Fall 2004

Impacts of Exotic Invasive Vines on the Ecology and Reproduction of the Endangered Trillium Reliquum

Christopher D. Heckel

Recommended Citation

Heckel, Christopher D., "Impacts of Exotic Invasive Vines on the Ecology and Reproduction of the Endangered Trillium Reliquum" (2004). *Electronic Theses and Dissertations*. 692.
https://digitalcommons.georgiasouthern.edu/etd/692

IMPACTS OF EXOTIC INVASIVE VINES ON THE ECOLOGY AND REPRODUCTION OF THE ENDANGERED *TRILLIUM RELIQUUM*

by

CHRISTOPHER D. HECKEL

(Under the Direction of Lissa M. Leege)

ABSTRACT

Biological invasions are the second largest threat to biodiversity next to habitat loss. Invaders imperil 42-49% of all species listed as threatened or endangered in the U.S., yet few studies have examined invasions from the viewpoint of endangered species. Rare species may be intrinsically more susceptible to invader impacts due to their already small global population sizes. Understanding the ecology of the interactions between invaders and rare plants is crucial to the conservation of native systems.

Trillium reliquum Freeman is an endangered plant restricted to 20 counties in three states that is imperiled by habitat loss and encroachment from the exotic invasive vines, kudzu (*Pueraria montana* Ohwi) and Japanese honeysuckle (*Lonicera japonica* Thunb.). My study examines the impacts of kudzu and honeysuckle on native plant communities, *T. reliquum* population dynamics and *T. reliquum* reproduction. In 2003 and 2004 I used vegetation sampling methods, demographic models, and a honeysuckle removal experiment to determine invader impacts on community structure and composition and *T. reliquum* population dynamics. I used supplemental pollen treatments to determine if pollen availability limited reproduction in 2004.

Invasive vines were associated with higher understory cover and lower overstory cover in invasive vine habitats, and with lower species richness in the kudzu habitat. Invasive vines were also associated with low *T. reliquum* population density, and stage structure differed among habitats. When honeysuckle was removed, trillium populations increased in size and recruited more new individuals. These results suggest that invasive vines affect trillium population dynamics by decreasing recruitment and altering reproductive and non-reproductive transition dynamics. Projections of trillium populations over time size further suggest that trillium populations will be extinct in kudzu habitat in 15 years.

Pollen availability did not limit trillium reproduction in 2004. Invasive vines were found to be associated with reduced seed set and seed size in trillium. The resource limitation experienced by *Trillium reliquum* coupled with hypothesized invasive vine limitations on trillium recruitment may put this species at a serious reproductive disadvantage. My results suggest that invasive vines play an important role in shaping community structure and *T. reliquum* population dynamics. Management efforts should focus on controlling invaders, monitoring reproductive and non-reproductive transition rates, and improving reproduction of this species.

KEYWORDS: *Trillium reliquum*, invasive species, rare specis, population dynamics, matrix models, seed production, breeding system, plant community, *Pueraria montana, Lonicera japonica*

IMPACTS OF EXOTIC INVASIVE VINES ON THE ECOLOGY AND REPRODUCTION OF THE ENDANGERED *TRILLIUM RELIQUUM*

by

CHRISTOPHER D. HECKEL

B.S., Hillsdale College, 1999

M.S., Georgia Southern University, 2004

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

2004

IMPACTS OF EXOTIC INVASIVE VINES ON THE ECOLOGY AND REPRODUCTION OF THE ENDANGERED *TRILLIUM RELIQUUM*

by

CHRISTOPHER D. HECKEL

Major Professor:	Lissa M. Leege
Committee:	Alan W. Harvey Lorne M. Wolfe

Electronic Version Approved:
December 2004

ACKNOWLEDGEMENTS

I thank Lissa Leege for being an excellent advisor and for all her help with my research. Her enthusiasm for research has been a great inspiration. I also thank my committee members, Alan Harvey and Lorne Wolfe, for sharing their insights and knowledge to help improve my research. All members of my committee provided outstanding advice on making it through graduate school and continuing on to the next level, for which I am grateful. Thanks to Ray Chandler for his aid with statistics and Don Drapalik for assisting me with plant identifications. My gratitude is also extended to Eric VandeGenachte, Tom Patrick, and Joe Murphy of the Georgia Department of Natural Resources for their assistance over the past two years. The Georgia Southern University College of Graduate Studies Graduate Student Professional Development Research Fund, Garden Club of America's Catherine H. Beattie Fellowship, Sigma Xi Grant in Aid of Research, Georgia Botanical Society Marie Mellinger Field Botany Research Grant, and in-kind support from the Georgia DNR made this research possible by providing funding.

Apart from scholastic aid, I thank Lissa, Frank, and Micah for allowing Pam and I into their family and making our stay in Statesboro a memorable experience. My parents and Pam's parents have been unwavering in their support of our endeavors and for this we are eternally grateful. Lastly, I thank the world's best research assistant/field photographer, my wife Pam. I can never really thank her enough; completion of this degree would not have been possible without her love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Literature Review

Impacts of invasions

In his book, *The Ecology of Invasions by Plants and Animals*, Charles Elton (1958) predicted an “ecological explosion” that would lead to a simpler and poorer world biota. Elton could not have been more right; invasive species are the second largest threat to biodiversity next to habitat loss in the United States (Wilcove et al. 1998). In fact, depending on the source of information, invaders put at risk between 42% (Pimentel et al. 2000) and 49% (Wilcove et al. 1998) of all species listed as threatened or endangered in the U.S. In addition to the peril to biodiversity, invasive species cause billions of dollars in damages in the U.S. each year (Pimentel et al. 2000). Protecting biodiversity from habitat loss requires only the purchase of land for preservation; however, to protect native species from invaders there is no simple solution. To further prevent biodiversity loss from biological invasions, managers and researchers must learn to control current invaders, predict species likely to invade, predict which species or communities may be more susceptible to invasion, and prevent the entry of new invaders.

The term “invasive species” can lead to some confusion (for review see Williams and Meffe 1998, for review see Davis and Thompson 2000). In general, a non-native or exotic species becomes invasive when it is introduced to areas outside of its native range, becomes established and spreads (Sakai et al. 2001). There have been up to 50,000 exotic species introduced into U.S. ecosystems (Pimentel et al. 2000), and an estimated 5%-10% of all introductions become established in natural systems (Williams and Meffe 1998). Once established, ecological interactions between invasive and native species can result in changes to the population dynamics, genetics and evolution of native plants (Williams and Meffe 1998, Mooney and Cleland 2001, Sakai et al. 2001). In this paper, the term "invasive species" will refer to those species whose introduction and establishment into habitat outside their native range could cause ecological or economic harm.

Invasive plants can affect native plants at ecosystem, community, population, or individual levels (Gordon 1998, Williams and Meffe 1998). Ecosystem-level effects may occur when invasions result in changes to nutrient cycling (Vitousek and Walker 1989), fire regimes (Holmes and Cowling 1997), physical structure of the ecosystem (e.g. increased litter (Olson and Wallander 2002) or erosion levels (Mack and D'Antonio 1998)). Invasive plants can further affect native plant communities by opening new habitat niches for other colonizers or invaders (Gordon 1998, Gill and Burke 1999), or filling previously unfilled niches (Fargione et al. 2003), and consequently changing community dynamics. Invasive plants directly impact native plants by competing for resources (such as light (Woods 1993, Yamashita et al. 2000), soil nutrients (Huenneke and Thomson 1995, Callaway and Aschehoug 2000), or water (Westbrooks 1998, Ewe and Sternberg 2002), suppressing native recruitment (Equihua and Usher 1993, Woods 1993, Olson and Wallander 2002), or slowing plant growth (Dillenburg et al. 1993, Hager 2004, Miller and Gorchov 2004). Indirectly, native plants may be affected by invasive plants that alter the behavior of pollinators (Kearns et al. 1998, Parker and Haubensak 2002) and seed dispersers (Bond and Slingsby 1984, Porter and Savignano 1990) or change the grazing patterns of herbivores (Trammell and Butler 1995). These direct and indirect interactions between invasive and native plants can change population dynamics of a species and ultimately lead to the complete displacement of a native species (Westbrooks 1998, Williams and Meffe 1998, Sakai et al. 2001).

Invasion effects on rare plants

Wilcove et al. (1998) reported that nearly half of the 723 plant species listed as threatened, endangered, or proposed for listing in the U.S. were reported to be declining at least in part, due to invasive species. Due to their intrinsic nature, rare plants may be more susceptible to the negative effects of invasion. Rabinowitz (1986) classified forms of rarity based on three criteria, including geographic range, habitat specificity, and local population size; Fiedler and Ahouse (1992) added temporal persistence of the taxon as a fourth criterion for evaluating rarity. Based on Rabinowitz's criteria, rare plants can be those that 1) have a wide geographic range but occur in only small localized populations, 2) have a narrow geographic range with large localized populations, or 3) are restricted to specific and unique habitats.

These different classes of rarity pose a unique problem in understanding the interactions between rare and invasive plant species. Consider two fictional rare species: one with a widespread geographic range but is locally sparse, and another with a narrow geographic range but a dense local population. The addition of invasion pressure has a negative effect on both species, increasing the speed of their decline toward extinction. Managers often must choose how to protect a species (Menges 1992), and the question here becomes, which species should receive priority for protection? On one hand, both species are rare and likely closer to extinction than a common species. On the other hand, the coupling of causes of rarity plus invasion effects may allow prioritization. Since the second species above had a more narrow geographic range it should receive more focus because even though it has larger populations it is more globally rare. The first of the two hypothetical species would have more areas to which it could survive that may be invader-free. Therefore, knowledge of the class of rarity may be useful to help determine where to aim conservation efforts.

