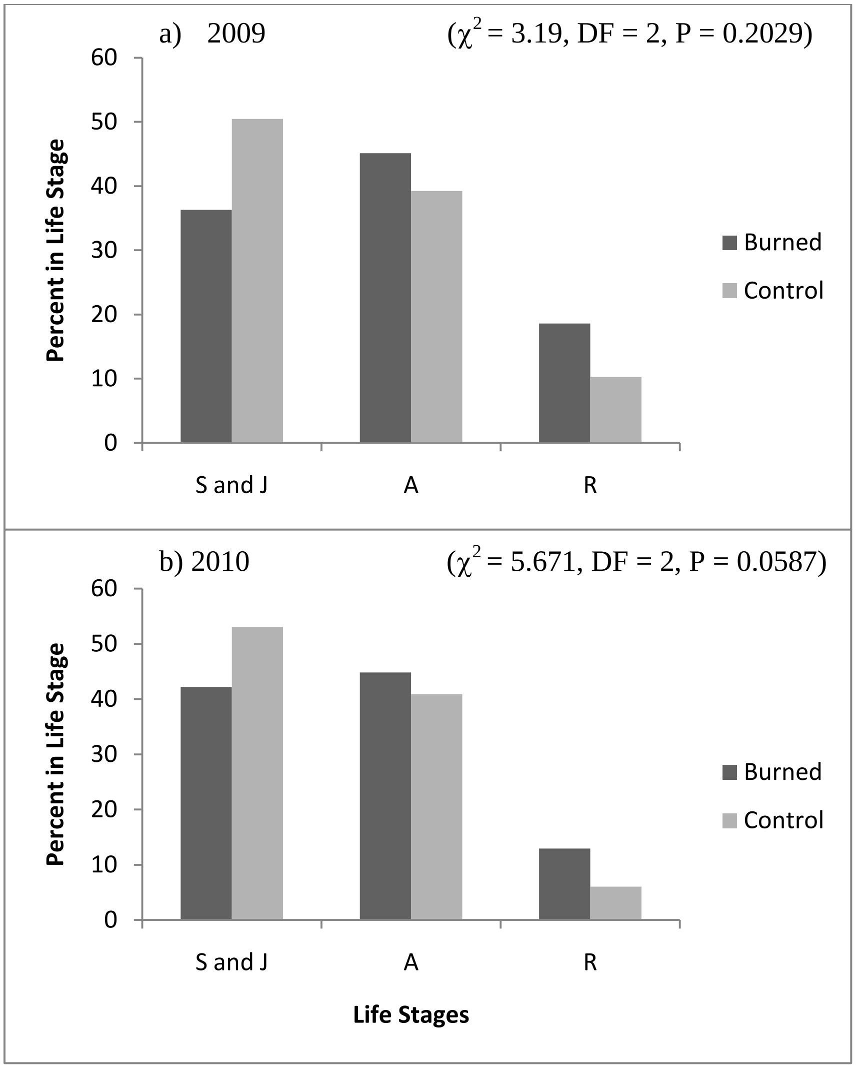


Figure 2.4. Stage structure presented as percent in life stage by site treatment at Moody Creek for a) 2009 and b) 2010. S = seedling, J = juvenile, A = subadult, and R = reproductive. S and J life stages were combined due to low S number.

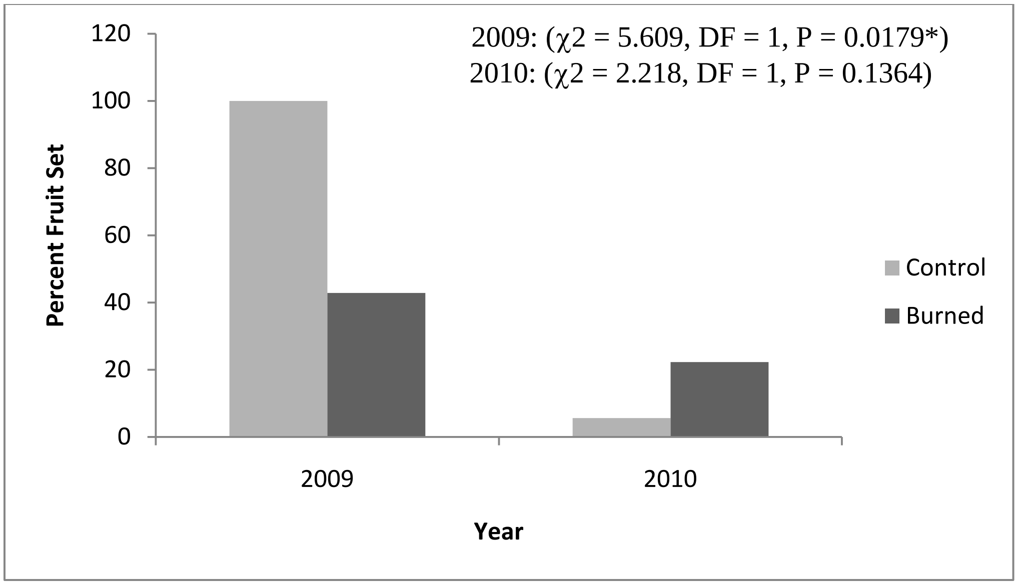


Figure 2.5. Percent fruit set for 2009 and 2010 between burned and control plots at Moody Creek.

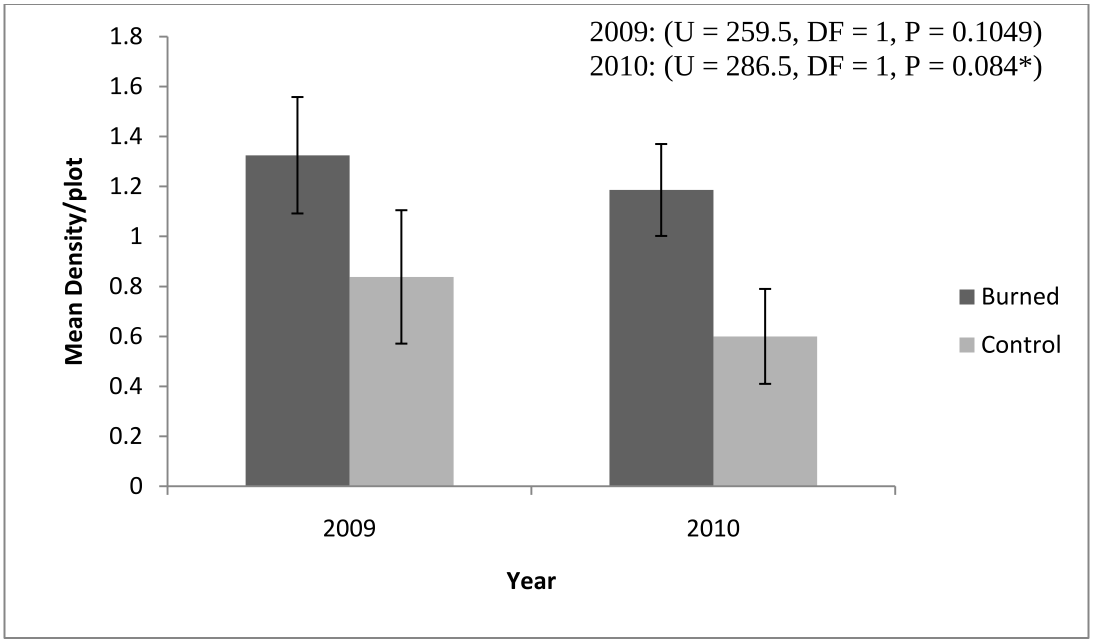


Figure 2.6. *Trillium persistens* density within plots for 2009 and 2010 between the burned and control plots at Moody Creek.

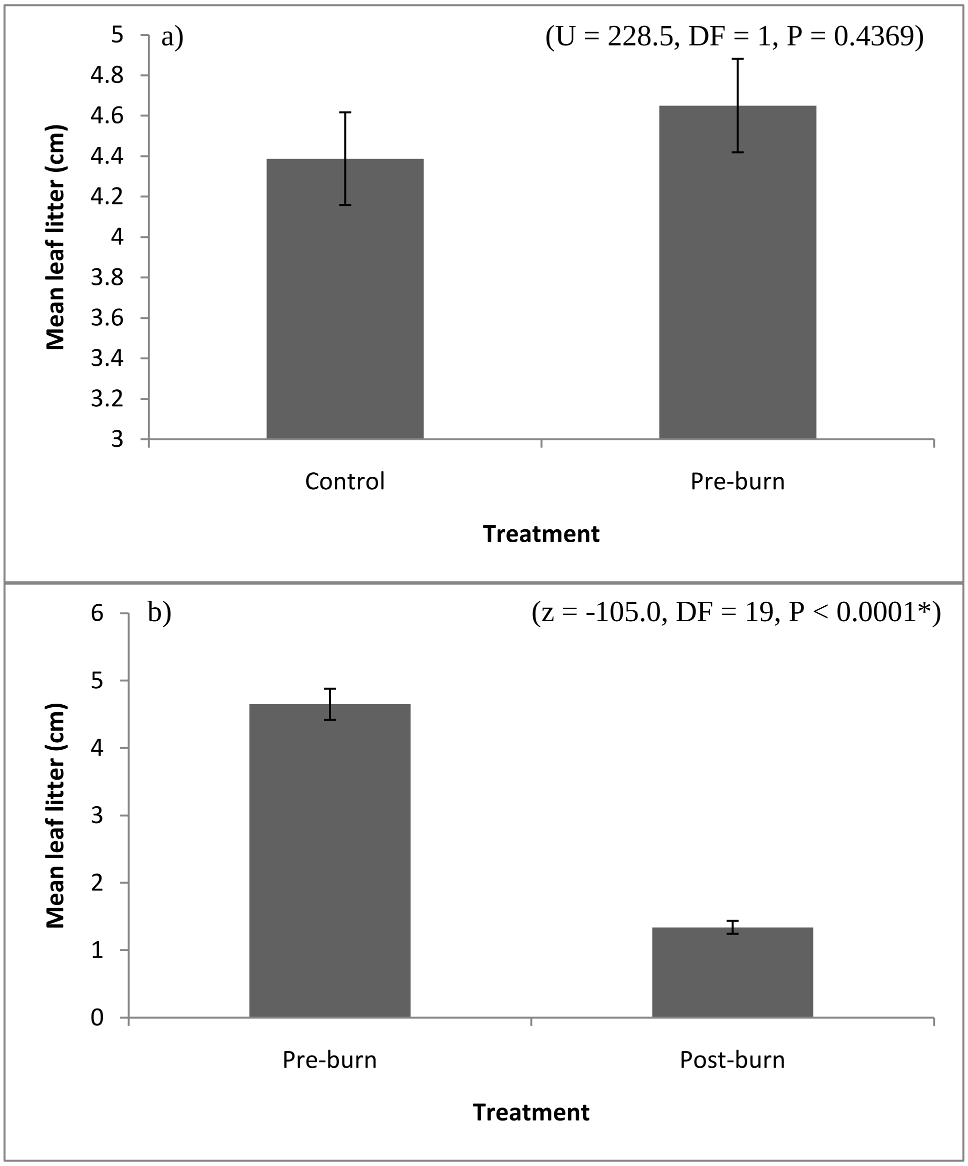


Figure 2.7. Mean leaf litter (cm) (±SE) at Moody Creek for the a) pre-burn and control and b) pre-burn and post-burn.

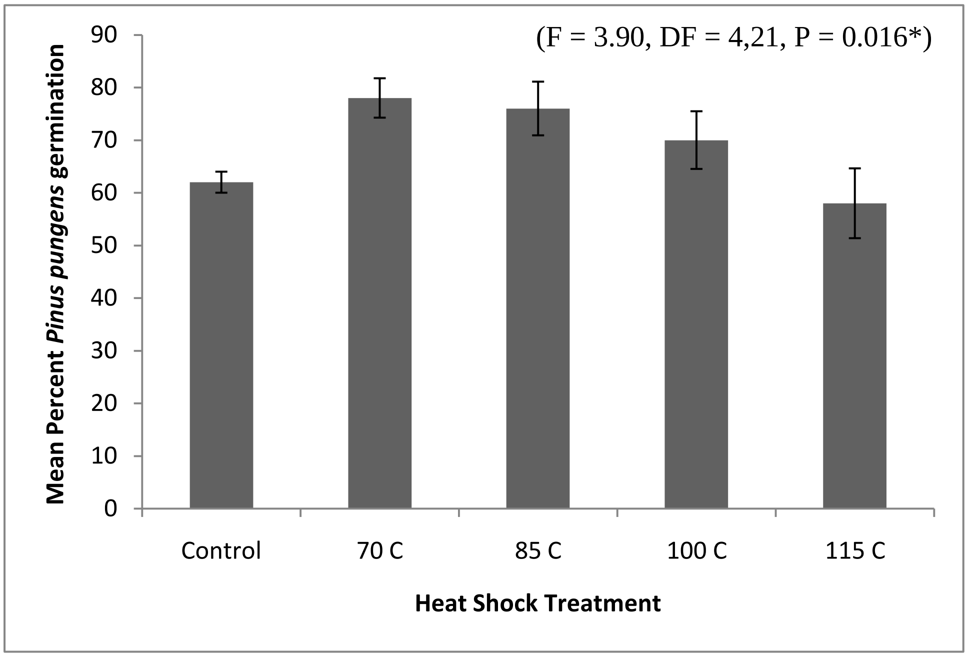


Figure 2.8. Mean percent *P. pungens* germination in response to heat shock treatments. Values represent mean ±SE.

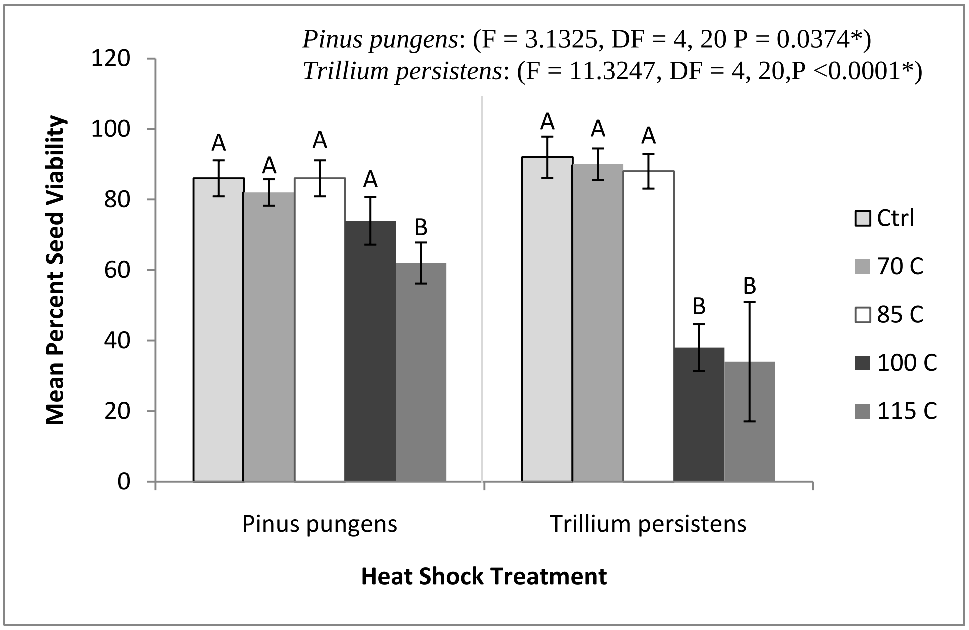


Figure 2.9. Mean total viability (±SE) of *Trillium persistens* and *Pinus pungens* for each heat treatment of the heat shock analysis.

CHAPTER III

Population Demography of *Trillium persistens*: Implications for Management

Demographic matrix models

Understanding life history traits of rare plants can provide information to help maintain populations (Ohara et al. 2006). The persistence of rare plants is often related to demographic traits and population dynamics, therefore it is important to characterize the demography of a species (Bevill and Louda 1999). An example of an endangered plant is in need of conservation is *Trillium persistens*. Demographic traits to evaluate include vital rates such as annual survivorship, population growth, changes between life stages, and fecundity (Menges 1990). Documentation of these traits can help project how population structure will change over time (Bierzychudek 1982).

While demographic information such as life stages and reproductive success can characterize *T. persistens* across its range, the use of demographic models can project vital rates into the future. Demographic models are used frequently in studies to assess imperiled plants and help determine where conservation efforts should be focused (Menges 1986b, 1990, Schemske et al. 1994, Pino et al. 2007). Demographic models examine the birth and death rates of individuals of each life stage within a population over time (Schemske et al. 1994, Barbour et al. 1999) and are an excellent method of assessing whether the population of a rare plant is stable, growing, or shrinking (Schemske et al. 1994). One example of a demographic model is the projection matrix model, which is based on the vital rates of stage or age classes of a population (Bierzychudek 1999). Projection matrix model techniques were first applied in studies by Leslie (1945) and Lefkovitch (1965), who examined age-class and stage-class structured populations, respectively.

A study by Bierzychudek (1999) illustrated that using projection matrix models can determine the health of a population of plants by investigating the changes in growth, survival, and reproductive schedules. Projection matrices are especially useful for understanding the dynamics of threatened and endangered species because they simulate population changes within age or stage structure over time (Menges 1990, McPeek and Kalisz 1993). These matrices can be used to investigate of life history traits (patterns of growth, reproduction and longevity) and can also be used to examine life stage-classified information of a plant's population (Bierzychudek 1982) because they use stage duration and initial rate of population change to calculate the probability of the growth to the next stage for each year (Crowder et al. 1994).

Elasticity and sensitivity analyses derived from projection matrix models have been used to determine the life stage or age transition that most affects λ (population growth rate) (Vellend et al. 2006). Conservationists can then work towards protecting the most sensitive life stage. Demographic models may be one of the best ways to assess the risks of rare plants (Schemske et al. 1994). By using stage based matrix models, life stages can be examined without studying individual plants over their life span, which is beneficial the case of long-lived rare plants such as *T. persistens*.

The goal of this study was to characterize demographic traits as well as construct projection matrix models of *T. persistens* across its range. Demographic traits include stage structure and reproductive success. From the projection matrix models, life stages that have the greatest impact on λ can be determined, which can help land managers conserve *T. persistens*.

METHODS

I established study sites in and around Tallulah Gorge State Park in northern Georgia within the counties of Habersham, Rabun, and Stephens in Georgia, and Oconee County, South Carolina, U.S.A. (Figure 3.1). One study site was established in each of the four *T. persistens* fragments at Battle Creek (34°42'25.14"N 83°20'43.73"W), Moccasin Creek (34°42'10.62"N 83°21'14.43"W), Moody Creek 34°42'29.83"N 83°22'16.69"W) and Panther Creek (34°40'37.07"N 83°20'20.13"W) (Figure 3.1, Appendix A). I used each of these sites to document the demography of *T. persistens* over two consecutive growing seasons between 2009 and 2010.

In Spring 2009 at Battle Creek and Moccasin Creek, I haphazardly selected 1x1 meter plots containing at least one individual *T. persistens* (Cox 1990). I added plots until at least 100 *T. persistens* were censused at each site. I set up sixty-nine plots at Battle Creek and 32 at Moccasin Creek. In each plot at all sites, I marked each individual with a metal numbered tag and recorded life stage. Because *T. persistens* were so dense at Panther Creek, I used two haphazardly-selected 10x10 m plots 10 m apart from one another. I gridded each 10x10 m plot into 100 1x1 m plots, then, using a random numbers table, I selected 1x1 m plots until 100 individuals were censused for each 10x10 m plot. At the first 10x10 m plot, I censused stage structure and reproductive success 25 1x1 m plots, and at the second 10x10 m plot, I censused 50 1x1 m plots. See Chapter 2 for census methods. To determine if stage structure varied by site, I used a chi-square analysis for both 2009 and 2010. I compared fruit set between sites with a chi-square analysis for 2009 and 2010. See Chapter 2 for Moody Creek sampling methods, stage structure, and fruit set results.

To establish the relationship between leaf size and fruit size, I measured leaf width and length for each *T. persistens.* I used leaf length and width measurements to calculate leaf area. To determine leaf area, I used the formula for an oval, which was then multiplied by the number of leaves (one for juveniles, three for subadults and reproductives).

$$\text{Leaf area} = \pi\left(\frac{\text{length}}{2}\right)\left(\frac{\text{width}}{2}\right)(\text{number of leaves})$$

To examine if there was a relationship between leaf area of reproductives and fruit size, I regressed fruit diameter against the leaf area of the parental plant for each site within 2009 and 2010. I analyzed all sites except Battle Creek in 2009 because no fruit were present at the census. Outliers were tested with either a Dixon's test for data series where n > 25, otherwise Grubbs' test was used (Sokal and Rohlf 1995).

Dormancy occurs for other trilliums, both common and rare (Hanzawa and Kalisz 1993, Gonzales and Hamrick 2005, Heckle and Leege 2006, Leege et al. 2008). New individuals in larger life stages were present in 2010 that were not present in 2009, so I could calculate proportion of *T. persistens* re-emergence in 2010. To calculate proportion of re-emergence for each life stage by site, I divided the number of re-emerged individuals in 2010 for each life stage by the total number of individuals within that life stage. I excluded seedlings because these individuals would be new to the population, and could therefore have not been dormant in the previous year. To determine if the proportion of re-emergence differed by for each life stage I used a chi-square At Battle Creek, no individuals re-emerged from dormancy in 2010, so I could not include it in the analyses.

Projection matrix models

To estimate population dynamics of *T. persistens* over time, I developed stage-based projection matrix models from *T. persistens* data collected at Battle Creek, Moccasin Creek, and Panther Creek as well as in the control and burned plots in Tallulah Gorge at Moody Creek. I censused the *T. persistens* demography at each site for life stages over two consecutive growing seasons (2009-2010) in order to develop the model. I identified stage classes for each individual at each site, and the matrix was formalized by the categories of life stage (seedling (S), juvenile (J), subadult (A), reproductive (R)). To determine the transition probabilities of *T. persistens* based on its life cycle I used census data from 2009 and 2010 (Table 3.1). I calculated the transition probabilities of marked individuals by dividing the total number of each transition type from year one to year two by the total number of individuals in a life stage in year one. Transition type included forward transition, stasis, or back-transition. *Trillium persistens* that were dormant in 2009 and 2010 were not included the matrix. Table 3.1 depicts the population transition matrix **A**, where a_{ij} represents the probability of an individual in stage i to transition to stage j in the next year (McPeek and Kalisz 1993).

In order to take into account multiple parameters that can influence fecundity, I calculated both a low estimate and high estimate for fecundity. I calculated the low estimate of fecundity by dividing the number of seedlings in a site in 2010 by the number of reproductives in 2009. For Moody Creek in the burned treatment and Panther Creek, this calculated value was 0, so I inputted the estimated value of 0.0001 make these matrices functional. I determined the high estimate of fecundity with the equation

$$\text{high estimate of fecundity} = \left(\frac{\#\ \text{fruit}}{\text{plant}}\right)\left(\frac{\text{average}\ \#\ \text{seeds}}{\text{fruit}}\right)(\%\ \text{germinable})$$

where # fruit/plant was the number of fruits in 2009 divided by the number of reproductives in 2009 for each site, the average # seeds/fruit was taken from separate censuses at Panther Creek and Moody Creek (Appendix B), and % germinable was determined by germination trials in the laboratory at Georgia Southern University, Statesboro, GA (See Chapter 2).

I conducted sensitivity and elasticity analyses on the stage-based projection models. The projection model was also used to determine λ, which is the dominant eigenvalue of a matrix and the population growth rate. I also determined population growth rate was by dividing the 2010 population size by the 2009 population size. When $\lambda > 1$, the population is growing, $\lambda = 1$ the population is stable, and $\lambda < 1$ the population is shrinking. The sensitivity of λ to a change in the a_{ij}th element of **A** is given by the equation

$$\frac{\delta\lambda}{\delta a_{ij}} = \frac{v_i w_j}{< \mathbf{w}, \mathbf{v} >}$$

where w represents stable stage distribution as the right eigenvector of **A**, and v is the left eigenvector corresponding to λ and represents the reproductive value of an individual, or the relative number of offspring an individual in a stage class will contribute in its lifetime to the population (McPeek and Kalisz 1993). Elasticity analyses quantify the change in λ to proportional changes in **A**

$$e_i = \frac{\delta(\ln\lambda)}{\delta(\ln a_{ij})} = \left(\frac{a_{ij}}{\lambda}\right)(\delta a_{ij})$$

with e_{ij} being the elasticity of each element (Kalisz and McPeek 1992). Elasticities are proportional sensitivities that determine which element within a projection matrix most affects λ.

Sensitivity and elasticity analyses and λ calculations were conducted with PopTools version 3.1.0 (2009).

Projection matrix models make assumptions about the conditions of populations. These assumptions include the dependency on current as opposed to previous life stages of each individual (Bierzychudek 1982). Also, results depend on conditions that define the matrix staying the same (Menges 1986). Projection matrix models must be modified in order to examine density-dependent population dynamics, therefore these models are otherwise based on density-independent growth (Leslie 1948). These assumptions require that the results be interpreted with caution. Finally, projection matrix models do not include additions to the population through dormancy.

RESULTS

Stage structure of *T. persistens* among the three sites differed for both 2009 and 2010 (Figure 3.2 a, 3.2 b). For both years, seedlings were the least represented of all life stages. Reproductives were the most represented among sites except for at Panther Creek in 2010, where the number of reproductives dropped and juveniles and subadults increased, compared to 2009. In 2009, the number of juveniles/plot ranged from 1-14 individuals. Subadults/plot ranged from 1-15, and reproductives ranged from 1-5. In 2010, juveniles/plot and subadults/plot ranged from 1-18, and reproductives/plot ranged from 1-9.

Percent fruit set differed among sites for 2009 however it did not differ for 2010 (Figure 3.3). In 2009, no fruit were found at Battle Creek, however Moccasin Creek and Panther Creek had nearly the same proportion. In 2010, percent fruit set increased from 2009.

Fruit diameter did not increase with leaf area in 2009 for any of the sites. In 2010, Moccasin Creek was the only site with a positive relationship between leaf area and fruit diameter (Figure 3.4). When one outlier in the Battle Creek data was removed, however, the slope became positive and the results were significant, which was not the case when one outlier was removed from Moccasin Creek. Fruit diameter from 2009-2010 ranged from 2.25-4.74 mm at Moccasin Creek, 6.59-5.09 mm at Panther Creek, and 5.42-5.25 mm at Moody Creek's burned treatment. Battle Creek and Moody Creek's control treatment only had one year's worth of data, however the average fruit diameter at Battle Creek in 2010 was 6.09 mm and at Moody Creek's control it was 3.36 mm. At Moody Creek, there was no relationship between leaf area and fruit diameter between treatments.

Proportion of re-emergence within larger life stages did not differ among sites (Figure 3.5). Individuals were more likely to re-emerge in the juvenile or subadult stage rather than the reproductive stage. The proportion of juveniles to re-emerge ranged from 0.12-0.37, and subadult re-emergence ranged from 0.036-0.25. Reproductive re-emergence ranged from 0-0.25.

The population growth rate (λ), calculated from the projection matrix model, estimates whether a population is growing, stable, or shrinking. Using the high estimate of fecundity in the matrix model, Battle Creek was the only population with $\lambda > 1$, predicting that the population is growing (Table 3.2). The other sites including Battle Creek's low estimate of fecundity had a $\lambda < 1$, which project shrinking populations. The population growth rate calculated from empirical data showed λ ranged from 0.85-1.6 with a mean of 1.08, which was higher than the means from the other calculation methods.

Forward transition indicates the proportion of individuals that progressed from a smaller life stage in year one to a larger life stage in year two. Back-transition refers to an individual in a larger life stage in year one becoming a smaller life stage in year two. Stasis is the maintenance of a life stage between years one and two. Rate of forward transition ranged from 0.02 to 0.39 for juveniles and subadults (Table 3.3). Some juveniles at Moccasin Creek, Moody Creek in the burned treatment, and Panther Creek were noted to have transitioned to reproductive, which is unusual for trilliums. Rate of back-transition ranged from 0.03 to 0.24 for subadults and reproductives.

Sensitivity and elasticity analyses were based off projection matrices using the low estimate and high estimate of fecundity (Table 3.4, 3.5). The matrix element that had the greatest impact on λ within both the sensitivity and elasticity analyses varied between sites and between fecundities used. For the sensitivity analyses at low estimate of fecundity, stasis as a reproductive had the greatest impact on λs for all sites except Moody Creek with plots receiving the control treatment and Panther Creek. For Moody Creek, progression from seedling to juvenile had the greatest impact on λ. At Panther Creek, the back-transition of reproductive to subadult had the greatest effect on λ. The elasticity analyses based on the low estimate of fecundity were in agreement with the sensitivity analyses except for one site. For Moody Creek control plots, the matrix element that most affected λ was stasis as a juvenile. At high estimate of fecundity, many of the matrix elements that were found to affect λ were the same compared to low estimate of fecundity. The only sites with different results were Battle Creek, Moccasin Creek and Panther Creek. For the sensitivity analysis at Battle Creek, the matrix element that most affected λ was the transition from subadult to reproductive; the elasticity analysis results were the same with low estimate of fecundity with reproductive stasis affecting λ the most. At

Moccasin Creek, progression from seedling to juvenile was the matrix element that most affected λ for the sensitivity analysis; the elasticity analysis was unchanged with stasis as a reproductive most affecting λ. Finally, the matrix elements for both the sensitivity and elasticity of Panther Creek changed, with seedling to juvenile and reproductive stasis affecting λ the most, respectively.

DISCUSSION

This study shows that among sites, *T. persistens* varies in stage structure and reproductive success between years. Stage structure for 2009 and 2010 differed significantly between Battle Creek, Moccasin Creek, and Panther Creek. The largest proportion of individuals was in the reproductive stage in 2009 which suggests there is the potential for a large contribution for the future generation. This is further supported in 2010 because the number of seedlings appears to increase, presuming the seedlings in 2010 are from the seeds reproductives deposited in 2009. This would not be the case if *T. persistens* undergoes double dormancy, which has not been determined. Stage structure differed in 2010 because the largest groups were the juveniles and subadults at Battle Creek and Panther Creek, specifically. These groups could have increased because of transition from a subsequent life stage, back-transitioning from a previous life stage, or dormancy. Dormancy, which is a trait noted amongst other trillium and forest herb species (Thompson 2007, Shefferson 2009) , was observed for *T. persistens* because individuals in larger life stages that were not present in 2009 emerged in 2010. Also, if the number of reproductive individuals decreased, then the juveniles and subadults would increase proportionally.