Elucidation of the causes of rarity requires a great deal of historical, biological, and demographic data for a species (Fiedler and Ahouse 1992); data that are also essential to developing plans for the conservation of rare species (Schemske et al. 1994). Due to the nature of the defining criteria of rarity, the causes of rarity are wide ranging. Fiedler and Ahouse (1992) list thirteen classes of causes of rarity including ecological factors, life history strategies, population dynamics, evolutionary history, and taxonomic history among others. The patterns of growth, reproduction, and longevity make up a species' life history traits, and the right life history traits in appropriate conditions promote survival (Barbour et al. 1987). Invasive species can impact ecological factors, life history strategies, and population dynamics (Gordon 1998, Mack and D'Antonio 1998) and therefore may also be a cause of rarity.

Population dynamics of rare species

Examining the population dynamics of rare species can provide insight into whether a population is growing, declining, or stable (Werner and Caswell 1977, Bierzychudek 1982, Schemske et al. 1994, Horvitz and Schemske 1995, Byers and Meagher 1997, Parker 1997, Caswell 2001). Demographic information can be used to develop models to determine what part of a species' life history contributes the most to lifetime fitness (Crouse et al. 1987, Kalisz and McPeek 1992), identify vital rates that are most affected by environmental factors (Calvo and Horvitz 1990, Schemske et al. 1994, Caswell 2001, Knight 2004), and examine stochastic effects on population viability (Menges 1992, Cortes 1999, Kaye and Pyke 2003). Understanding population dynamics, not just individual responses to interactions with pollinators, herbivores and invaders, allows better planning of management strategies for sustaining and increasing populations (Crouse et al. 1987, Schemske et al. 1994, Caswell 2001).

***Trillium reliquum*, a rare endemic herb**

Trillium reliquum Freeman (relict trillium) is a perennial herb that occurs in undisturbed moist hardwood forests of the Piedmont regions of Georgia, Alabama and South Carolina (U.S. Fish and Wildlife Service 1988, Case and Case 1997). It has only 21 populations throughout its range and was placed on the Federal Endangered Species list in 1988 (U.S. Fish and Wildlife Service 1988). The primary threat to the survival of *T. reliquum* is habitat loss (U.S. Fish and Wildlife Service 1990), but the species recovery plan also lists encroachment by invasive vines, including kudzu (*Pueraria montana* (Lour.) Merr.) and Japanese honeysuckle (*Lonicera japonica* Thunb.), as serious threats to the species (U.S. Fish and Wildlife Service 1990). No formal studies have been conducted to describe the ecology of this species. Recent studies of other trillium species have shown that deer (Augustine and Frelich 1998, Knight 2003, Vellend et al. 2003, Knight 2004), seed dispersers (Ohara and Higashi 1987, Smith et al. 1989, Kalisz et al. 1999), and habitat fragmentation (Jules 1998, Jules and Rathcke 1999, Kalisz et al. 2001, Tomimatsu and Ohara 2002) impact trillium population dynamics. Routhier and Lapointe (2002) suggest that the length of exposure to the high light period before the forest canopy closes in the spring is very important to the survival and growth rates of early spring flowering plants like trilliums. Therefore, the impact of invasive vines, which have the potential to change canopy structure (Gordon 1998), may be significant.

The invaders

Kudzu has become a well-known invasive species throughout the southeastern U.S. (Winberry and Jones 1973, Kidd and Orr 2001, Allison 2003). Kudzu was first introduced into the U.S. at the World Exposition in Philadelphia in 1876 (Wechsler 1977). A leguminous, twining vine, native to east Asia (Wechsler 1977, Tsugawa and Kayama 1985), kudzu primarily spreads through asexual reproduction (Winberry and Jones 1973, Susko et al. 2001). About 3 million hectares of land in the southeast are infested with kudzu and its range increases by approximately 50,000 hectares each year (Pappert et al. 2000). Due to its rapid rate of growth (up to 0.3m per day) and dense foliage, kudzu can out-compete native plants (Westbrooks 1998). Kudzu can affect native plant communities by decreasing recruitment, altering stand structure, decreasing available light, and altering the nitrogen cycling of the ecosystem (Gordon 1998).

Japanese honeysuckle is an aggressive invasive vine introduced from Asia into the U.S. in the late 19th century (Schierenbeck et al. 1995, Allison 2003). It is a semi-evergreen woody vine with a rapid rate of growth that exhibits both climbing and trailing habits (Schierenbeck and Marshall 1993). This invader can grow under a variety of habitats including thickets, old fields, riparian zones, forests, and undisturbed natural communities (Robertson et al. 1994, Schweitzer and Larson 1999). Honeysuckle reproduces sexually, with bird-dispersed seeds, or clonally using stolons that may spread as much as 4.5m per year (Cain 1984). *Lonicera japonica* can form dense mats of vegetation on the forest floor which can affect native plant communities by altering the stand structure, decreasing recruitment, and competing for light (Cain 1984, Gordon 1998).

Objectives

The goal of my research was to describe the impacts of kudzu and Japanese honeysuckle on populations of *T. reliquum* by comparing demographic parameters, community characteristics, and seed production of *T. reliquum* in the presence and absence of invasive vines. My research asked several questions about the impacts of kudzu and Japanese honeysuckle. 1) Does the structure of the local plant community differ in the presence of invasive vines? 2) Do population dynamics of *T. reliquum* differ in the presence of invasive vines? 3) How are population dynamics of *T. reliquum* affected by the removal of honeysuckle? 4) Is *T. reliquum* reproduction resource- or pollen-limited and does the presence of invasive vines affect seed production?

This study is one of the first to quantify the impacts of two notorious invasive species on natural plant communities of the southeastern U.S. Few published studies have documented the impacts of these two invaders on natural communities (see Slezak 1976, Dillenburg et al. 1993). Prior to this study the ecological consequences of kudzu and honeysuckle invasion were inferred from studies focused on other aspects of the biology of these two invaders (Wechsler 1977, Cain 1984, Carter and Teramura 1988, Schierenbeck and Marshall 1993, Schweitzer and Larson 1999). My research emphasizes the impacts of invaders on the ecology and reproduction of an endangered species and provides critical information about its population dynamics.

METHODS

Study site

I conducted my study at Montezuma Bluff Natural Area (MBNA) (N32°20' W84°1') in Macon County, GA. This 202 hectare natural area lies along the east bank of the Flint River and is characterized by limestone outcroppings and a mixture of beech-magnolia hardwood and coniferous forests growing on steep, moist slopes. MBNA encompasses large populations of endangered *Trillium reliquum* in habitats of varying degrees of kudzu and honeysuckle encroachment. This site was chosen for the study as it contained a *T. reliquum* population with thousands of individuals, and it is likely that more than half of the total population at this site grows in the presence of either kudzu of Japanese honeysuckle (personal observation).

Study system

Trillium reliquum is a perennial spring ephemeral herb, native to only three states in the southeastern U.S (Appendix 2). It overwinters as an underground rhizome that puts out one or more new shoots each spring (Appendix 1). In MBNA, individuals first emerge in late February or early March, and flowers bloom in mid-March for a period of 2-3 weeks. The above-ground parts are identified by one leaf (in juveniles) or a whorl of three leaves (in non-reproductives and reproductives) at the end of a decumbent stem. The leaves are mottled with three distinct shades of green, and a silvery stripe down the leaf mid-vein. Reproductive plants produce a sessile flower with three petals that are purplish in color. This species is most easily identified by its distinctive beaked anthers (Appendix 3, Freeman 1975, Patrick et al. 1995). This species is not self-compatible

(Eva Gonzales, personal communication) but will produce fruit and seed via apomixis (Appendix 1). The ecology of pollination and seed dispersal remains unknown for this species. I observed only two candidate pollinators during > 250 hours in the field. Fruit development continues until maturation in June when the fruits break open and release seeds. Eliaosomes attached to the seeds are a reward for ants that serve as dispersal agents.

Trillium reliquum has a life cycle similar to other *Trillium* species (see Patrick 1973, Kawano et al. 1986, Jules 1998). *Trillium reliquum* individuals go through four distinct morphological stages in their lifetime (Appendix 4). An individual spends its first season as a seedling and emerges the next season as a juvenile, with only one true leaf. As the rhizome accumulates enough photosynthate, the individual will transition into a three-leaf non-reproductive stage followed by a reproductive (flowering) stage, often with several years between transitions. Individuals experiencing physical damage or other stressful conditions may also back-transition to an earlier stage. In addition to the four above-ground stages, *T. reliquum* may also remain in a dormant stage with no above-ground shoots during the growing season (Appendix 1). The life span of *T. reliquum* is not currently known; other species in the genus can have life spans > 20 years (Case and Case 1997).

Experimental Design

To examine local plant community structure and composition and to determine population dynamics of *Trillium reliquum* in the presence and absence of invasive vines, I randomly selected and permanently marked four 15 x 15m sites of each of three pre-existing habitats: 1) no-vine: no invasive vines present, 2) kudzu: *T. reliquum* and kudzu present, and 3) honeysuckle: *T. reliquum* and honeysuckle present. After emergence of *T. reliquum* in the spring of 2003, I randomly selected 1x1 m plots within each 15x15m site until I had 20 plots containing *T. reliquum* (Appendix 5).

I mapped the location of each *T. reliquum* individual within a plot and recorded the GPS coordinates (Appendix 6) of each reference flag with a Garmin GPS 48 handheld GPS unit (Garmin, USA). To permanently mark the location of each individual I nailed a uniquely numbered aluminum tag into the soil ~3 cm from the base of each plant. I recorded the tag number, life stage and leaf size (length and width of one leaf) for every *T. reliquum* in a plot. Leaf size may be a good indicator of biomass (Kawano et al. 1986) which can in turn lead to predictions about plant age (Hanzawa and Kalisz 1993), however since seedlings have no true leaves and must either transition or die in the next season, the seed leaf was not measured.