Percent fruit set differed among sites for 2009 however it did not in 2010. One reason for is because I censused reproductive success approximately two weeks earlier in 2010 than I did in

2009. While previous literature suggests that *T. persistens* sets fruit between June and July, most of the fruits had fallen away in 2009 in early July. Therefore, I censused earlier in 2010 so I could capture the measurements of more fruits before they fell away or the whole fruit was lost due to deer herbivory, which is common among trillium (Rooney and Gross 2003, Vellend et al. 2006, Thompson 2007, Leege et al. 2010).

Because leaves are major photosynthetic organs, it was expected that as leaf area increased in parental plants, fruit size would increase because larger-leaved plants would be able to store more carbohydrates, which could then be invested in fruit production. Sites differed in fruit sizes, with fruit diameter relatively similar between small and large plants in 2009. In 2010, as plant size increased, so did fruit size. However, there was variability between leaf size and fruit diameter between years, perhaps other variables are acting to perturb this relationship, such as herbivory and variation in resources across the range of sites.

While the proportion of re-emergence within larger life stages among sites did not differ significantly, this study shows that dormancy plays a large role in the life cycle of *T. persistens* because the proportion of re-emergence was as high as 0.37. The role of re-emergence from dormancy can be further illustrated by comparing *T. persistens* with another rare trillium, *T. reliquum.* In a study by Thompson (2007), the highest proportion of 0.1, nearly ¼ that than found for *T. persistens*.

Demographic matrix models

Matrix models suggest that *T. persistens* is in decline within each of the four sites sampled for both the low and high estimates of fecundity (Table 3.3). The λ calculated with empirical data was >1, however, indicating the populations are growing. This calculation takes into account dormant individuals, whereas matrix projections do not. Sites where many dormant individuals re-emerged in 2010 were added have a higher empirical λ than estimated by matrix models, which excludes individuals re-emerging from dormancy. For example, Moody Creek's empirical λ is about twice as high than the other two estimates, which is a result of many dormant individuals from 2009 re-emerging to the population in 2010. Because this study shows that dormancy plays a significant role in the life cycle of *T. persistens*, adding a dormant stage to future matrices will give a more realistic interpretation with which to project *T. persistens* population demographics.

The results of the sensitivity and elasticity analyses show that subadults and reproductives most affect population growth of each site. The matrix models can be manipulated, however, to alter the results. Manipulating matrix models is useful in cases where there is a non-functioning matrix. For instance, I inserted a low estimate of the S to J transition 0.0001 at Moccasin, Moody Creek for both treatments, and at Panther Creek in order to prevent non-functional matrices. The highest measured seedling to juvenile transition rate was 1.00 at Battle Creek. If the estimated values at each of these sites is replaced with what was actually at Battle Creek, along with setting fecundity to the highest value, Moccasin Creek and Panther Creek exhibit healthier populations, which was not the case for Moody Creek. Therefore, if efforts are made to maintain those individuals that contribute seeds to the population along with protecting the seedling life stage, populations may exhibit growth.

Population decline may be a result of alteration to demographic traits that cause population density to change. Stage transitions in an individual can be affected by resource allocation. A study by Hanzawa and Kaliz (1993) showed that there is a trade-off in allocating resources between growth, reproduction, and storage. Therefore, if a plant allocates resources in one year to reproduce, the next year it may not have enough resources to remain reproductive, so it will become a subadult. For growing seasons where resources are exceptionally limited, an individual may even be dormant for the next growing season.

Management implications

From the empirical λs, the populations appear to be stable from year-to-year. However, more research can help determine threats to *T. persistens* population growth. For example, sites where 1.00 was substituted in the S-J transition, populations were still in decline, however models with the substituted S-J transition plus the higher fecundity appeared healthier. The substituted value, originating from Battle Creek's S-J transition, only represented the transition of a total of 3 individuals from seedlings to juveniles. With that in mind, land managers should be aware that the recruitment and survivorship of a few individuals could have an impact on a population.

The most represented life stage among sites between 2009 and 2010 was reproductives. Despite a large number of reproductives, recruitment was poor between years. One explanation for low seed establishment is that *T. persistens* may require double dormancy, meaning seeds that germinated during this study were produced from reproductives from several seasons ago. I observed that *T. persistens* seeds planted out in the field in 2009 for another study had not germinated in 2010, which further supports this species requiring double dormancy. Future studies should examine why establishment and recruitment of seeds is so low despite a large proportion of reproductives. One way to increase seedling number is to supplement the population is to germinate seeds in the lab and plant them at each site. Germination of *T. persistens* seeds did not occur in the lab for this study (see Chapter 2). One reason is because some trilliums are found to be skotomorphic, meaning seed growth does not require direct sunlight (Gyer 2007). Therefore, one way to improve germination in the lab is to germinate seeds in the dark.

Fruit set was highly variable among sites. One explanation for differences in fruit set could be deer browsing, which is common among trilliums. Research on deer browsing of the endangered *T. reliquum* has illustrated deer herbivory can have a negative impact on plant populations, unless deer are excluded from plants (Thompson 2007, Leege et al. 2010). Future research could include exclusion of deer from *T. persistens* to see if herbivory is reduced and if fruit set is affected.

This study examined how fruit diameter related to plant size, however another relationship to study is if fruit diameter relates to seed number. Other studies on trilliums have related fruit diameter to seed number (Heckle 2004). From data at Panther Creek and Moody Creek, the relationship between fruit diameter and seed number was positive (Appendix B). These relationships can combine so plant size can be used as an indicator of seed number.

Because this study only captures data from 2009 and 2010, it does not count for the individuals that were present in 2009, but non-emergent in 2010. These individuals may emerge in 2011, so adding data from an extra year can better depict population dynamics of *T. persistens* and increase the reliability of the matrix models. Until dormancy is added to the models, the empirical λs illustrate more accurately the population growth rate of *T. persistens*. Empirical λs, however, only take into account conditions experienced by *T. persistens* in 2009 and 2010, so if stochastic events occur after 2010, empirical λs could change.

Finally, future research should focus on recording the number of *T. persistens* among sites. The number present is not the only important factor, however, because fruit and plant size can also indicate the health of the species. If individuals are reducing in size and reproductive success, it could indicate decay of the species, despite the number of individuals per site (Thompson 2007). Also, future research should focus on collecting another census year for *T. persistens* and add dormancy to the matrices in order for these matrices to be more accurate.

Table 3.1. Stage-based transition matrix model depicting possible transitions for *Trillium persistens.* Pij represents the probability of an individual in stage i to transition to stage j in the next year. F is fecundity, calculated as the number of seedlings in year t+1 contributed by reproductive individuals in year t. S = seedling, J = juvenile, A = subadult, R = reproductive.

Life Stage in Year 2	**Life Stage in Year 1**			
	S	**J**	**A**	**R**
S	-	-	-	*F*
J	*Psj*	*Pjj*	*Paj*	-
A	-	*Pja*	*Paa*	*Pra*
R	-	*Pjr*	*Par*	*Prr*

Table 3.2. Population growth rate (λ) for each study site using fecundity calculated with a low estimated value or a high estimated value.

Population Growth Rate			
Site	**λ (with low estimate of fecundity)**	**λ (with high estimate of fecundity)**	**Empirical λ (N_{t+1}/N_t)**
Battle Creek	0.81	1.14	0.85
Moccasin Creek	0.97	0.97	1.02
Moody Creek-Burned	0.80	0.80	0.97
Moody Creek-Control	0.76	0.76	1.60
Panther Creek	0.87	0.87	0.98
Mean λ (±SE)	0.84 (±0.04)	0.91 (±0.07)	1.08 (±0.13)

Table 3.3. Projection matrix models. S = seedling, J = juvenile, A = subadult, R = reproductive. fecundity is depicted as a range from the low estimated value to the high estimated value.

.

Battle Creek	**Life Stage in Year 1**			
Life Stage in Year 2	**S**	**J**	**A**	**R**
S	0.00	0.00	0.00	0.068-3.545
J	1.00	0.40	0.06	0.00
A	0.00	0.29	0.53	0.03
R	0.00	0.00	0.19	0.74
N in 2009	3	35	101	34

Moccasin Creek	**Life Stage in Year 1**			
Life Stage in Year 2	**S**	**J**	**A**	**R**
S	0.00	0.00	0.00	0.048-4.483
J	0.00001	0.60	0.06	0.00
A	0.00	0.20	0.59	0.24
R	0.00	0.07	0.29	0.76
N in 2009	2	15	95	17

Moody Creek-Burned	**Life Stage in Year 1**			
Life Stage in Year 2	**S**	**J**	**A**	**R**
S	0.00	0.00	0.00	0.796-1.236
J	0.00001	0.43	0.06	0.00
A	0.00	0.20	0.53	0.14
R	0.00	0.02	0.13	0.71
N in 2009	0	49	53	14

Moody Creek-Control	**Life Stage in Year 1**			
Life Stage in Year 2	**S**	**J**	**A**	**R**
S	0.00	0.00	0.00	0.125-0.309
J	0.00001	0.67	0.10	0.00
A	0.00	0.24	0.50	0.00
R	0.00	0.00	0.17	0.75
N in 2009	0	33	30	4

Panther Creek	**Life Stage in Year 1**			
Life Stage in Year 2	**S**	**J**	**A**	**R**
S	0.00	0.00	0.00	0.866-4.051
J	0.00001	0.64	0.16	0.00
A	0.00	0.15	0.40	0.11
R	0.00	0.06	0.39	0.73
N in 2009	0	80	102	44

Table 3.4. Sensitivities and elasticities from all sites using low estimate of fecundity. S = seedling, J = juvenile, A = subadult, R = reproductive, F = seeds (fecundity). The highest value by site is bolded.

	Transition									
	S-J	**J-J**	**J-A**	**J-R**	**A-J**	**A-A**	**A-R**	**R-A**	**R-R**	**R-S**
Site	***Sensitivity***									
Battle Creek	0.0316	0.0986	0.1406	0.0000	0.1439	0.2053	0.2525	0.5343	**0.6569**	0.4879
Moody Creek-Burned	0.0000	0.0252	0.0386	0.0674	0.1635	0.2506	0.4379	0.4145	**0.7243**	0.0000
Moody Creek-Control	**1.8872**	0.7345	0.2826	0.0000	0.6850	0.2636	0.0000	0.0000	0.0019	0.0002
Moccasin Creek	0.0192	0.0449	0.0605	0.0698	0.2643	0.3560	0.4049	0.5267	**0.5991**	0.0000
Panther Creek	0.0001	0.1489	0.1692	0.1386	0.2095	0.2380	0.1949	**0.7487**	0.6130	0.0000
Site	***Elasticity***									
Battle Creek	0.0392	0.0488	0.0497	0.0000	0.0106	0.1359	0.0588	0.0197	**0.5981**	0.0364
Moody Creek-Burned	0.0000	0.0136	0.0099	0.0017	0.0116	0.1663	0.0726	0.0744	**0.6499**	0.0000
Moody Creek-Control	0.0000	**0.6443**	0.0902	0.0000	0.0901	0.1734	0.0000	0.0000	0.0019	0.0000
Moccasin Creek	0.0000	0.0277	0.0125	0.0047	0.0172	0.2160	0.1228	0.1276	**0.4715**	0.0000
Panther Creek	0.0000	0.1096	0.0293	0.0100	0.0393	0.1105	0.0882	0.0982	**0.5148**	0.0000

Table 3.5. Sensitivities and elasticities from all sites using high estimate of fecundity. S = seedling, J = juvenile, A = subadult, R = reproductive, F = seeds (fecundity).

	Transition								
	S-J	**J-J**	**J-A**	**J-R**	**A-J**	**A-A**	**A-R**	**R-A**	**R-R**	**R-S**
Site	***Sensitivity***									
Battle Creek	0.1513	0.2136	**0.5511**	0.0000	0.1037	0.2675	0.8241	0.1252	0.3858	0.0427
Moody Creek-Burned	0.4200	0.0252	0.0386	0.0675	0.1635	0.2506	0.4379	0.4144	**0.7242**	0.0000
Moody Creek-Control	**4.6401**	0.7324	0.2819	0.0000	0.6830	0.2629	0.0003	0.0000	0.0047	0.0002
Moccasin Creek	**1.8039**	0.0450	0.0606	0.0689	0.2642	0.3560	0.4049	0.5267	0.5990	0.0000
Panther Creek	**3.0813**	0.1490	0.1693	0.1387	0.2094	0.2380	0.1949	0.7485	0.6129	0.0000
Site	***Elasticity***									
Battle Creek	0.1330	0.0751	0.1385	0.0000	0.0054	0.1258	0.1363	0.0033	**0.2495**	0.1331
Moody Creek-Burned	0.0000	0.0136	0.0099	0.0017	0.0116	0.1663	0.0726	0.0744	**0.6499**	0.0000
Moody Creek-Control	0.0001	**0.6425**	0.0899	0.0000	0.0899	0.1730	0.0001	0.0000	0.0046	0.0001
Moccasin Creek	0.0000	0.0278	0.0125	0.0047	0.0172	0.2160	0.1228	0.1275	**0.4715**	0.0000
Panther Creek	0.0000	0.1097	0.0293	0.0100	0.0393	0.1105	0.0882	0.0982	**0.4715**	0.0000

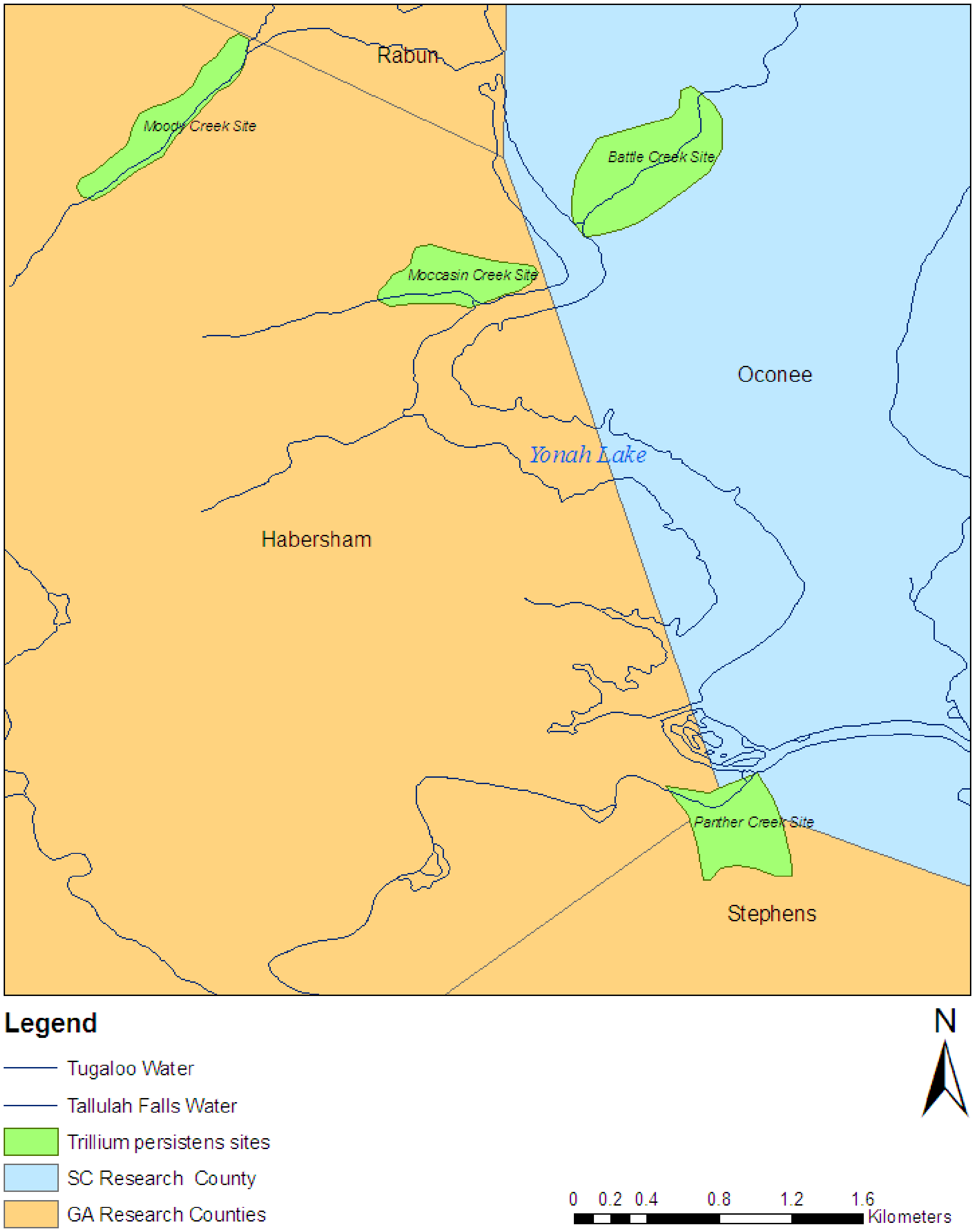


Figure 3.1. The distribution of *Trillium persistens* between Georgia and South Carolina. Panther Creek, Battle Creek, Moccasin Creek and Tallulah Gorge fragments are outlined.

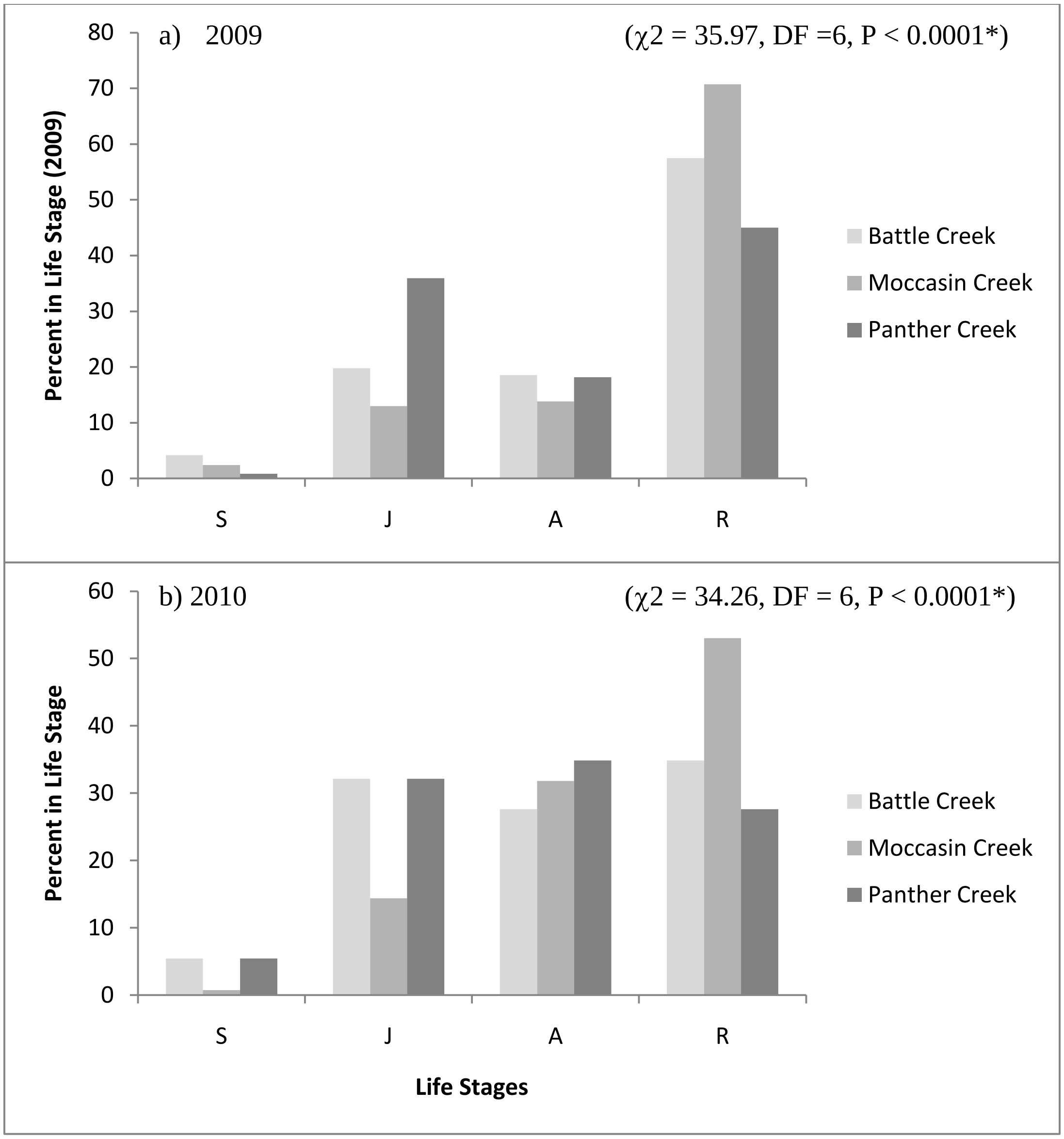


Figure 3.2. Stage structure presented as percent in life stage by site for a) 2009 and b) 2010. S = seedling, J = juvenile, A = subadult, and R = reproductive.

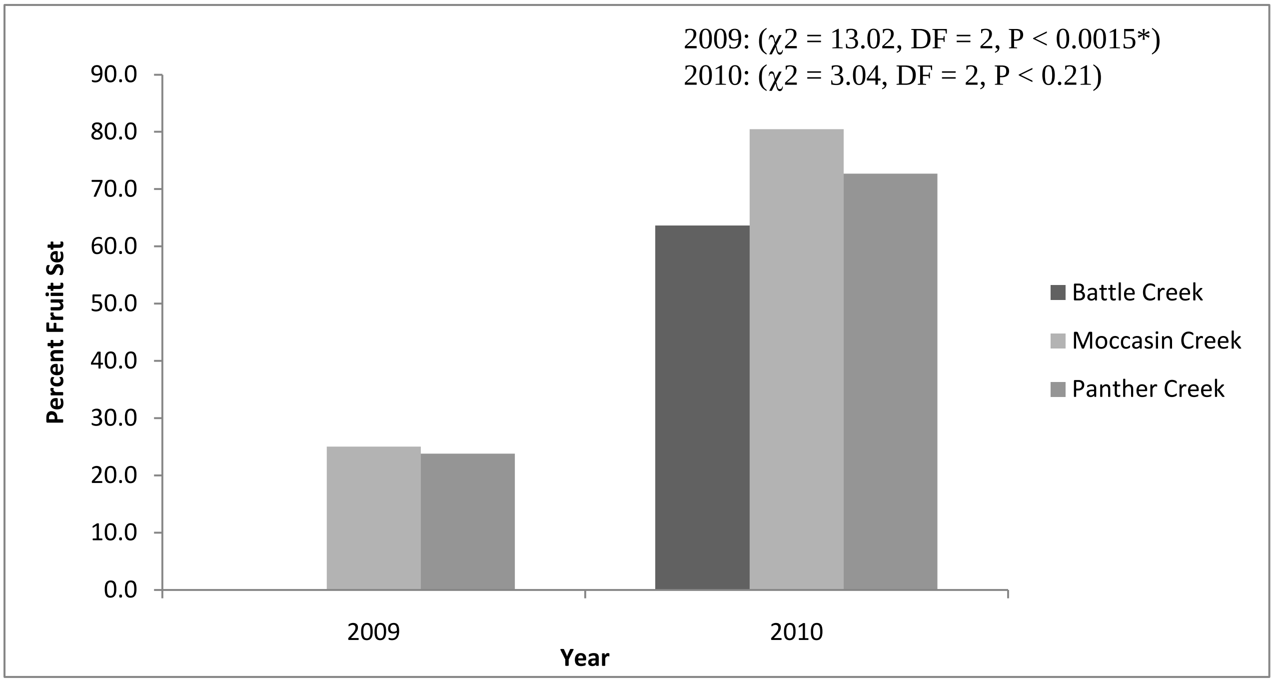
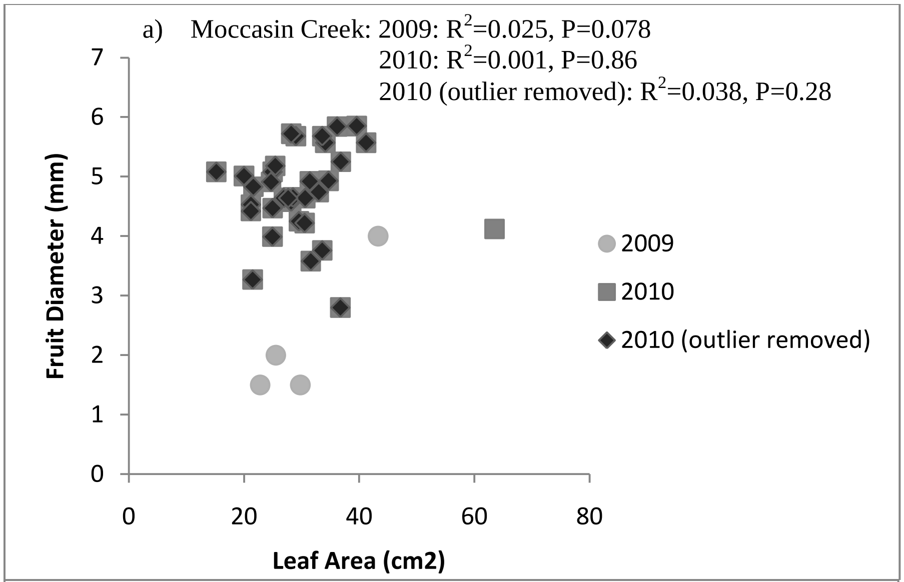
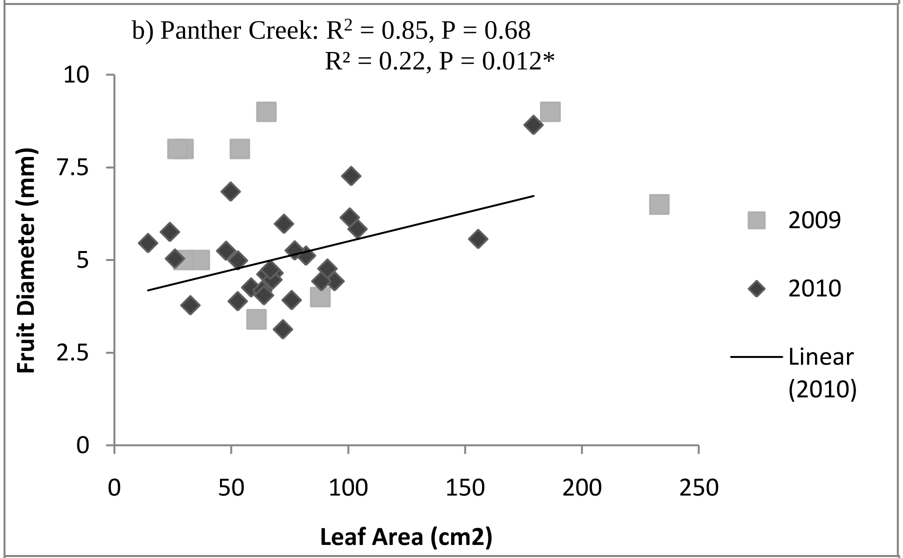


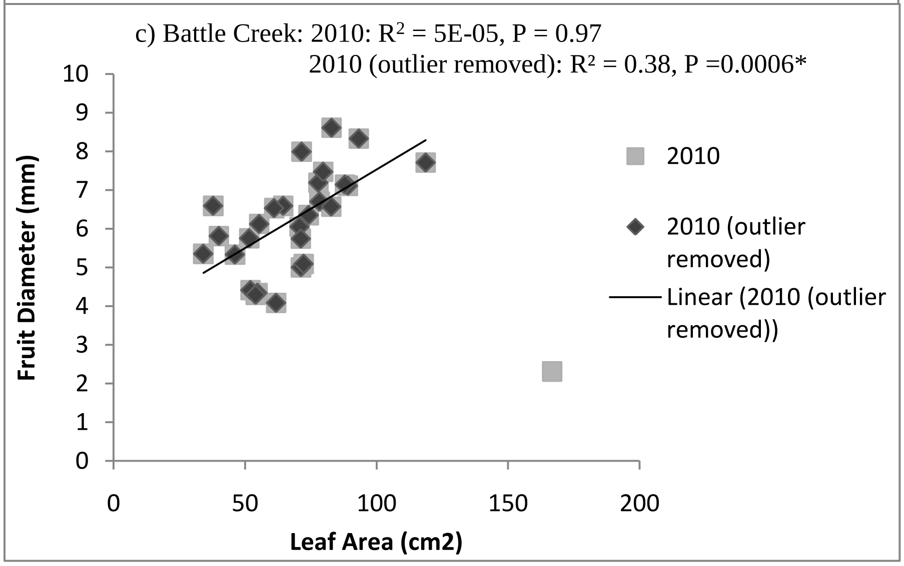
Figure 3.3. Percent fruit set for 2009 and 2010 across sites.



a) Moccasin Creek: 2009: R^2=0.025, P=0.078
2010: R^2=0.001, P=0.86
2010 (outlier removed): R^2=0.038, P=0.28



b) Panther Creek: R^2 = 0.85, P = 0.68
R^2 = 0.22, P = 0.012*



c) Battle Creek: 2010: R^2 = 5E-05, P = 0.97
2010 (outlier removed): R^2 = 0.38, P =0.0006*