I censused plots in all habitats from March 18-23 and May 5-31 in 2003 (to assess single season survivorship) and March 13 – April 3, 2004 (Appendix 5). Any surviving/emergent individuals not tagged in the first year's census received tags in 2004; individuals whose tags could not be found in 2004 received new tag numbers. Also, in cases where seedlings grew in dense clumps, seedlings did not receive permanent tags because tags could potentially interfere with emergence in the next season. Due to similarities in morphology between *T. reliquum* and *T. maculatum* and the fact that the two grow in complete sympatry, it can sometimes be difficult to accurately identify the species individuals in non-reproductive stages. Consequently, estimates of the proportion of the individuals in *T. reliquum* populations of non-reproductive stages may be slightly positively biased.

Honeysuckle removal experiment

In addition to the 12 sites in the demographic study, I randomly selected and permanently marked four 15 x 15m sites (removal sites) in honeysuckle habitat for use in a honeysuckle removal experiment. This manipulative experiment allowed me to determine the effect of honeysuckle on population growth of *Trillium reliquum*. A kudzu removal experiment was not conducted because: 1) efficient methods of kudzu removal are not generally agreed upon (Zidac and Backman 1996, Kidd and Orr 2001) and 2) funding and available manpower did not permit it. I used Roundup™ Poison Ivy and Tough Brush Killer (27% glyphosate) to remove honeysuckle from plots following the first *T. reliquum* demography assessment. Before applying herbicide, I first covered all *T. reliquum* individuals in the sites with 16 ounce plastic cups to protect them from herbicide. On April 12, 2003 I used a 11L garden compression sprayer to apply herbicide at the manufacturers recommended rate (1:2 ratio of herbicide:water) (Appendix 5).

I applied herbicide to all honeysuckle inside the 1m^2 area of each plot and to all honeysuckle within buffer zone with a 1m radius around each plot. Species other than honeysuckle were not the target of herbicide application, however, due to the density of honeysuckle in the habitat some non-target species received treatment. Treatment occurred early in the growing season, so many non-target species with late emergence avoided treatment. In March 2004 I re-censused the *T. reliquum* population in removal sites (Appendix 5).

CHAPTER II

Invasive Vine Impacts on the Plant Community at Montezuma Bluffs Natural Area, Macon County, GA

A wide variety of abiotic factors and biotic interactions help to shape plant community dynamics. Many studies of plant community assemblage focus on the availability of resources such as water, light, soil nutrients (Carson and Pickett 1990, Stevens and Carson 2002, Baer et al. 2004). Biotic interactions, however, can be equally important in determining community level processes (Wootton 1994). The addition of invasive plants to a community is one such biotic interaction that can have a great impact on plant communities (Vitousek and Walker 1989, Trammell and Butler 1995, Gordon 1998, Dukes 2001, Shea and Chesson 2002). Community-level effects of invaders can include decreased species richness and altered physical community structure, for example changes to understory and canopy cover (Vitousek and Walker 1989, Holmes and Cowling 1997, Miller and Gorchov 2004). Once established, some invaders can cause ecosystem-level disturbances such as changes to nutrient cycling (Vitousek and Walker 1989) or hydrology (Tickner et al. 2001) that may further facilitate invasion by other non-natives.

Invasive plants may directly or indirectly affect native plant communities. Direct effects may include decreased recruitment (Holmes and Cowling 1997, Olson and Wallander 2002, Miller and Gorchov 2004), allelopathic effects (Callaway and Aschehoug 2000), or competitive exclusion (Huenneke and Thomson 1995, Bockelmann and Neuhaus 1999, Hager 2004). Indirect effects may be ecosystem-level changes to soil quality (Vitousek and Walker 1989, Kourtev et al. 1998) and hydrology (Tickner et al.

2001). Invaders may also indirectly affect native species via competition for pollinators (Parker 1997, Parker and Haubensak 2002) or by apparent competition for predators (Trammell and Butler 1995).

Kudzu, *Pueraria montana* Ohwi., is an invasive aggressive vine that can alter the composition and structure of a plant community (Gordon 1998, Allison 2003). Due to its dense foliage and rapid growth, kudzu has strong potential to shade out other plant species, by presenting a dense physical barrier that prevents the growth and survival of native plants (Wechsler 1977, Westbrooks 1998). Kudzu may have a competitive advantage over natives in resource acquisition, as kudzu is a leguminous vine that fixes nitrogen (Slezak 1976, Dillenburg et al. 1993, Fujita et al. 1993). Kudzu is thought to affect geomorphology by decreasing erosion rates (Winberry and Jones 1973).

Similar to kudzu, the rapid growth of Japanese honeysuckle, *Lonicera japonica* Thunb., may afford this species a competitive advantage in garnering resources like light, water, and nutrients (Slezak 1976, Dillenburg et al. 1993). Honeysuckle may also be at a competitive advantage because, unlike kudzu, it has its leaves year-round (Slezak 1976), allowing continuous growth. Additionally, Japanese honeysuckle may change the structure of the communities it invades by climbing trees, and thus increasing canopy cover (Dillenburg et al. 1993, Schweitzer and Larson 1999). Japanese honeysuckle has been especially problematic on tree plantations, where the vine is known to overtake seedling trees, ultimately resulting in seedling death (Slezak 1976, Cain 1984). In a natural environment, this ability to overtake seedling trees may result in significant changes in the age structure of a forest.

It is well known that both kudzu and Japanese honeysuckle are major ecological pests in the southeastern U.S. (Allison 2003). However, few studies document differences in community composition and structure associated with their presence (but see Slezak 1976, but see Dillenburg et al. 1993), and those that do are often conducted in a silvicultural context (Cain 1984). Instead, the focus of most studies is to elucidate the mechanisms behind their success as invaders (Wechsler 1977, Forseth and Teramura 1987, Carter and Teramura 1988, Schierenbeck and Marshall 1993, Schweitzer and Larson 1999), to find methods for controlling these vines (Zidac and Backman 1996, Boyette et al. 2002), or to examine the relatedness of populations across the range of invasion (Schierenbeck et al. 1995, Pappert et al. 2000, Jewett et al. 2003, Belote et al. 2004). The objective of my research is to determine community-level impacts of kudzu and Japanese honeysuckle on a mature forest ecosystem by comparing species richness, vegetative cover and stand structure of habitats with and without invasive vines.

METHODS

Plant Community Measurements

Ground Layer Vegetation – I compared the plant community structure and composition among three different pre-existing habitats (no-vine, kudzu, and honeysuckle). In April 2003, I sampled all vegetation < 200 cm tall rooted within each of the 20 randomly chosen 1x1m plots in each site that contained *Trillium reliquum* (Appendix 5). Measurements in these plots determined the habitat characteristics in plots where trillium grew. To determine overall characteristics of the three different habitats, in April 2004, I sampled all vegetation < 200 cm tall in 25 completely randomized plots within each site. In both sampling years, I identified and counted the number of all species present (species richness) and their abundance (% cover, # stems), including invasive vines and *T. reliquum* within each plot. In the removal experiment, post-herbicide-treatment measurements were made one year after application. I identified specimens in the field and in the lab using field guides and taxonomic keys (Radford et al. 1968, Duncan and Duncan 1988, 1999, Porcher and Rayner 2001) and placed voucher specimens in the Georgia Southern University Herbarium.

I calculated modified importance values (IV) for all species in each site by summing the relative frequency and the relative cover where:

$$\text{Relative Frequency} = \frac{\text{Absolute frequency of species}}{\sum \text{Absolute frequency for all species}}$$

and

$$\text{Relative Cover} = \frac{\sum \text{\% cover of species in site}}{\sum \text{\% cover all species in site}}$$

Calculation of importance values normally would include the relative species density (Brower et al. 1998), but because the number of individuals per plot was indeterminable for many of the vine species, this part of the formula was omitted. The importance values allowed an assessment of which plant species are the most influential in the local plant community. I determined the 15 species with the highest mean IV within each habitat, and pooled these species into one list of 27 most important species. I omitted values for kudzu and honeysuckle, as their presence was necessary for definition of habitat types; all other species had equal chance of being found in any habitat. To indicate similarity in community composition, I ranked the importance values within habitat type of each of the 27 species and calculated Kendall's coefficient of concordance, which tests whether the species of highest importance were in agreement among habitats (Sokal and Rohlf 1995).

To determine habitat effect on species richness and understory cover, I used nested ANOVA with habitat as a fixed effect and site[habitat] as a random effect. In order for data sets to meet normality and homoscedasticity assumptions of the tests, I transformed data using a variety of transcendental and trigonometric functions to obtain the best fit. When significant differences were found, I used Student's t-tests to make pairwise comparisons of habitats. I compared pre- and post-herbicide treatment species richness, cube root-transformed total understory cover, square root-transformed honeysuckle cover, and log-transformed cover from all species excluding honeysuckle with paired t-tests. All statistical analyses were performed using JMP-IN 5.1 (SAS Institute, Cary, NC).

<u>Forest stand structure</u> – To determine forest stand structure, I measured the basal area of canopy trees and canopy cover in each site. After leaf-out in the spring of 2003 (Appendix 5), I measured overstory cover from the center of each site in four cardinal directions with a spherical densiometer (Forestry Suppliers Inc., Jackson, MS). In 2004, I determined the stand density and basal area of trees contributing to the forest canopy in each of the 16 sites by measuring the diameter at breast height (DBH) of each tree in the site (Appendix 5). I considered canopy trees to be any tree that whose crown was not covered by the branches of a neighboring tree. I identified each tree to at least the genus level. As a measure of invasive vine impact on mature trees, I recorded the number of standing dead trees and compared between natural, kudzu, and honeysuckle habitats

using a G-test. I compared log-transformed canopy cover data and log-transformed basal area data among habitats with nested ANOVA with habitat as a fixed effect and site[habitat] as a random effect. To determine if there were differences in the structure of the forest community, I compared canopy tree density within habitat I used one-way ANOVA.