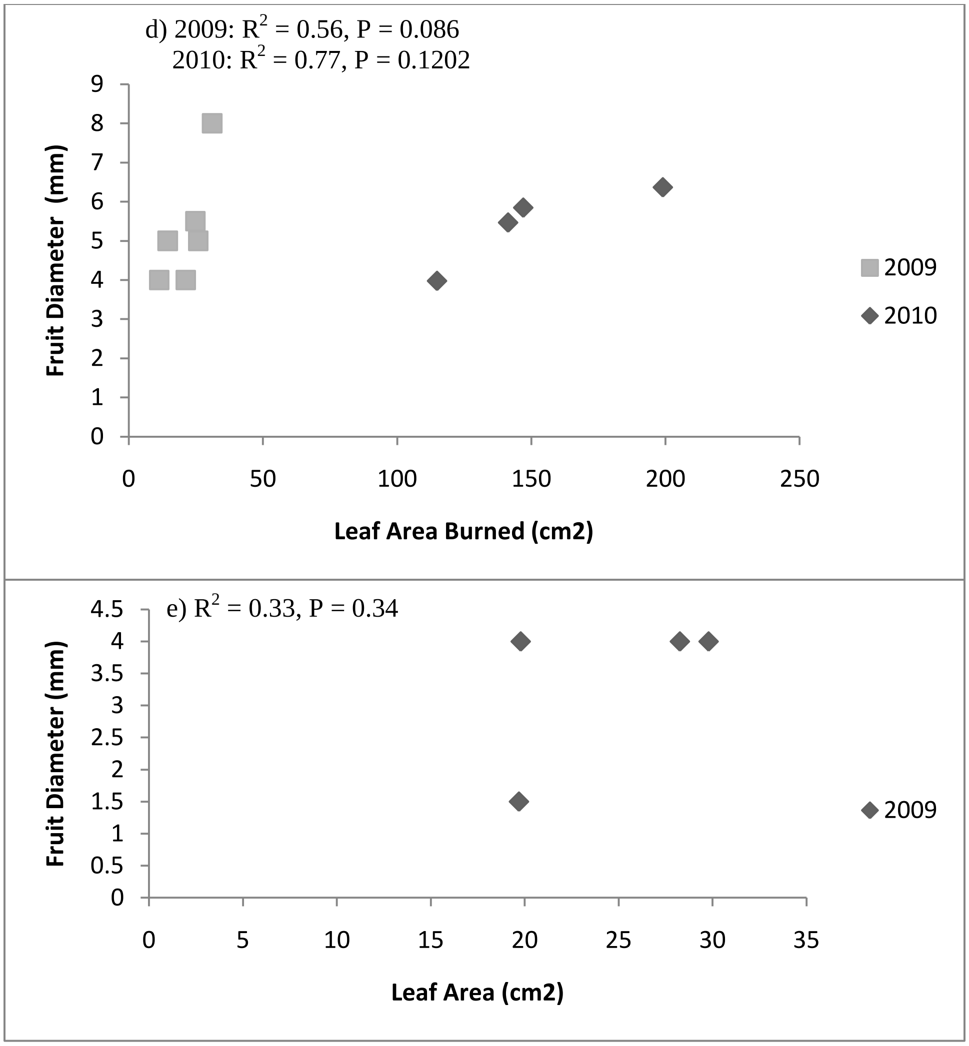


Figure 3.4. Linear regression of leaf area versus fruit diameter by treatment by site for 2009 and 2010 at a) Moccasin Creek and b) Panther Creek and 2010 c) Battle Creek and by treatment at Moody for d) 2009 and 2010 burned and e) 2009 control. No fruit were censused in 2009 at Battle Creek, so no data were reported.

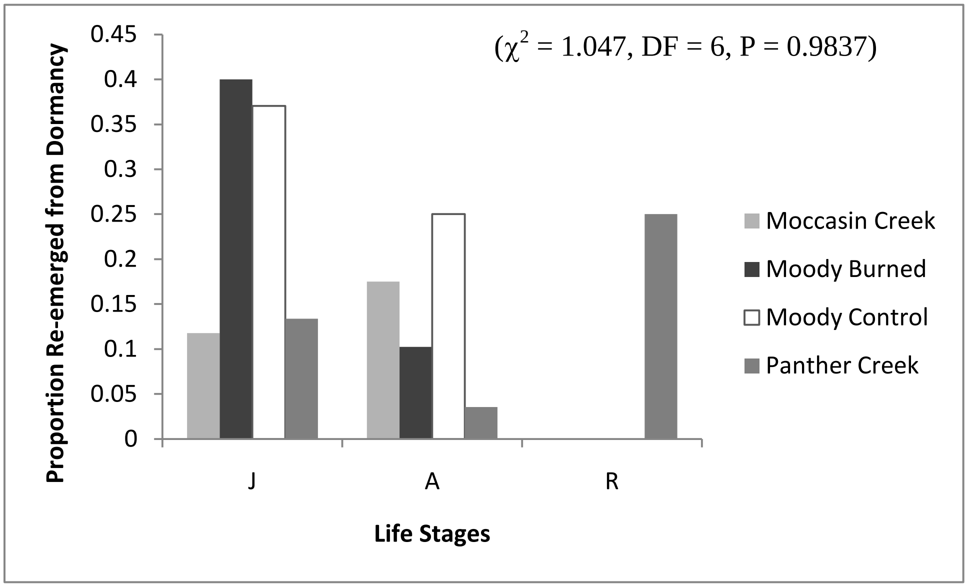


Figure 3.5. Proportion of individuals re-emerged from dormancy across sites in 2010. J = juvenile, A = subadult, R = reproductive.

REFERENCES

Aber, J. and J. M. Melillo. 1991. Terrestrial Ecosystems. Saunders College Publishing, Philadelphia.

Aguilar, R., M. Quesada, L. Ashworth, Y. Herrerias-Diego, and J. Lobo. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology **17**:5177-5188.

Augustine, D. J. and L. E. Frelich. 1998. Effects of white-tailed deer on populations of an understory forb in fragmented deciduous forests. Conservation Biology **12**:995-1004.

Barbour, M. G., J. H. Burk, W. D. Pitts, F. S. Gilliam, and M. W. Schwartz. 1999. Terrestrial Biology of Plants. 3rd edition. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Barden, L. S. 1979. Serotiny and seed viability of *Pinus pungens* in the Southern Appalachians. Castanea **44**:44-47.

Bevill, R. L. and S. M. Louda. 1999. Comparisons of related rare and common species in the study of plant rarity. Conservation Biology **13**:493-498.

Bierzychudek, P. 1982. The demography of jack-in-the-pulpit, a forest perennial that changes sex. Ecological Monographs **52**:335-351.

Bierzychudek, P. 1999. Looking backwards: assessing the projections of a transition matrix model. Ecological Applications **9**:1278-1287.

Boerner, R. E. J. 2000. Effects of fires on the ecology of the forest floor and soil of central hardwood forests. U.S. Department of Agriculture, Forest Service, Northeastern Research Station, Newtown Square, PA: U.S.

Bomhard, B., D. M. Richardson, J. S. Donaldson, G. O. Hughes, G. F. Midgley, D. C. Raimondo, A. G. Rebelo, M. Rouget, and W. Thuiller. 2005. Potential impacts of future land use and climate change on the Red List status of the Proteaceae in the Cape Floristic Region, South Africa. Global Change Biology **11**:1452-1468.

Bossuyt, B. and O. Honnay. 2008. Heat shock increases the reliability of a temperate calcareous grassland seed bank study. Plant Ecology **199**:1-7.

Bradstock, R. A., M. Bedward, and J. S. Cohn. 2006. The modelled effects of differing fire management strategies on the conifer *Callitris verrocosa* within semi-arid mallee vegetation in Australia. Journal of Applied Ecology **43**:281-292.

Brose, P. H. and T. A. Waldrop. 2006. Fire and the origin of Table Mountain pine - pitch pine communities in the southern Appalachian Mountains, USA. Canadian Journal of Forest Research **36**:710-718.

Brown, J. K. and J. K. Smith. 2000. Wildland fire in ecosystems: Effects of fire on flora. USDA Forest Service, Rocky Mountain Research Station, Ogden, UT.

Burns, R. M. and B. H. Honkala. 1990. Silvics of North America: 1. Conifers; 2. Hardwoods. U.S. Department of Agriculture, Washington, D.C.

Carvalheiro, L. G., E. R. M. Barbosa, and J. Memmott. 2008. Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study. Journal of Applied Ecology **45**:1419-1427.

Caswell, H. 2009. Stage, age and individual stochasticity in demography. Oikos **118**:1763-1782.

Chapin III, F. S., O. E. Sala, I. C. Burke, J. P. Grime, D. U. Hooper, W. K. Laurenroth, A. Lombard, H. A. Mooney, A. R. Mosier, S. Naeem, S. W. Pacala, J. Roy, W. L. Steffen, and D. Tilman. 1998. Ecosystem consequences of changing biodiversity. BioScience **48**:45-52.

Chapin III, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature **405**:234-242.

Clarke, P. J. and K. Dorgi. 2008. Are trade-offs in plant resprouting manifested in community seed banks? Ecology **89**:1850-1858.

Cook, R. E. and P. M. Dixon. 1987. A review of recovery plans for threatened and endangered plants. Report to the World Wildlife Fund, Ithaca, New York, USA.

Cox, G. W. 1990. Laboratory Manual of General Ecology. 6th edition. Wm. C. Brown Publishers, Dubuque, IA.

Crowder, L. B., D. T. Crouse, S. S. Heppell, and T. H. Martin. 1994. Predicting the impact of turtle excluder devices on loggerhead sea turtle populations. Ecological Applications **4**:437-445.

Dumas, S., H. S. Neufeld, and M. C. Fisk. 2007. Fire in a thermic oak-pine forest in Linville Gorge Wilderness Area, North Carolina: importance of the shrub layer to ecosystem response. Castanea **72**:92-104.

Duncan, W. H., J. F. Garst, and G. A. Neece. 1971. *Trillium persistens* (Liliaceae), a new pedicellate-flowered species from northeastern Georgia and adjacent North Carolina. Rhodora **73**:244-248.

Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002. Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecology Letters **5**:558-567.

Esque, T. C., J. A. Young, and C. R. Tracy. 2010. Short-term effects of experimental firest on a Mojave Desert seed bank. Journal of Arid Environments **74**:1302-1208.

Facelli, J. M. and T. A. Pickett. 1991. Plant litter: its dynamics and effects of plant community structure. Botanical Review **57**:1-32.

Fiedler, P. L. and S. K. Jain. 1992. Conservation Biology. Routledge, Chapman & Hall, Inc., New York.

Garten Jr., C. T. 2006. Predicted effects of prescribed burning and harvesting on forest recovery and sustainability in southwest Georgia, USA. Journal of Environmental Management **81**:323-332.

Gilliam, F. S. and W. J. Platt. 1999. Effects of long-term fire exclusion on tree species composition and stand structure in an old-growth *Pinus palustris* (Longlead pine) forest. Plant Ecology **140**:15-26.

Glasgow, L. S. and G. R. Matlack. 2007. Prescribed burning and understory composition in a temperate deciduous forest, Ohio, USA. Forest Ecology and Management **238**:54-64.

Gonzales, E. and J. L. Hamrick. 2005. Distribution of genetic diversity among disjunct populations of the rare forest understory herb, *Trillium reliquum*. Heredity **95**:306-314.

Grabe, D. F. 1970. Tetrazolium testing handbook for agricultural seeds. Contribution no. 29 to the Handbook of seed testing. Association of Official Seed Analysts. North Brunswick, New Jersey, USA.

Gyer, J. F. 2007. The dark side of trillium.

Hamrick, J. L. 2006. Conservation of genetic diversity in old-growth forest communities of the southeastern United States. Applied Vegetation Science **9**:51-57.

Hanzawa, F. M. and S. Kalisz. 1993. The relationship between age, size, and reproduction in *Trillium grandiflorum* (Liliaceae). American Journal of Botany **80**:405-410.

Hartley, S. and W. E. Kunin. 2003. Scale dependency of rarity, extinction risk, and conservation priority. Conservation Biology **17**:1559-1570.

Heckle, C. D. 2004. Impacts of exotic invasive vines on the ecology and reproduction of the endangered *Trillium reliquum*. Georgia Southern University, Statesboro.

Heckle, C. D. and L. M. Leege. 2006. Life history and reproductive biology of the endangered *Trillium reliquum*. Plant Ecology.

Heuberger, K. A. and F. E. Putz. 2003. Fire in the suburbs: ecological impacts of prescribed fire in small remnants of Longleaf pine (*Pinus palustris*) sandhill. Restoration Ecology **11**:72-81.

Heyward, F. 1938. Soil temperatures during forest fires in the longleaf pine region. Journal of Forestry **36**:478-491.

Hobbs, R. C. and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology **6**:324-337.

Horvitz, C. C. and D. W. Schemske. 1995. Spatiotemporal variation in demographic transitions of a tropical understory herb: projection matrix analysis. Ecological Monographs **65**.

Hutchinson, T. F. 2006. Fire and the herbaceous layer of easter oak forests.*in* M. B. Dickinson, editor. Fire in eastern oak forests: delivering science to land managers, proceedings of a conference. U.S. Department of Agriculture, Forest Service, Northern Research Station, Columbus, OH.

Iverson, L. R. and T. F. Hutchinson. 2002. Soil temperature and moisture fluctuations during and after prescribed fire in mixed-oak forests, USA. Natural Areas Journal **22**:296-304.

Jules, E. S. 1998. Habitat fragmentation and demographic change for a common plant: trillium in old-growth forest. Ecology **79**:1645-1656.

Jules, E. S. and B. J. Rathcke. 1999. Mechanisms of Reduced Trillium Recruitment along Edges of Old-Growth Forest Fragments. Conservation Biology **13**:784-793.

Kalisz, S. and M. A. McPeek. 1992. Demography of an age-structure annual: resampled projection matrices, elasticity analyses, and seed bank effects. Ecology **73**:1082-1093.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pllinator interactions. Annual Review of Ecology, Evolution, and Systematics **29**:83-112.

Keeley, J. E. and W. J. Bond. 1997. Convergent seed germination in South African fynbos and Californian chaparral. Plant Ecology **133**:153-167.

Knapp, E. E., D. W. Schwilk, J. M. Kane, and J. E. Keeley. 2007. Role of burning season on initial understory vegetation response to prescribed fire in a mixed conifer forest. Can. J. of For. Res. **37**:11-22.

Knight, T. M. 2003. Floral density, pollen limitation, and reproductive success in Trillium grandiflorum. Oecologia **137**:557-563.

Kobziar, L., J. Moghaddas, and S. L. Stephens. 2006. Tree mortality patterns following prescribed fires in mixed conifer forest. Can. J. of For. Res. **36**:3222-3228.

Leege, L. M., J. S. Thompson, and D. J. Parris. 2008. The responses of a rare and a common trillium (*Trillium reliquum* and *Trillium maculatum*) to white-tailed deer herbivory and invasive Japanese honeysuckle removal. Castanea.

Leege, L. M., J. S. Thompson, and D. J. Parris. 2010. The responses of a rare and a common trillium (*Trillium reliquum* and *Trillium maculatum*) to white-tailed deer herbivory and invasive Japanese honeysuckle removal. Castanea.

Lefkovitch, L. P. 1965. The study of population growth in organisms grouped by stages. Biometrics **21**:1-18.

Leslie, P. H. 1948. Some further notes on the use of matrices in population mathematics. Biometrika **35**:213-245.

Lubbers, A. E. and M. J. Lechowicz. 1989. Effects of leaf removal on reproduction vs. belowground storage in *Trillium grandiflorum*. Ecology **70**:85-96.

Lyons, K. G., C. A. Brigham, B. H. Traut, and M. W. Schwartz. 2005. Rare species and ecosystem functioning. Conservation Biology **19**:1019-1024.

Lyons, K. G. and M. W. Schwartz. 2001. Rare species loss alters ecosystem function-invasive resistance. Ecology Letters **4**:358-365.

Maina, G. G. and H. F. Howe. 2000. Inherent rarity in community restoration. Conservation Biology **14**:1335-1340.

McPeek, M. A. and S. Kalisz. 1993. Population Sampling and Bootstrapping in Complex Designs: Demographic Analysis. Page 445 *in* S. M. Scheiner and J. Gurevitch, editors. Design and Analysis of Ecological Experiments. Chapman and Hall, New York.

Menges, E. S. 1986a. Predicting the furute of rare plant populations: demographic monitoring and modeling. Naturals Areas Journal **6**:13-20.

Menges, E. S. 1986b. Predicting the future of rare plant populations: demographic monitoring and modeling. Natural Areas Journal **6**:13-20.

Menges, E. S. 1990. Population viability analysis for an endangered plant. Conservation Biology **4**:52-62.

Miller, W., S. Hassan, and B. Jackson. 2010. Lab for Environmental Analysis Department of Crop & Soil Sciences University of Georgia.

Mirkin, B. M., P. S. Shirokikh, V. B. Martynenko, and L. G. Namova. 2010. Analysis of trends inthe formation of species richness of plant communities using sytaxonomy and ecological scales. Russian Journal of Ecology **41**:279-283.

Molina, M., R. Fuentes, R. Calderon, M. Escudey, K. Avendano, M. Gutierrez, and A. C. Chang. 2007. Impact of forest fire ash on surface charge characteristics of andisols. Soil Science **172**:820-834.

Mulligan, M. K. and L. K. Kirkman. 2002. Burning influences on Wiregrass (*Aristida beyrichiana*) restoration plantings: natural seedling recruitment and survival. Restoration Ecology **10**:334-339.

Mulligan, M. K., L. K. Kirkman, and R. J. Mitchell. 2002. *Aristida beyrichiana* (Wiregrass) establishment and recruitment: implications for restoration. Restoration Ecology **10**:68-76.

Murphy, J. D., D. W. Johnson, W. W. Miller, R. F. Walker, and R. R. Blank. 2006. Prescribed fire effects on forest floor and soil nutrients in a Sierra Nevada forest. Soil Science **171**:181-199.

Neary, D. G., C. C. Klopatek, L. F. DeBano, and P. F. Ffolliot. 1999. Fire effects on beloground properties of soils and aqueous suspensions. Forest Ecology and Management **122**:51-71.

Ohara, M. and S. Higashi. 1987. Interference by ground beetles with the dispersal by ants of seeds of trillium species (Liliaceae). Journal of Ecology **75**:1091-1098.

Ohara, M., H. Tomimatsu, T. Takada, and S. Kawano. 2006. Importance of life history studies for conservation of fragmented populations: A case study of the understory herb, *Trillium camschatcense*. Plant Species Biology **21**:1-12.

Outcalt, K. W. 2008. Lightning, fire and longleaf pine: using natural disturbance to guide management. Forest Ecology and Management **255**:3351-3359.

Patil, V. N. and M. Dadlani. 2009. Tetrazolium test for seed viability and vigour. Handbook of Seed Testing.

Pavek, D. S. 1993. Fire effects information system, [Online]. U.S. Department of Agriculture, Fire Sciences Laboratory (Producer).

Pino, J., F. X. Pico, and E. de Roa. 2007. Population dynamics of the rare plant *Kosteletzkya pentacarpos* (Malvaceae): a nine-year study. Botanical Jounal of the Linnean Society **153**:455-462.

Rabinowitz, D. S., S. Cairns, and T. Dillon. 1986. Seven forms of rarity and their frequency in the flora of the British Isles. Sinauer, Sunderland, Massachusetts, U.S.A.

Ramsey, G. W., C. H. Leys, R. A. S. Wright, D. A. Coleman, and A. O. Neas. 1993. Vascular flora of the James River gorge watersheds in the central Blue Ridge mountains of Virginia. Castanea **58**:260-300.

Rayner, D. A. 1984. Persistent trillium (*Trillium persistens*) recovery plan. United States Fish and Wildlife Service, Atlanta, Georgia:69.

Rooney, T. P. and K. Gross. 2003. A demographic study of deer browsing impacts on *Trillium grandiflorum*. Plant Ecology **168**:267-277.

Sackett, S. S., S. M. Haase, and G. M. Burke. 1992. Soil temperatures related to prescribed burning in Ponderosa Pine stands in Yosemite Valley that include California Black Oak. Pages 144-146 *in* Symposium on Ecology and Management of Oak and Associated Woodlands: Perscpectives in the Southwestern United States and Northern Mexico, Sierra Vista, Arizona.

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology **75**:584-606.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. Journal of Ecology **97**:1000-1009.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and Company, New York.

Sullivan, B. T., C. J. Fettig, W. J. Otrosina, M. J. Dalusky, and C. W. Berisford. 2003. Association between severity of prescribed burns and subsequent activity of conifer-infesting beetles in stands of longleaf pine. Forest Ecology and Management **185**:327-340.

Theodose, T. A., C. H. Jaeger, and J. C. Schardt. 1996. Uptake and allocation of 15N in alpine plants: implication for the importance of competitive ability in predicting community structure in a stressful environment. Oikos **75**.

Thomas, P. B., E. C. Morris, and T. D. Auld. 2003. Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. Austral Ecology **2003**:674-683.

Thompson, J. S. 2007. Impacts of deer and honeysuckle on the endangered *Trillium reliquum* and its associated plant community. Georgia Southern University Statesboro, GA.

Thomson, D. 2005. Measuring the effects of invasive species on the demography of a rare endemic plant. Biological Invasions **7**:615-624.

Tomimatsu, H. and M. Ohara. 2002. Effects of forest fragmentation on seed production of the understory herb *Trillium camschatcense*. Conservation Biology **16**:1277-1285.

Tomimatsu, H. and M. Ohara. 2004. Edge effects on recruitment of *Trillium camschatcense* in small forest fragments. Biological Conservation **117**:509-519.

Tomimatsu, H. and M. Ohara. 2010. Demographic response of plant populations to habitat fragmentation and temporal environmental variability. Oecologia **162**:906-911.

Torang, P., J. Ehrlen, and J. Agren. 2010. Linking environmental and demographic data to predict future population viability of a perennial herb. Oecologia **163**:99-109.

Tozer, M. 1998. Distribution of the soil seedbank and influence of fire on seedling emergence in *Acacia salignai* growing on the central coast of New South Wales. Australian Journal of Botany **46**:743-755.

Varner III, J. M., J. K. Hiers, R. D. Ottmar, D. R. Gordon, F. E. Putz, and D. D. Wade. 2007. Overstory tree mortality resulting from reintroducing fire to long-unburned longleaf pine forests: the importance of duff moisture. Can. J. of For. Res. **37**:1349-1358.

Vellend, M., T. M. Knight, and J. M. Drake. 2006. Antagonistic effects of seed dispersal and herbivory on plant migration. Ecol Lett **9**:319-326.

Vermeire, L. T. and M. J. Rinella. 2009. Fire alters emergence of invasive plant species from soil surface-deposited seeds. Weed Science **57**:304-310.

Vitt, P., K. Havens, B. E. Kendall, and T. M. Knight. 2009. Effects of community-level grassland management on the non-target rare annual *Agalinis auriculata*. Biological Conservation **142**:798-805.

Wang, X., T. Wiegand, Z. Hao, B. Li, J. Ye, and F. Lin. 2010. Species associations in an old-growth temperate forest in north-eastern China. Journal of Ecology **98**:674-686.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998a. Quantifying threats to imperiled species in the United States. BioScience **48**:807-815.

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

Williams, C. E. and W. C. Johnson. 1990. Age structure and maintenance in Pinus pungens in pine-oak forests of southwestern Virginia. Am. Midl. Nat. **124**:130-141.

Williams, P. R., R. A. Congdon, A. C. Grice, and P. J. Clarke. 2003. Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. Austral Ecology **28**:507-514.

Young, A. S., S. M. Change, and R. R. Shartiz. 2007. Reproductive ecology of a federally endangered legume, *Baptisia arachnifera*, and its more widespread congener, *B. lanceolata* (Fabaceae). American Journal of Botany **94**:228-236.

Zietsman, J., L. L. Dreyer, and K. J. Esler. 2008. Reproductive biology and ecology of selected rare and endangered *Oxalis* L. (Oxalidaceae) plant species. Biological Conservation **141**:1475-1483.

Zobel, D. B. 1969. Factors affecting the distribution of *Pinus pungens*, an Appalachian endemic. Ecol. Monogr. **39**:303-333.

Appendix A

Directions to study sites

From Statesboro:

<u>Sites near Tallulah Falls, GA:</u>

Turn left at US-25 N/Northside Dr. W. Continue to follow US-25 N. Turn left at GA-121 N. Turn left at GA-24 N/GA-80 N/W 6th St. Continue to follow GA-24 N/GA-80 N. Turn left at GA-80 N/GA-88 N. Continue to follow GA-80 N. Turn right at GA-17 N/GA-80 N and continue to follow GA-17 N. Continue onto GA-17 BUS N. Turn left at GA-8 W/US-29 S/Franklin Springs St. Continue to follow GA-8W/US 29 S. Turn right at GA-145 N/Toccoa Carnesville Rd. Continue to follow GA-145 N. Turn right at Athens St. Take the first left onto Central Ave. Turn left at Lavonia Rd. Contineu onto GA-59 W/Commerce Rd. Turn right at GA-320 N. Turn left at GA-106 N/Mize Rd. Turn left at GA-17 N/Toccoa Bypass. Turn right to stay on GA-17 N. Turn right at GA-15 N/US-23 N/US-441 N.

<u>Moody Creek:</u>

From GA-15 N/US-23 N/US-441 N, take a right turn onto Tugalo Short Cut Rd./Co. Rd. 225. Turn left at Tugalo Village Road. Follow Tugalo Village Rd. until coming to a sign on the right that says “Tallulah Gorge Wildlife Management Road.” Follow the dirt road until coming to the first power line clearing, passing a gate halfway through*. Site is on the right-hand slope after the power line clearing.

*Gate may be open by Tallulah Gorge park service, upon request
Danny Tatum, Park Superintendent
Tallulah Gorge State Park
PO Box 248
Tallulah Falls, GA 30573
Phone (706) 754 7970
Fax (706) 754 7974
E-mail: tallulah@stc.net

Moccasin Creek:

Turn right at Tugalo Village Rd. and follow until coming to a chain-linked fence with a sign indicating Yonah Dam. Follow the gravel road. Park next to the first paved road on the right, then by foot, follow the paved road until coming to a small access road on the right, which will be a forest path. Follow the forest path, crossing one power line clearing. Population is on the left of the path, bordering a creek at the bottom-most stretch of the road.

Battle Creek:

Pass the Moccasin Creek site and follow the gravel road to the bottom, ending at Yonah Dam and a camp ground. By boat, cross the lake and go towards the creek (Battle Creek). Population is on the right side of the creek.

Site near Toccoa, GA, from Tugalo Village Short Cut Rd:

Head southwest of GA-15/US-23 S/US-441 S. Turn left at GA-17 Alt S./Talmadge Dr. Continue to follow GA-17 Alt St. Turn right at Broad St./ State Route 17 Alt.

To Panther Creek:

From Broad St., take second left onto E. Tugalo St. Turn left at GA-184 N/Prather Bridge Rd. Follow Prather Bridge Rd. The road will come to a small concrete parking area, before a bridge and small picnic area at the right; park in the concrete area. Walk along the side of the creek, going up-stream, until coming to a small stream; cross the stream and head uphill. You should start seeing *Trillium persistens* as you head uphill, with main population near the next stream you come across and also around a fallen water tower, also uphill.

Appendix B

Methods

Two populations, Moody Creek and Panther Creek, were censused for *Trillium persistens* in the reproductive life stage in March-April, 2010. Each population was divided into two groups: the core group, or the central 1 x 1 m area of a population and fringe, which was about 100-150 m outside the core. At Moody Creek, 33 individuals were found in the core and 73 in the fringe. At Panther Creek, 24 were found in the core and 76 in the fringe. Once fruit began to develop by May, 2010, the fruiting-ends of the plants were bagged with bridal veil to retain fruits so seed count could be observed later in the season. Fruits were ripe by June 16, 2010. The distance between the carpel ridges of the ripe fruits were measured to determine fruit diameter, then they were removed and opened so the seeds could be counted. Seed numbers were used to determine the relationship between fruit size and seed number with a linear regression, including the average number of seeds per fruit. Missing fruits were also noted. Fruit diameter data were also used to examine difference between core and fringe individuals.

Results

The relationship between fruit diameter and seed number was positive ($R^2 = 0.72$, $P=0.0001$, seed count = -8.54 + 2.60*Fruit Diameter (mm). . No difference was found between core individuals and fringe individuals, however the core individuals had a greater number of seeds per fruit.

Site	Number Plants Sampled	Mean Fruit Diameter (mm) (± SE)	Mean Seed Number/Fruit (± SE)
Moody Creek	106	5.26 (± 0.16)	6.74 (± 0.34)
Panther Creek	100	4.82 (± 0.23)	6.21 (± 0.38)

APPENDIX C

Community structure has been altered due to natural and anthropogenic disturbances, which has led to floral decline worldwide (Hamrick 2006). Community structure also becomes disrupted when natural disturbances are interrupted, such as suppression of fire (Varner III et al. 2007). Because herbaceous plants contribute significantly to plant species diversity and richness in forests, it is important to conserve herb layer communities (Ramsey et al. 1993). *Trillium persistens* is an herb species that has become endangered across its range, however no study has examined the deciduous forest habitat or quantified species richness across its range. Because species richness in a habitat can maximize resource acquisition and retention at each trophic level (Chapin III et al. 1998), it is important to conserve threatened and endangered species within communities.

Community sampling

To examine the habitat of *T. persistens,* I conducted a census of the community vegetation at the herb, shrub, and tree layers in Summer 2009. At each of the four sites, I established twenty plots for each of the layers, with the herb layer plots being 1x1 m, the shrub layer being 5x5 m plots, and the tree layer being 10x10 m plots (Figure C.1). To determine where the plots would be placed, I used transect lines (Figure C.2). I placed a meter tape along the top of the slope over the area to be censused. Five transect points at least 10 m apart were selected along the meter tape using a random numbers table. At each point, I laid another measuring tape down the slope, perpendicular to the original transect tape. With a random numbers table, I then selected four points along each of the five transect lines at least 10 m apart. At each point, the herb, shrub, and tree plots were set up (Figure C.1).

At the herb layer, percent cover of all plants less than 1 m tall including tree and shrub seedlings, forbs and grasses was estimated within each of the 20 1x1 m plots. Each species was identified and for each tree seedling, herb seedling, and forb, cross sectional area and height were measured. Ocular estimates were made of percent grass cover. Mean percent cover of species at the herb layer per plot was calculated by adding the area of the individual plants per plot, then averaging total area per plot per site. To determine whether percent herb cover differed by site, a non-parametric Kruskal-Wallis test was used because the data did not transform to fit a normal distribution. Mean species richness was determined by counting the number of different species per plot, which was then averaged per site and was analyzed non-parametrically using a Kruskal-Wallis test because the data did not transform to a normal distribution. Mean *T. persistens* density at the herb layer was calculated by averaging the total number of trillium per plot. To determine whether *T. persistens* density differed by site, a Kruskal-Wallis test was used because the variances were not equal and the data did not transform to a normal distribution. Variation among means were examined with a post-hoc Tukey HSD tests.

At the shrub layer, all species including trees and shrubs with a height ≥ 1 m tall and with a diameter at breast height (DBH) of < 3 cm were measured. The number of stems was counted for each shrub and each species was identified. Shrub stem density was calculated based on the number of stems per plot area (25 m^2). The plot shrub stem density was averaged for each of the four sites, then sites were compared using a Kruskal-Wallis test. Mean species richness was determined for shrubs as it was for herbs.