Abiotic measures – To determine differences in the abiotic factors associated with the different habitats, I measured available light and soil quality in all habitats in spring 2004 (Appendix 6). I measured the intensity of available light in each plot using the AccuPAR-80 ceptometer (Decagon Devices, Inc., Pullman, WA). To measure light intensity in each plot, I stood at the NW corner of each plot and held the ceptometer at waist level (~1m high) across the plot toward the SE. I configured the ceptometer to take 3 light samples in each plot 500 ms apart. I conducted all light sampling on days with clear sky conditions between 11 am and 1 pm. On March 19, 2004, before forest canopy closure, I sampled available light in each plot in all sites of the natural, kudzu, and honeysuckle habitats; I sampled the removal habitat the next day. I re-sampled available light on April 17 and 18, 2004 in all habitats after full closure of the forest canopy to compare seasonal differences within the forest understory. I compared cosine-transformed pre- and post-canopy closure light intensity data among habitats using one-way ANOVA. To compare differences in light intensity before and after canopy closure I used paired t-tests.

I collected soil samples from all sites in each habitat in April 2004. I took soil cores from a depth of 10cm from 5 different points in each site (the four corners and the center point). All five soil cores for each site were homogenized and samples were sent to the University of Georgia Soil Science Department for analysis of total nitrate, P, K, Ca, Mg, Zn, Mn and soil pH. Kudzu is a nitrogen fixer (Fujita et al. 1993), so I compared total nitrate among habitats with ANOVA to determine if the kudzu habitat was more nitrogen rich. I compared soil fertility among habitats using Principal Component analysis. Principal components 1 and 2 accounted for 73% of the variation and were compared among habitats with ANOVA.

RESULTS

Understory Vegetation

Plant communities in the kudzu, honeysuckle and no-vine habitats differed in structure and composition. In 2003, understory cover did not differ among habitats in plots that contained trillium (Table 2.1, Figure 2.1). In 2004, understory cover in kudzu and honeysuckle habitat was 45% to 95% higher than that of the no-vine habitat (Table 2.1, Figure 2.1).

In both sampling years, species richness in the honeysuckle and no-vine habitats was higher than in the kudzu habitat (Table 2.1, Figure 2.2). In 2004 species richness in the kudzu habitat was less than one-half that of the other two habitats (Figure 2.2). The understory cover and species richness of sites within habitats were similar based on the variance component of the nested effect in all tests.

Both kudzu and honeysuckle had the highest importance values in their namesake habitats (Table 2.2), and *Hexastylis arifolia* had the highest importance value in the no-vine habitat. In the no-vine habitat over half of the species were forbs typical of southeastern forest understory communities, one-third were woody species (all seedling tree species) and only two of the species were lianas. The kudzu habitat was similar to the no-vine habitat in that over half of top species were forbs; however almost one-third were lianas and only two were woody plants, a reversal in comparison with no-vine habitat (Table 2.2). The honeysuckle habitat had the opposite complexion in that almost half of the 15 species of highest importance listed were lianas, one-third were woody species, and only four forb species were represented in the top 15 (Table 2.2). Kendall's coefficient of concordance showed that there is no agreement among habitats in the ranking of species within habitats (W_c=0.314, df=25, χ^2=23.57, P>0.50).

In the removal habitat species richness, total cover and cover from honeysuckle decreased in the year after herbicide treatment; however, there was no change in the total understory cover excluding honeysuckle (Paired t-test, t=0.36, df=80, P=0.72). Pre- and post-treatment species richness in removal habitat plots decreased from 7.5 to 5 species per plot (±SE) (Paired t-test, t=-10.21, df=80, P<0.0001). Figure 2.3 shows that overall cover decreased by almost one-third after herbicide treatment (Paired t-test, t=-4.62, df=80, P<0.0001) and cover from honeysuckle decreased by one order of magnitude (Paired t-test, t=-13.60, df=80, P<0.0001).

Forest Canopy

In 2003, canopy cover in the honeysuckle and no-vine habitats was found to be almost one-third greater than the canopy cover in kudzu habitat (Figure 2.4). No difference was found in mean basal area of living trees (Honeysuckle: 619.0 cm^2 ±133.0, Kudzu: 679.5 cm^2 ±388.6, No-vine: 436.9 cm^2 ±87.41) among habitats (Table 2.1). Also, in the kudzu habitat about 23% of all canopy trees were dead, whereas in the other two habitats only 2% of canopy trees were dead (G-test, G=8.98, df=2, P=0.011). Canopy tree density (±SE) did not differ among habitats (Honeysuckle: 10.25/15m^2 ±2.17, Kudzu: 4.5/15m^2 ±1.19, No-vine: 11/15m^2 ±2.12; F=3.56, df=2, 9, P=0.073).

Abiotic measures

No differences were found in below-canopy light intensity (±SE) among honeysuckle, kudzu and no-vine habitats (Table 2.1) before or after canopy closure. High variation in the light intensity in the kudzu habitat, a factor which may explain why there was no difference among habitats even though it seems the kudzu habitat receives more intense light (Figure 2.5). Available light post canopy closure dropped to < one-quarter its pre-closure intensity in the understory of the honeysuckle and no-vine habitats (Figure 2.5, Paired t-test, Honeysuckle: t=-8.44, df=3, P<0.01; No-vine: t=-6.82, df=3, P<0.01), yet there was no difference in the kudzu habitat (Kudzu: t=-2, 78, df=3, P=0.07).

Nitrate levels did not differ among habitats (Table 2.3, F=2.93, df=2, 9, p=0.11). Soil nutrients levels were lower in the honeysuckle habitat for all nutrients measured (Table 2.3) and soil pH was found to be acidic in all habitats (Table 2.3). The soil nutrient composition differed among habitats, with kudzu and no-vine habitats likely to have richer soils than honeysuckle habitat (F=7.77, df=2, 9, p=0.011) based on principal component 1. This component explained 51.2% of the variation in soil measurements (Table 2.4, Figure 2.6).

DISCUSSION

Kudzu and Japanese honeysuckle are associated with differences in community structure and composition in comparison with habitat lacking these invasive vines. In 2003, plots containing *Trillium reliquum* had similar amounts of cover in all three habitats, yet in 2004 the random plots in invasive vine habitats were higher in cover compared with the no-vine habitat. This suggests invasion of exotic vines may be decreasing the amount of habitat patches suitable for trillium survival.

Higher cover is likely directly attributable to the presence of the invasive vines themselves. Both species of invaders are known to have high growth rates and to produce dense foliage (Slezak 1976, Wechsler 1977). This is also supported by results from the honeysuckle removal experiment. Significant change in understory cover between years was due to a reduction in honeysuckle cover because there was no reduction in the cover from other species. High cover associated with invaders may increase competition for light and soil nutrients, ultimately thinning out less effective competitors. Native plants that lack phenotypic plasticity or that grew slowly may be at a competitive disadvantage when invaders change community structure. Displacement may already be occurring in these habitats, as evidenced by the low species richness in the kudzu habitat in comparison to the no-vine habitat.

In comparison to the kudzu and honeysuckle habitats, the most important species in the no-vine habitat were typical to the forest understory species in the spring, i.e. spring ephemerals and tree recruits. The greatest difference in importance values of species was in the kudzu habitat, where five of the ten most important species (*P. montana, G. aparine, S. graminea, L. japonica, G. carolinianum*) were ruderals, including the two invasive vines, typical to frequently disturbed or waste areas. A second focal aspect of the importance values of species in the kudzu habitat was the lack of woody species in the understory. This seems to suggest that there is a lack of tree recruitment in that habitat. Couple this with the nearly significant (P=0.073) lower canopy tree density in kudzu habitat and that 23% of the overstory trees in the kudzu habitat were dead, and it suggests that kudzu's largest impact on the community is to alter the vertical structure of the community and become the dominant canopy species.

A larger proportion of lianas had high importance values in the honeysuckle habitat compared to the no-vine and kudzu habitats. Vines may alter the successional patterns of a habitat. Dillenburg et al. (1993) showed that honeysuckle and *Parthenocissus quinquefolia* have slowed growth of host tree species, and Schnitzer et. al (2000) found lianas to inhibit non-pioneer tree survival and slow gap-succession in tropical forests. Differences in species composition may also reflect differences in soil quality among the habitats. The trailing habit of lianas may afford them a higher mobility and allow them to reach more nutrient- or light-rich areas more quickly than forb or woody species.

Also in the kudzu habitat, trends in light intensity data suggested more light was able to reach the understory kudzu habitat, but these trends were not significant. High variability between light measurements in the kudzu habitat most likely accounted for the non-significance. The kudzu habitat was the most heterogenous in vertical structure because it is located in a large forest canopy gap. This gap may have allowed the initial colonization of this area by kudzu. The gap can account for high variability among light measurements in this habitat because sample recorded along the edge of the habitat were under the forest canopy and samples from the center were in full sun. The increased light availability may further affect the community structure by altering species richness. Carson and Pickett (1990) found that light enrichment without increased soil moisture resulted in the decline of species richness in old-field communities. Future studies may examine differences in soil moisture available among the kudzu, no-vine, and honeysuckle habitats.

The soil nutrient and pH profiles of the honeysuckle, kudzu, and no-vine habitats were distinct for each habitat. The kudzu and no-vine habitats were the most nutrient rich. In the honeysuckle habitat, the low soil nutrient levels together with the higher cover compared to the no-vine habitat suggests that trillium in this habitat may face the most intense competition for resources. I hypothesized that kudzu habitat would have the highest amounts of nitrate because kudzu fixes nitrogen. However, nitrate levels did not differ among sites.

In MBNA, differences in observed community structure and composition could be associated with the presence of invasive vines. The honeysuckle removal experiment results suggest that honeysuckle may have a major role in restructuring the local plant community. Further research is needed to address the mechanisms behind the changes that occur when an invader enters a community. Additional studies are needed to examine the impacts of these vines on other communities at other sites to determine if the differences observed in this study are a common feature of invasion. This study provides further evidence that invasive species can facilitate community-wide changes.

Table 2.1. ANOVA results for tests of plant community characteristics on data collected in 2003 and 2004. All variables were tested using a nested ANOVA except for 2004 pre-canopy closure below-canopy photosynthetically actively radiation (PAR) which was tested using one-way ANOVA due to lack of replicates within sites. df = degrees of freedom, MS = mean square, F = F-statistic, P = probability. Data transformations are listed under each variable.

Variable	***Source of Var.***	***df***	***MS***	***F***	***P***
2003 Understory Cover	Habitat	2	0.800	2.767	0.1156
(cos*x*)	Site[Habitat]	9	0.289	0.605	0.7927
	Plot	11	0.381	0.798	0.6425
	Error	230	0.478		
2004 Understory Cover*	Habitat	2	75.996	6.307	**0.0194**
($\sqrt{x}$)	Site[Habitat]	9	12.051	4.411	**<0.0001**
	Plot	11	23.651	8.658	**<0.0001**
	Error	288	2.730		
2003 Species Richness	Habitat	2	0.250	0.200	0.8222
(cos*x*)	Site[Habitat]	9	1.247	2.667	**0.0058**
	Plot	11	1.063	2.274	**0.0119**
	Error	230	0.468		
2004 Species Richness*	Habitat	2	14.002	7.482	**0.0122**
($\sqrt{x}$+0.05)	Site[Habitat]	9	1.872	9.003	**<0.0001**
	Plot	11	4.083	19.642	**<0.0001**
	Error	288	0.208		
2003 Canopy Cover	Habitat	2	0.118	7.341	**0.0129**
$\log_{10}x$	Site[Habitat]	9	0.016	3.642	**0.0026**
	Plot	11	0.035	7.842	**<0.0001**
	Error	36	0.004		
2004 Basal Area	Habitat	2	4.802	2.890	0.098
ln*x*	Site[Habitat]	9	1.731	1.437	0.183
	Plot	11	1.954	1.622	0.105
	Error	91	1.205		
2004 Below Canopy PAR	Habitat	2	0.243	1.041	0.3921
(Pre-Canopy Closure)	Error	9	0.233		
(cos*x*)					
2004 Below Canopy PAR	Habitat	2	0.699	1.288	0.3222
(Post Canopy Closure)	Site[Habitat]	9	0.543	1.013	0.4303
(cos*x*)	Plot	11	0.572	1.068	0.3877
	Error	231	0.536		

* Plots were re-selected in 2004 to remove bias due to using plots that were selected only if they contained trillium in 2003.

Table 2.2. The 15 most important species within each habitat. Modified importance values were calculated by summing the relative frequency and the relative cover. The table also shows the growth habit of each species where: F= forb, L= liana, W= woody.

Honeysuckle Habitat			**Kudzu Habitat**			**No-Vine Habitat**		
Species	**Mean IV**	**Habit**	**Species**	**Mean IV**	**Habit**	**Species**	**Mean IV**	**Habit**
Lonicera japonica	0.315	L	*Pueraria montana*	0.822	L	*Hexastylis arifolia*	0.201	F
Ostrya virginiana	0.261	W	*Galium aparine*	0.317	F	*Ostrya virginiana*	0.196	W
Parthenocissus quinquefolia	0.187	L	*Stellaria graminea*	0.160	F	*Trillium reliquum*	0.170	F
Polystichum acrostichoides	0.137	F	*Trillium reliquum*	0.087	F	*Serenoa repens*	0.148	W
Ipomea sp.	0.104	L	*Polygonatum biflorum*	0.085	F	*Mitchella repens*	0.135	F
Unknown Liana 3	0.098	L	*Lonicera japonica*	0.068	L	*Cardamine angustata*	0.106	F
Trillium reliquum	0.095	F	Unknown Poaceae	0.067	F	Unknown Liana 3	0.090	L
Acer sp.	0.087	W	*Geranium carolinianum*	0.055	F	Unknown Poaceae	0.087	F
Mitchella repens	0.078	F	*Parthenocissus quinquefolia*	0.050	L	*Parthenocissus quinquefolia*	0.087	L
Toxicodendron radicans	0.068	L	*Cardamine angustata*	0.048	F	*Sanicula* sp.	0.078	F
Fraxinus americana	0.068	W	*Hexastylis arifolia*	0.046	F	Unknown C5	0.071	F
Hexastylis arifolia	0.061	F	*Magnolia macrophylla*	0.041	W	*Acer* sp.	0.068	W
Quercus sp.	0.050	W	*Aesculus pavia*	0.035	W	*Fraxinus americana*	0.067	W
Fraxinus pennsylvanica	0.049	W	*Zephyranthes atamasca*	0.033	F	*Fagus grandifolia*	0.063	W
Vitis sp.	0.049	L	*Toxicodendron radicans*	0.033	L	*Polystichum acrostichoides*	0.062	F

Table 2.3. Mean soil nutrient and pH levels (±SE) for each habitat.

Nutrient	**Honeysuckle**	**Kudzu**	**No Vine**	**Removal**
P (kg/ha)	7.84(1.21)	17.36(2.07)	21.28(6.55)	10.92(1.15)
K (kg/ha)	75.04(5.73)	114.80(9.05)	103.04(8.34)	118.72(19.98)
Ca (kg/ha)	1194.76(176.57)	2505.44(358.11)	2068.92(129.82)	2005.08(496.64)
Mg (kg/ha)	68.6(6.77)	173.6(12.94)	126.28(10.38)	185.08(38.54)
Zn (kg/ha)	3.64(0.28)	4.20(0.84)	4.76(0.70)	5.04(0.97)
Mn (kg/ha)	29.96(2.16)	19.04(2.55)	35(5.08)	24.08(3.67)
Nitrate (kg/ha)	5.32(0.54)	12.32(1.37)	9.52(3.25)	7.56(0.96)
Soil pH	5.6(0.23)	5.6(0.23)	5.52(0.19)	5.65(0.25)

Table 2.4. Principal components from multivariate correlation of soil nutrients and soil pH for honeysuckle, kudzu and no-vine habitats.

	PC 1	**PC 2**	**PC 3**	**PC 4**
Eigenvalue	4.093	1.750	0.931	0.764
Percent	51.166	21.879	11.636	9.552
Cum Percent	51.166	73.045	84.681	94.233
Nutrient	**Eigenvectors**			
P	0.249	0.629	-0.002	-0.003
K	0.471	-0.065	0.185	0.134
Ca	0.471	0.015	-0.209	0.067
Mg	0.455	-0.148	0.026	0.001
Zn	0.270	-0.256	0.715	0.236
Mn	-0.185	0.444	0.083	0.798
Nitrate	0.292	0.519	0.061	-0.404
Soil pH	0.309	-0.213	-0.632	0.349

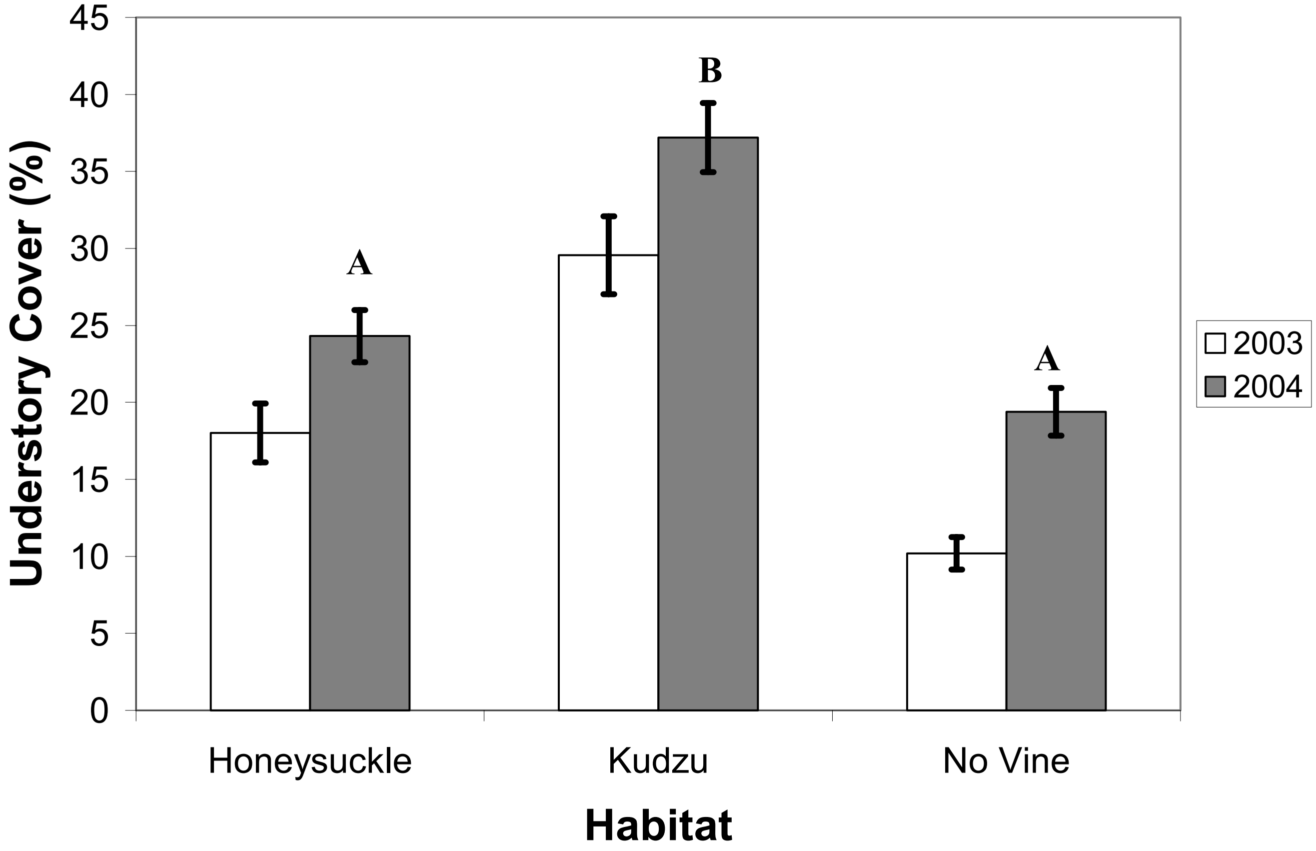


Figure 2.1. Mean understory cover (±SE) in honeysuckle, kudzu and no-vine habitats in 2003 and 2004. 2003 understory cover was determined using plots with *Trillium reliquum*. In 2004, understory cover was calculated from re-selected random plots. Different letters above bars denote significant differences within that year.