At the tree layer, individuals with a DBH of 3 cm or greater with a height > 1 m were counted within the 100 m^2 plots. The DBH of each tree was taken and the tree species was identified. The tree basal area (BA) was then determined from each DBH for all using the formula:

$$\text{Basal Area} = \pi\left(\frac{\text{DBH}}{2}\right)^2$$

Tree BA was averaged for each site, then mean tree basal area was square root transformed to meet the assumptions of normality and equal variance. To test is tree BA varied by site, a model I one-way ANOVA was used. Mean species richness was determined for trees as it was for herbs and compared among sites with a model I one-way ANOVA. Variation among means at the herb, shrub, and/or tree layer was examined with a Tukey HSD test. All statistical analyses were performed with JMP 8.0. (2008).

To examine the species that interact with one another in each community I determined importance values (IVs) for each site, which are the combined measures of the relative frequency, relative density, and relative dominance of species in a forest. To calculate IVs, I recorded all the species within the plots for each layer (herb, shrub, tree). For each species, I calculated relative frequency by the equation

$$\begin{aligned}\text{Relative frequency} &= \left(\frac{\text{\# plots species i found/total \# plots}}{\Sigma\ \text{frequency values for all species}}\right) * 100\\ &= \left(\frac{\text{frequency of species i}}{\Sigma\ \text{frequency values for all species}}\right) * 100\end{aligned}$$

Relative density was calculated by the following equation

$$\text{Relative density} = \left(\frac{\text{total \# i individuals/total area of plots}}{\Sigma\ \text{density for all species}}\right) * 100$$

$$= \left(\frac{\text{density of species i}}{\Sigma\ \text{density for all species}}\right) * 100$$

Finally, I calculated relative dominance for each of the three layers, using the I used the following equation

$$\text{relative dominance} = \left(\frac{\text{dominance for species i}}{\Sigma\ \text{dominance for all species}}\right) * 100$$

however the dominance for each layer was calculated differently. For the herb layer

$$\text{herb layer relative dominance} = \left(\frac{\text{\% cover for species i/total area of plots}}{\Sigma\ \text{dominance for all species}}\right) * 100$$

For the other two layers, instead of using percent cover, I used total stem number for shrubs

$$\text{shrub layer relative dominance} = \left(\frac{\text{total stem \# for species i/total area of plots}}{\Sigma\ \text{dominance for all species}}\right) * 100$$

and basal area (cm^2) for trees

$$\text{tree layer relative dominance} = \left(\frac{\text{basal area for species i/total area of plots}}{\Sigma\ \text{dominance for all species}}\right) * 100$$

instead of percent cover. Relative frequency, relative density, and relative dominance were summed to determine the IV for each species.

Community sampling

Twenty-four species at the herb layer were found across the range of sites, with species varying by site, with Panther Creek having the most species (Table C.3). Battle Creek had the fewest species however also had the greatest mean percent herb cover (Figure C.3, Table C.1). Moccasin Creek, which is the site closest to Battle Creek, had the second fewest species. Moody Creek and Panther Creek were the only sites where *T. persistens* were found within the herb layer plots. The only sites where *T. persistens* had an IV that ranked within the top five values were Moody Creek and Panther Creek with IVs of 23.9 and 57.4, respectively. The only herb species seen within all four sites that was within the top five for each site was the fern *Polystichum acrostichoides*, with a mean IV of 55.0. The species with the highest IV was *Galax urceolata*, which had an IV of 167.2 and was only found at Battle Creek. *Trillium persistens* density however, did not differ significantly by site at the herb layer (Figure C.3). Only two sites had *T. persistens* within the herb plot, so while sites did not differ statistically, the presence of *T. persistens* across sites was variable.

The mean stem density/plot in the shrub layer differed significantly between sites (Figure 4), with many of the fifteen species found present in all of the sites (Table C.2). While shrub stem density among these sites differed statistically, variation between means at sites could not be expressed with a Tukey HSD test. Panther Creek had the greatest shrub stem density, however Moccasin Creek and Moody Creek were approximately tied for the lowest. Battle Creek (Table C.2) had the fewest number of species compared to the other sites (Table 2 b, c, d) and Panther Creek (Table 2 d) had the most. Moccasin Creek and Moody Creek had intermediate values for the number of species (Table 2 b, c). *Acer rubrum* was at all sites, and had a mean IV of 17.5. However, its IV was only in the top five at Battle Creek and Moccasin Creek. *Kalmia latifolia*, with a mean IV of 36.8, was also at all four sites with its IV in the top five at Battle Creek and Panther Creek. *Tsuga canadensis* was found at all four sites and was the only species with an IV within the top five for all sites 74.5. The species with the highest IV at a singular location was

Rhododendron minus, which had an IV of 184.3 at Panther Creek. The only other site with *R. minus* was Moccasin Creek, where it had an IV of 52.6.

Approximately the same number of species were found at the tree layer compared to the number of species at the herb layer, with sites differing significantly from one another (F = 4.5183, DF = 3, 76, P = 0.0057; Figure 2.20). The mean BA was highest at Moody Creek, with Battle Creek having the lowest and Moccasin and Panther having an intermediate BA (Figure 2.20). Many of the twenty-three total species found were repeated between sites. Battle had the lowest number of tree species present, whereas Moody Creek had the highest (Figure 2.20). Moccasin Creek and Panther Creek had an intermediate number (Figure 2.20). *Acer rubrum* and *Tsuga canadensis* were the only two species found at all four sites, with a mean IV of 24.8 and 85.4, respectively (Table 2.7). *Tsuga canadensis* also had the highest singular IV of 126.3 at Moody Creek (Table 2.7).

Species richness was varied significantly for all the layers by site. The herb layer varied significantly by site, with means at all sites varying from one another (H = 14.1439, DF = 3, P = 0.0027; Figure 2.21 a). The shrub layer varied significantly, however Battle Creek was the only site with mean species richness differing from the other sites (H = 14.8241, DF = 3, P = 0.002; Figure 2.21 b). Finally, tree species richness varied significantly as well, with Battle Creek being the only site, with the mean species richness at Battle Creek differing among sites (F = 9.7888, DF = 3, 76, P < 0.0001; Figure 2.21 c) These differences illustrate the variability amongst the habitat range of *T. persistens* (Figure 2.21 a, b, c).

Table C.1. Importance Values for the herb layer at Battle Creek, Moccasin Creek, Moody Creek, and Panther Creek. IVs were calculated using relative frequency, relative density, and relative dominance. The maximum possible IV per site was 300. The top five importance values per site are bolded.

	Sites				**# of sites present**
Species	**Battle Creek**	**Moccasin Creek**	**Moody Creek**	**Panther Creek**	
Acer rubrum	-	8.85	**58.6**	5.93	3
Arundinaria appalachiana	-	16.74	-	-	1
Cercis canadensis	-	8.40	-	-	1
Chimaphila maculata	-	-	10.84	-	1
Erythonium umbilicatum	-	-	-	6.72	1
Fagus grandifolia	-	**28.42**	9.17	-	2
Galax urceolata	**167.20**	-	-	6.45	2
Hexastylis rhombiformis	14.51	-	10.30	**12.45**	3
Hexastylis shuttleworthii	**37.03**	-	-	-	1
Liriodendron tulipifera	-	-	-	5.93	1
Pinus strobus	-	-	7.01	-	1
Polystichum acrostichoides	**24.17**	**56.64**	**53.52**	**85.90**	4
Quercus alba	-	11.52	5.70	-	2
Rumex acetosella	-	-	-	6.32	1
Smilacina racemosa	-	**83.01**	**12.00**	**29.40**	3
Smilax bona nox	**30.80**	-	-	-	1
Smilax glauca	**26.30**	-	**64.25**	11.75	3
T. persistens	-	-	**23.92**	**57.36**	2
Toxicodendron radicans	-	**29.91**	5.84	-	2
Tsuga canadensis	-	-	-	5.90	1
Viola hastata	-	-	-	6.35	1
Viola rotundifolia	-	-	-	5.98	1
Vitis rotundifolia	-	-	**38.86**	**53.53**	2
Woodsia obtusa	-	**36.42**	-	-	1

Table C.2. . Importance Values for the shrub layer at Battle Creek, Moccasin Creek, Moody Creek, and Panther Creek. IVs were calculated using relative frequency, relative density, and relative dominance. The maximum possible IV per site was 300. The top five importance values are bolded, except for Battle Creek, where there were only four values higher than the least represented species.

	Sites				**# of sites present**
Species	**Battle Creek**	**Moccasin Creek**	**Moody Creek**	**Panther Creek**	
Acer rubrum	**26.58**	**26.29**	8.79	4.85	4
Aesclesus sylvatica	-	-	-	5.61	1
Carya glabra	-	-	-	4.85	1
Fagus grandifloria	-	**26.29**	-	4.85	2
Kalmia latifolia	**105.04**	19.12	8.79	**10.46**	4
Leucothoe fontanesiana	-	-	**18.69**	9.40	2
Liquidambar styraciflua	10.45	-	-	-	1
Quercus alba	-	**44.77**	8.79	-	2
Rhododendron minus	**104.50**	**52.57**	-	**179.08**	3
Rhododendron maximum	10.45	-	**13.74**	**35.78**	3
Smilax glauca	-	15.86	**21.04**	**12.43**	3
Tsunga canadensis	**43.01**	**102.5**	**86.39**	**24.06**	4
Vitis rotundifolia	-	12.60	**18.80**	-	2

Table C.3. Importance Values for the tree layer at Battle Creek, Moccasin Creek, Moody Creek, and Panther Creek. IVs were calculated using relative frequency, relative density, and relative dominance. The maximum possible IV per site was 300. The top five importance values per site are bolded.

	Sites				**# of sites present**
Species	**Battle Creek**	**Moccasin Creek**	**Moody Creek**	**Panther Creek**	
Acer rubrum	**22.49**	**27.78**	**31.13**	**17.95**	4
Acer saccharum	-	-	-	2.87	1
Aesclesus sylvatica	7.64	2.41	6.69	2.68	4
Carya glabra	**21.65**	10.71	2.86	15.14	4
Cornus florida	5.48	1.73	7.84	9.45	4
Fagus grandifolia	3.85	**52.65**	7.67	15.16	4
Fraxinus americana	-	2.32	**24.70**	4.42	3
Ilex opaca	-	1.77	1.75	-	2
Kalmia latifolia	**34.94**	22.36	1.75	10.28	4
Liquidambar styraciflua	10.48	4.10	-	6.21	3
Liriodendron tulipifera	1.91	**23.19**	4.95	11.04	4
Magnolia fraseri	9.25	13.58	2.31	2.13	4
Nyssa sylvatica	-	20.40	**23.25**	-	2
Pinus strobus	10.79	-	13.38	**38.84**	3
Quercus alba	20.82	**31.12**	**39.38**	3.93	4
Quercus coccinea	-	1.82	-	-	1
Quercus prinus	1.46	-	1.98	1.58	3
Rhododendron maximum	**31.59**	14.76	4.07	**21.01**	4
Rhododendron minus	21.44	10.42	-	**57.56**	3
Tilia americana	-	-	-	9.14	1
Tsuga canadensis	**85.82**	**58.88**	**126.30**	**70.61**	4

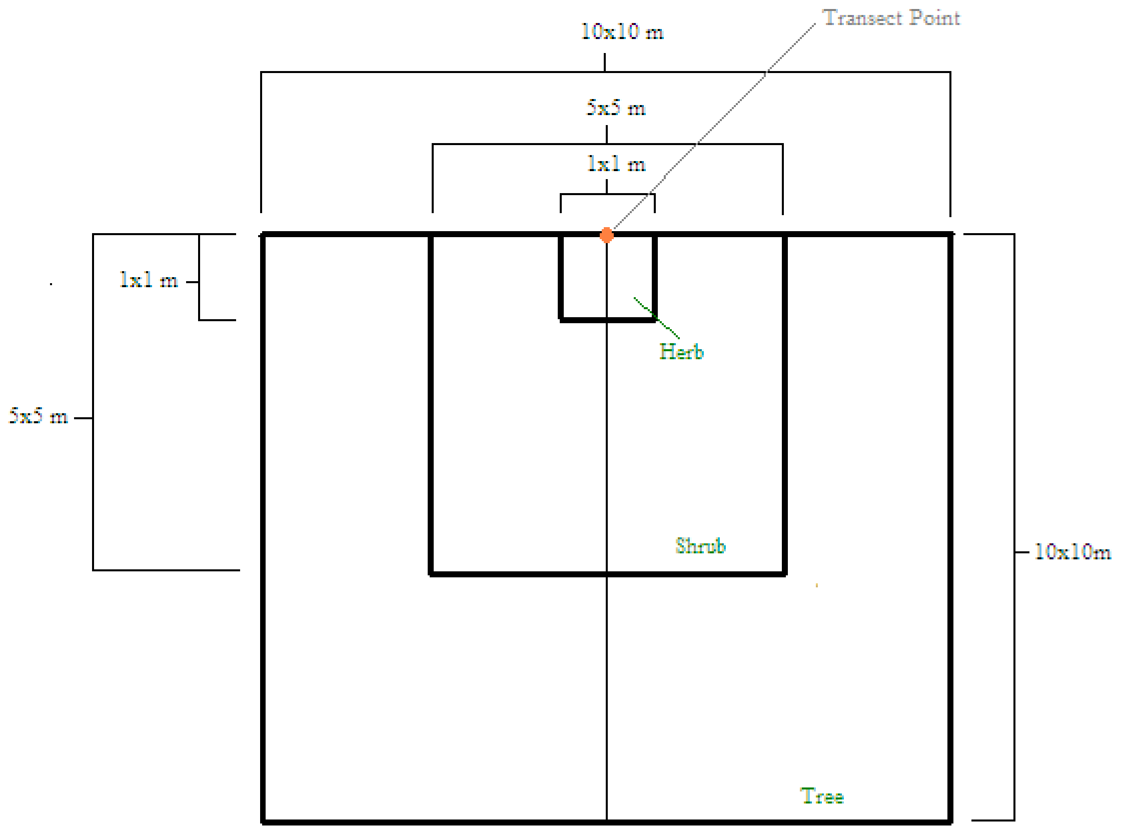


Figure C.1. Diagram of one transect point illustrating the herb, shrub, and tree layers.