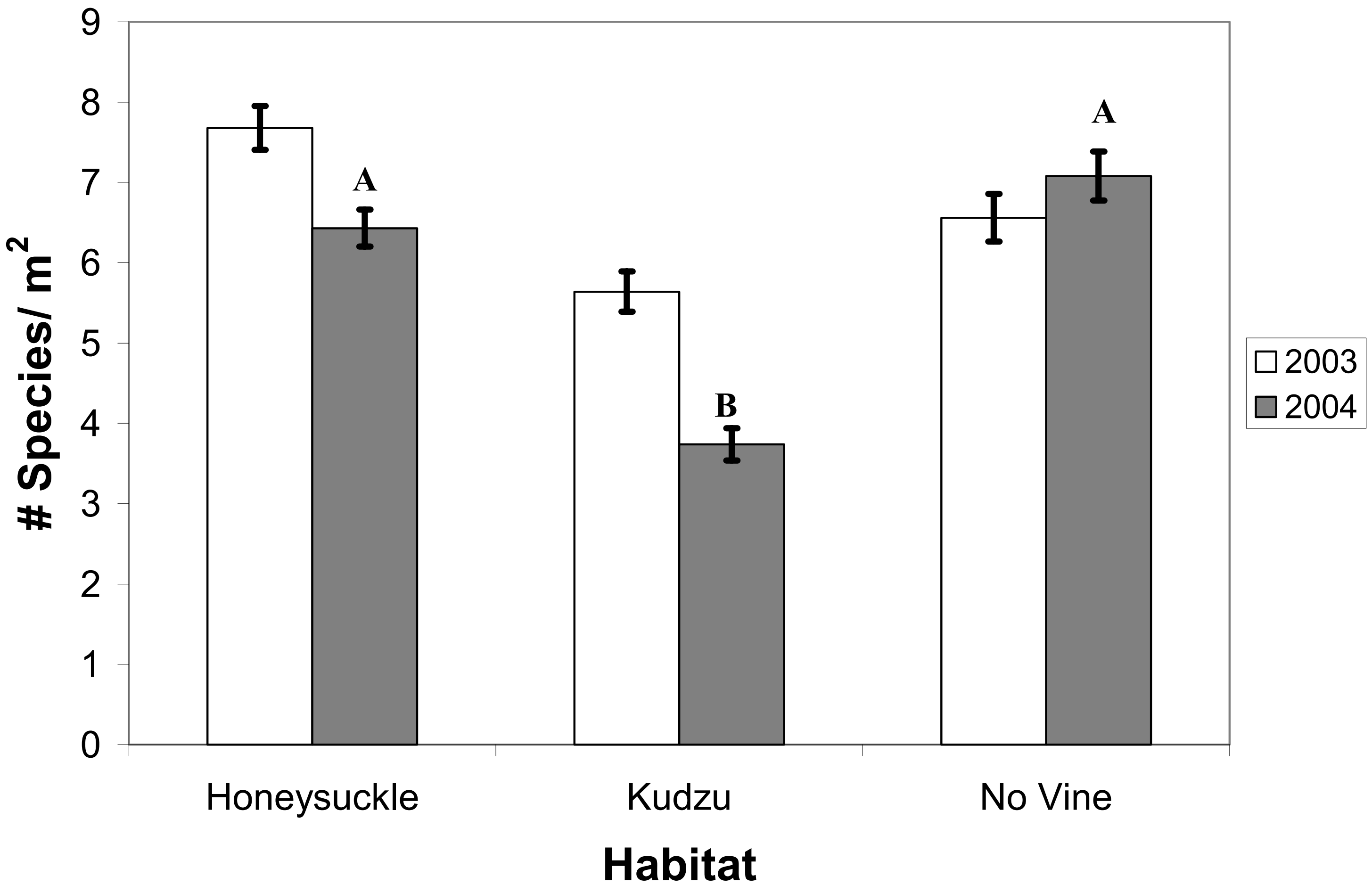


Figure 2.2. Mean species richness (±SE) in honeysuckle, kudzu and no-vine habitats in 2003 and 2004. Species richness in 2003 was determined using plots that were only selected if they contained *Trillium reliquum*. In 2004, species richness was calculated from re-selected random plots. Different letters above bars denote significant differences within that year.

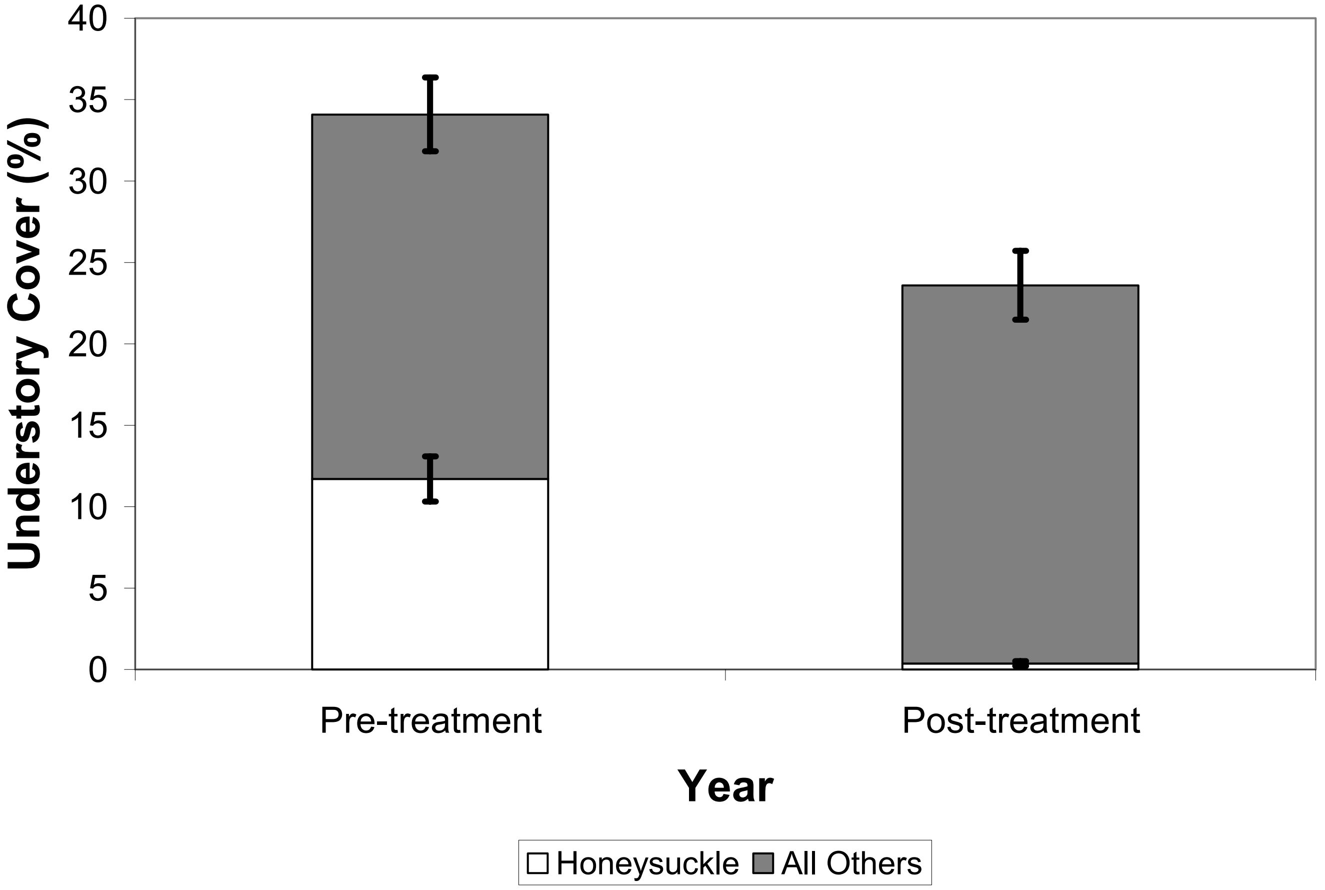


Figure 2.3. Mean understory cover (±SE) before (2003) and after (2004) herbicide treatment to remove honeysuckle.

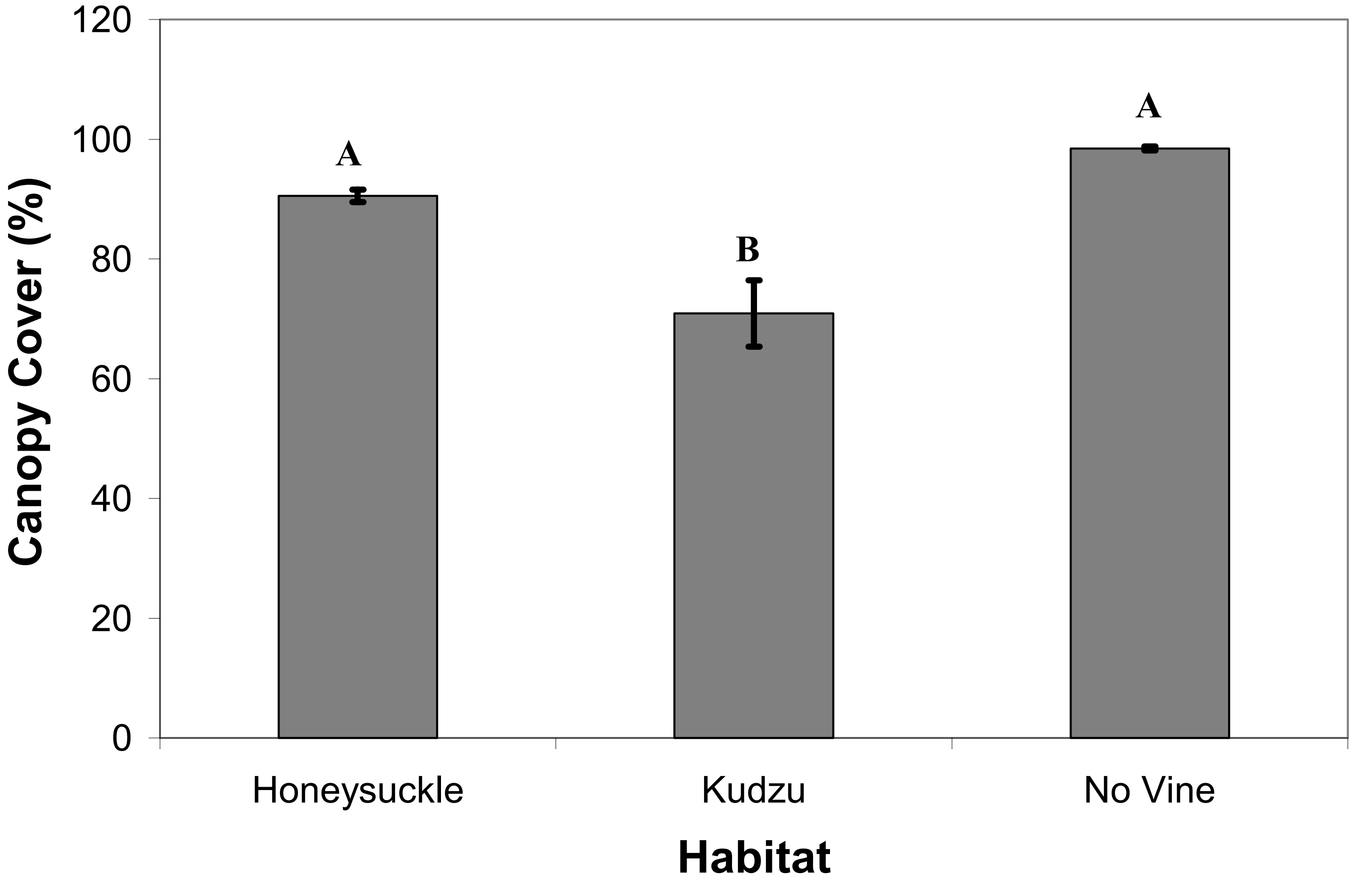


Figure 2.4. Mean canopy cover (±SE) in honeysuckle, kudzu and no-vine habitat in 2003. Letters above bars denote significance between habitats with different letters.

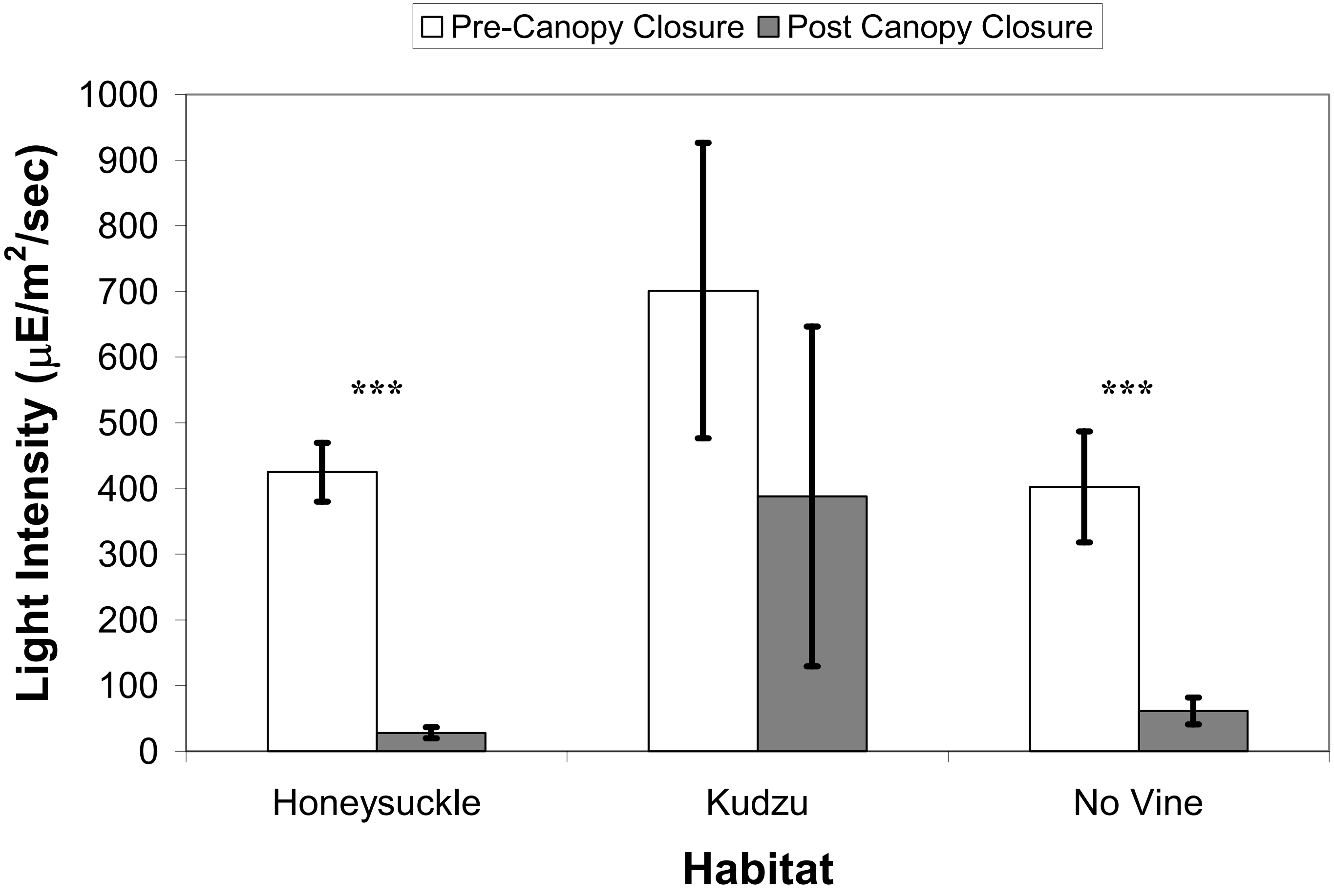


Figure 2.5. Mean light intensity (±SE), pre- and post canopy closing, of three different habitats in spring of 2004. Asterisks above bars denote a significant difference in pre- and post canopy closing light intensity within habitats.

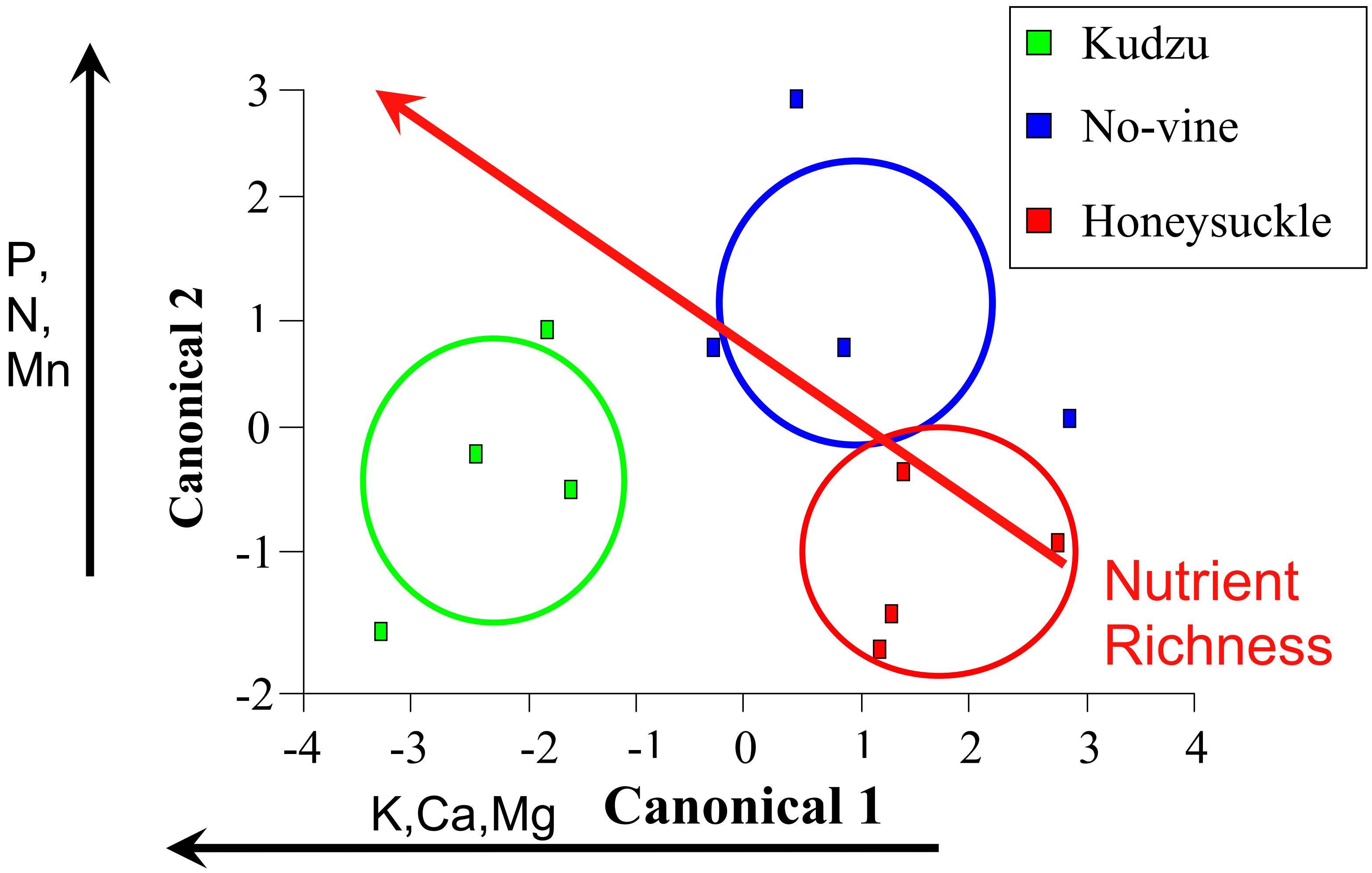


Figure 2.6. Discriminant function analysis of the first four principal components generated from multivariate correlation of soil nutrients of three habitats. Circles show the 95% confidence ellipse of the multivariate mean of each habitat. Moving right to left along canonical axis 1 represents increasing levels of K, Ca, and Mg. Moving up along canonical axis 2 represents an increase in P, Nitrate, and Mn.