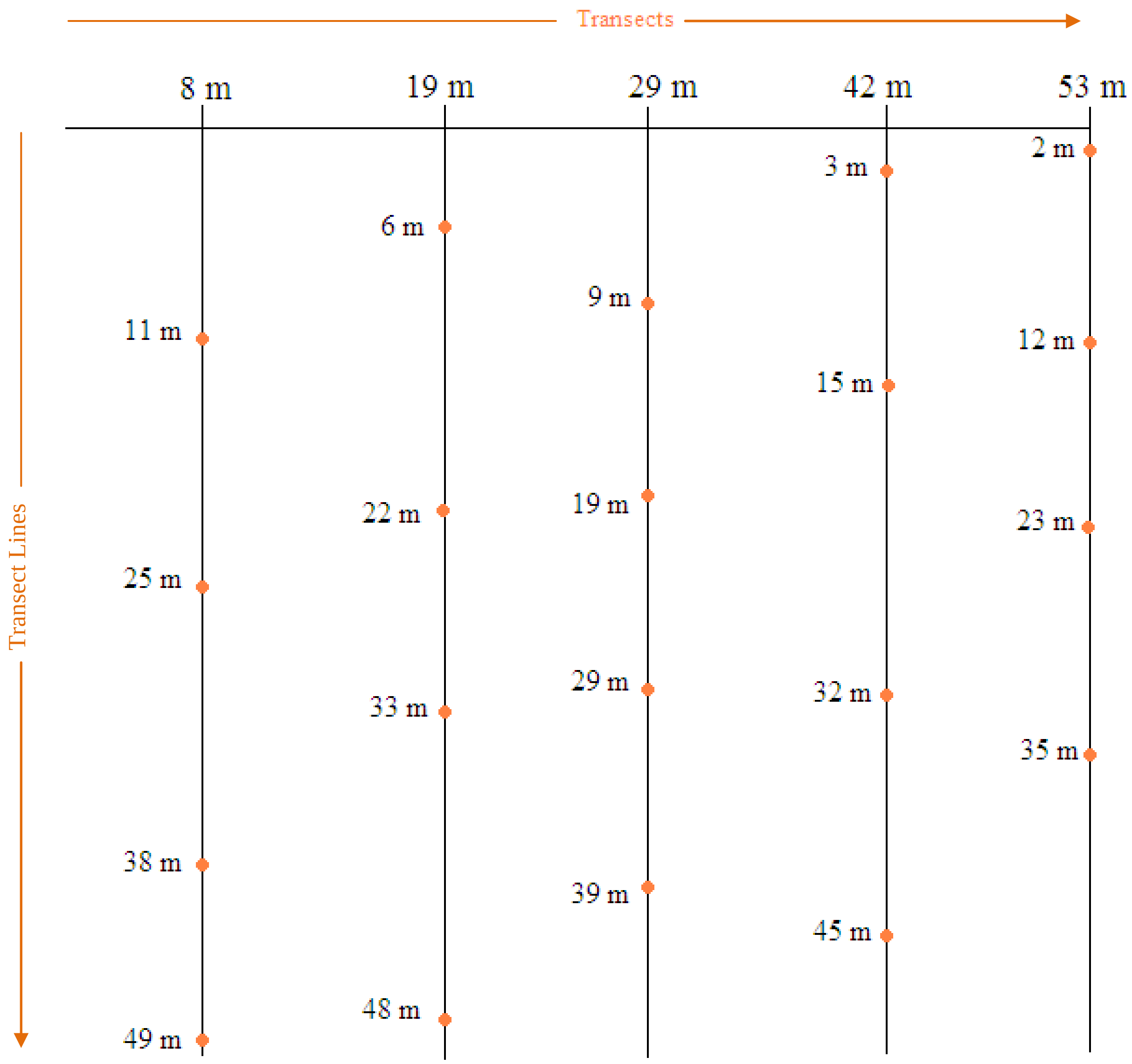


Figure C.2. Community sampling scheme used at each of the four sites. Measurements were derived from a random numbers table. The transect lines were set perpendicular to the top transects. Herbs, shrubs, and trees were measured at each transect point along the transect line.

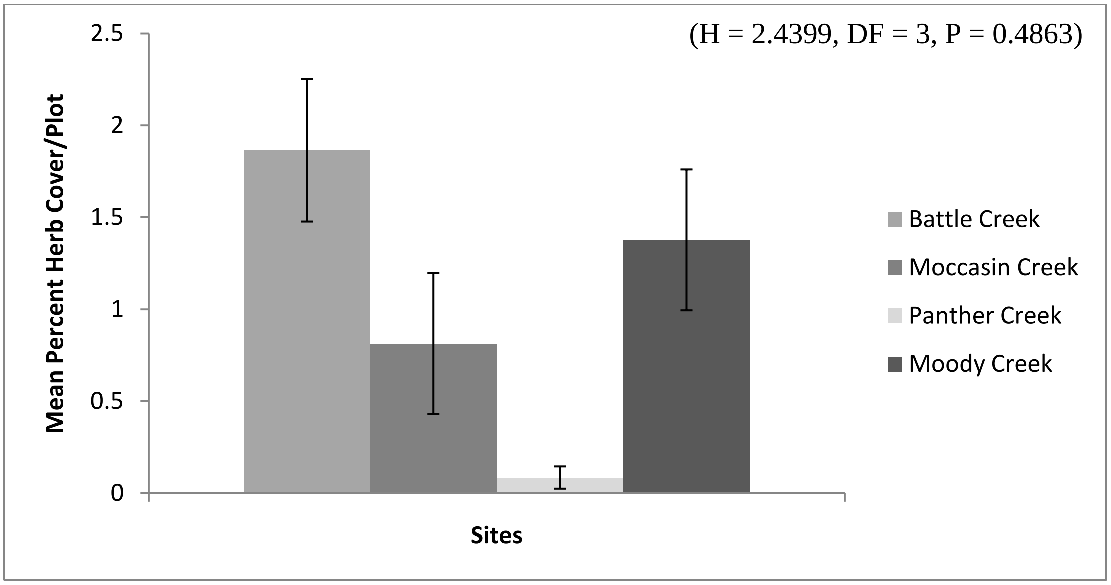


Figure C.3. Mean percent herb cover/plot for the four sites.

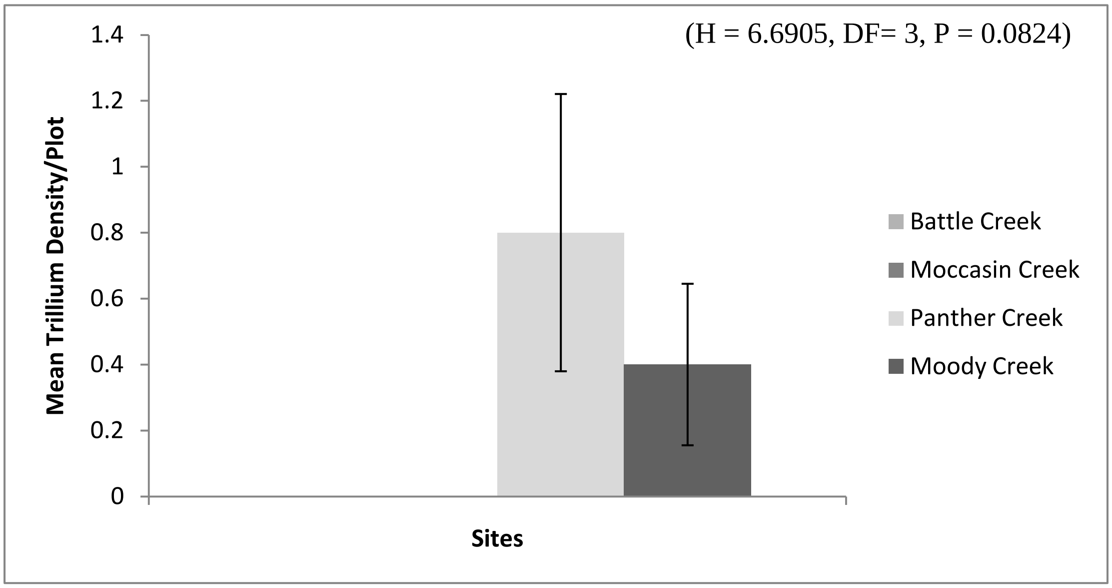


Figure C.4. Mean *T. persistens* density/plot for the four sites determined from transect data.

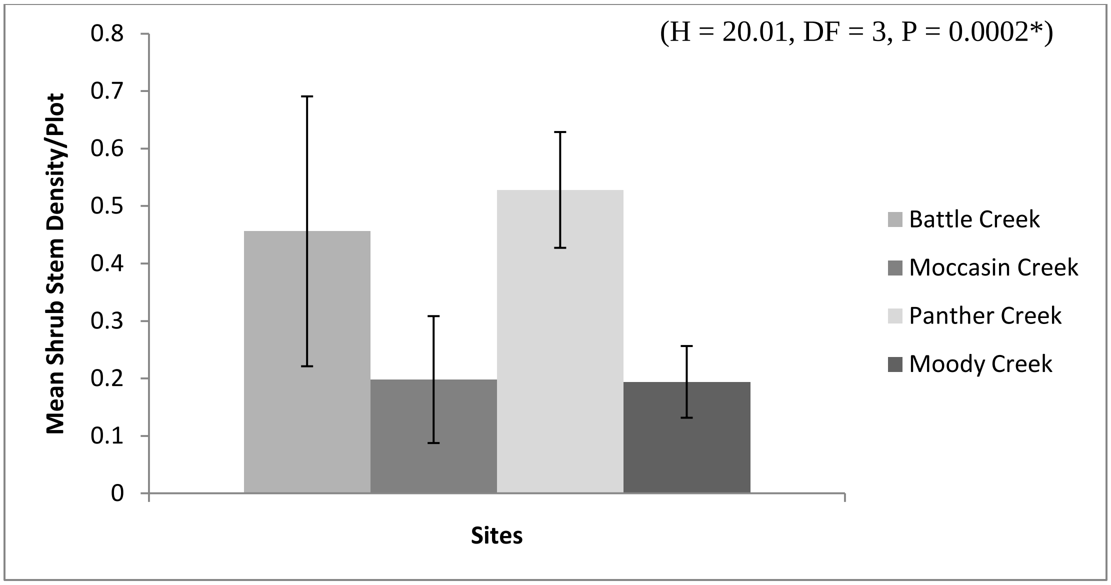


Figure C.5. Mean shrub stem density/plot for the four sites.

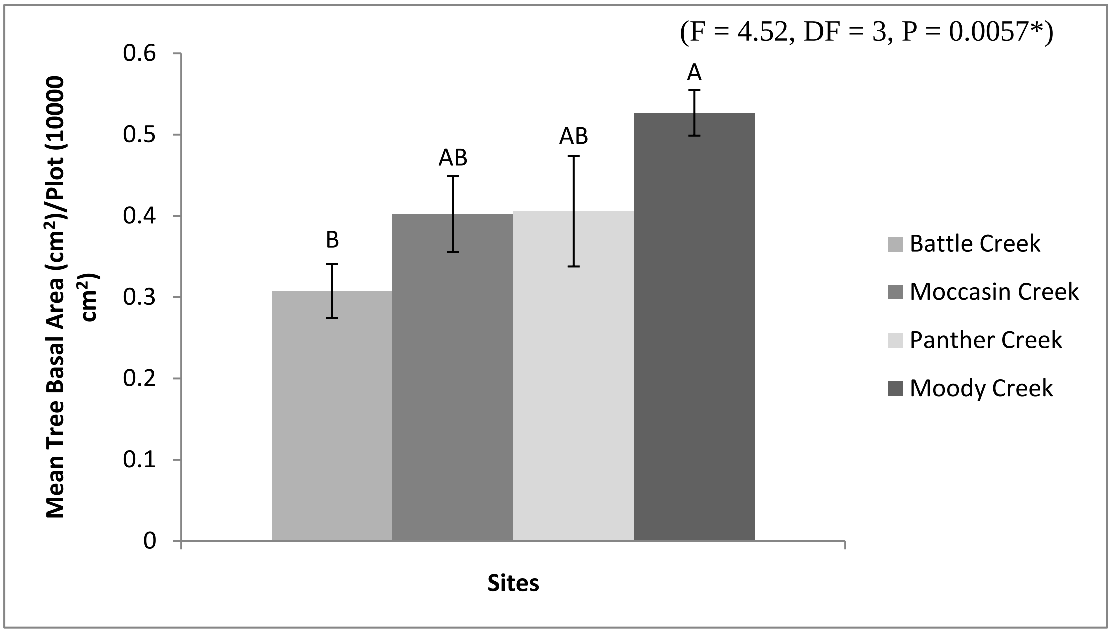


Figure C.6. Mean tree basal area (cm^2)/10000 cm^2 plot for the four sites.

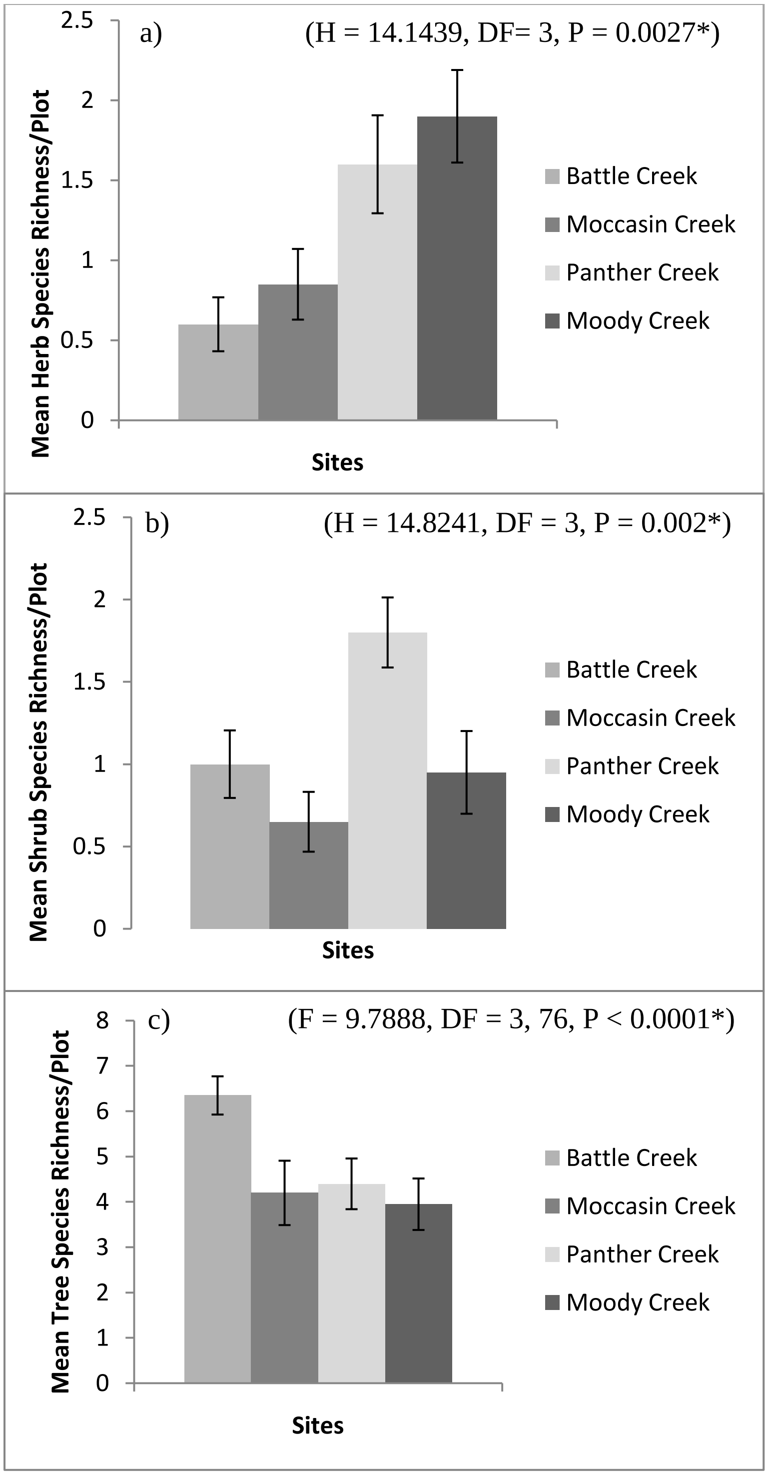


Figure C.7. Mean species richness for a) herb, b) shrub, and c) tree for the four sample sites.