CHAPTER III

Impacts of Invasive Vines on Population Dynamics in *Trillium reliquum*

Non-native invasions are widely recognized as one of the greatest threats to biodiversity in natural systems (Mooney and Drake 1986, Mooney and Cleland 2001, D'Antonio and Meyerson 2002). Populations of native species can be negatively impacted by invasive species via displacement (Bond and Slingsby 1984, Porter and Savignano 1990, Flecker and Townsend 1994), growth reduction (Miller and Gorchov 2004), competition for resources like light and soil nutrients (D'Antonio 1993, Equihua and Usher 1993, Scheu and Parkinson 1994, Bockelmann and Neuhaus 1999) and pollinators (Parker 1997, Kearns et al. 1998). These factors may have even greater effects on populations of rare species that may already be at risk due to anthropogenic changes to habitat (Schemske et al. 1994).

In their review, focused on evaluating the approaches toward the assessment and conservation of threatened plants, Schemske et al. (1994) suggest that studies utilizing demographic models may be the best approach to assessing rare plant status and determining where conservation efforts should be focused. Indeed, demographic models are essential tools in conservation and population management (Caswell 2001). One of the most versatile demographic tools is the projection matrix model. This model uses the vital statistics of an age- or stage-based population to determine the population growth rate λ and it can also be used to project future population sizes. Additionally elasticity and sensitivity analyses can be performed on matrices to determine the life stage where changes in vital rates would have the greatest impact on λ (Caswell 2001). Projection matrix models have been utilized by ecologists to project population growth (Werner and

Caswell 1977, Horvitz and Schemske 1995, Grosholz 1996, Cortes 1999), project extinction rates (reviewed in Menges 1992), determine the life stage with the greatest effect on population growth (Bierzychudek 1982, Caswell 1982, Crouse et al. 1987, Kalisz and McPeek 1992, Oostermeijer et al. 1996), or examine the effects of biotic interactions with pollinators (Calvo and Horvitz 1990) and herbivores (Knight 2003) on demography. My research uses population projection matrices to illustrate the impacts of two invasive species on an endangered forest herb.

Trillium reliquum is one of two endangered species of North American trilliums (Case and Case 1997). This species is native to only three states in the southeast and is threatened by habitat loss and the encroachment of the invasive vine species, kudzu (*Pueraria montana*) and Japanese honeysuckle (*Lonicera japonica*) (U.S. Fish and Wildlife Service 1990). Kudzu and Japanese honeysuckle (henceforth honeysuckle) are both aggressive, invasive vines, native to Asia that have escaped cultivation and become naturalized (Slezak 1976, Wechsler 1977, Carter and Teramura 1988). Both are considered major pests (Allison 2003). However, even though they are considered to be ecological threats (Winberry and Jones 1973, Carter and Teramura 1988, Pappert et al. 2000), few studies have actually quantified the ecological damage associated with their invasion (Slezak 1976, Cain 1984, Myster and Pickett 1992). Instead, most studies focus on the physiological characteristics that may allow their invasion (Wechsler 1977, Forseth and Teramura 1987, Carter and Teramura 1988, Sasek and Strain 1989, Schweitzer and Larson 1999), how to control them (Zidac and Backman 1996, Kidd and Orr 2001, Boyette et al. 2002) or how they may benefit wildlife (Ladine and Robert E. Kissell 1994).

Trillium reliquum rarely undergoes clonal growth, therefore its reproductive success depends on seed production. The biotic interactions and abiotic factors that affect seed production, including interactions with pollinators, seed dispersers, herbivores, and seed predators as well as the availability of soil nutrients, water, and light, can play important roles in determining the success of a population through growth or its decline toward extinction. The biological invasion of a natural system may affect seed production by altering biotic interactions and the local environment. An invasive plant may directly compete with native plants for soil nutrients, water, or light (Gordon 1998). Impacts of invaders on other aspects of a native plant's life history, such as reductions in growth, leading to low numbers of mature individuals, may indirectly limit reproductive success due to density-dependent reductions in pollinators (Knight 2003, Knight 2004).

The goal of my research is to determine the impacts of kudzu and honeysuckle on the population dynamics of *Trillium reliquum*. My research asks four questions about *T. reliquum* populations in habitats with varying degrees of invasive vine encroachment. 1) How does the presence of invasive vines in a habitat impact *T. reliquum* population density and λ? 2) For which life stage would changes to its vital rate have the greatest effect on λ? 3) Are there differences in seed production for *T. reliquum* individuals growing in habitats with invasive vines? 4) How does the removal of honeysuckle from a habitat affect *T. reliquum* population dynamics?

METHODS

Trillium reliquum Demography

To examine the population dynamics of *Trillium reliquum* in the presence and absence of invasive vines, I censused *T. reliquum* populations in three pre-existing habitats: 1) no-vine: no invasive vines present, 2) kudzu: *T. reliquum* and kudzu present, and 3) honeysuckle: *T. reliquum* and honeysuckle present (see Chapter 1 methods). To determine if differences in demography among habitats are directly related to the presence of honeysuckle I also censused a *T. reliquum* population prior to and after honeysuckle was removed from the local plant community (for details of removal see Chapter 1).

I used data from the 2003 and 2004 censuses to compare *T. reliquum* stand density (# individuals/m^2), population stage structure, population growth (λ), and projections of population size over time, based on 2003-2004 conditions. To calculate *Trillium reliquum* density, I divided the total number of *T. reliquum* individuals in a site by the total number of 1x1m plots sampled (26 to 115 plots sampled per site). The variances within habitats were not equal so I used non-parametric Kruskal-Wallis to test for an overall habitat effect on *T. reliquum* density and used a Mann-Whitney U-test to make pairwise comparisons of density by habitat. I pooled the data from sites within habitats because the site[habitat] effect accounted for only 3% of the total variation in a nested ANOVA when using habitat as a fixed effect and site[habitat] as a random effect. To compare *T. reliquum* density in a habitat between years, I used non-parametric Wilcoxon sign rank-sum test because the data did not transform to fit a normal distribution. I tested differences in population stage structures between habitats with goodness of fit tests (model I contingency table).

I used two methods to calculate λ in order to account for shoots occurring in demography plots that may have been in a dormant state in 2003 and could not be incorporated into projection matrix models. For comparisons of population growth rate among habitats, I calculated λ with the formula N_t / N_{t+1} where N_t is population size at time t and N_{t+1} is population size one time interval into the future. To calculate λ for the purpose of projecting future population sizes and for sensitivity and elasticity analyses I used stage-based population projection matrices. I used JMP IN 5.1 (SAS, Cary, NC, 2003) for all statistical tests and PopTools version 2.5.9 (2003) to analyze matrix models.

2003 Reproduction

To determine seed set for *Trillium reliquum* in 2003*,* I collected the fruits from all available reproductive plants (n=21) outside of the demography sites on June 26, 2003 (Table 3.1). Due to higher than anticipated early senescence of reproductive individuals prior to fruit collection, trillium fruits were difficult to find, resulting in low sample sizes. I counted the number of seeds, and recorded the presence or absence of elaiosomes. On some seeds, elaiosomes were missing, potentially due to the variety of insects in, on, or near the trillium fruits. I collected any insects I could capture by hand and stored them for future identification. The seeds dried at room temperature for six months.

I weighed all seeds from each fruit individually using an analytical balance (Denver Instrument Company TL-104, Denver CO). I also obtained a mean eliaosome mass per seed by measuring the mass of five arbitrarily chosen seeds from a fruit, removing the elaiosomes with a scalpel, and then re-massing the five seeds using the difference of the two masses as eliaosome mass. I recorded three replicates of five seeds for each fruit unless there were too few seeds for three replicates. To determine habitat effects on seed production I used one-way ANOVA to compare seed data collected in 2003. I used the $\ln\sqrt{x}$ transformed seed count data to meet normality and homoscedasticity assumptions. Seed mass data had equal variances but could not be transformed for normal distributions and eliaosome mass data was normal but could not be transformed to obtain equal variances; therefore, I used a non-parametric Kruskal-Wallis test on these data sets.

2004 Reproduction

To determine if *Trillium reliquum* reproduction in the study habitats was pollen or resource limited, I used supplemental pollen treatments in 2004 to compare fruit and seed set between open-pollinated flowers and flowers that received a supplemental dose of pollen applied by hand. Between the February 29 and March 6, 2004 I marked and numbered all reproductive plants I could find with the habitats: at least 97 reproductive individuals in each of the natural, kudzu, and honeysuckle habitats ($n = 321$) outside of the demography sites. I randomly assigned each individual to either a supplemental pollination or open pollination treatment.

I administered pollination treatments between March 16 and March 20, 2004 after the first signs of pollen dehiscal. To provide supplemental pollen, I collected anthers from flowering individuals not selected for this study and deposited their pollen on the stigmas of selected plants by rubbing the anther over the stigma until the entire receptive surface was saturated with pollen. *Trillium reliquum* reproductive parts are relatively large, so pollen saturation was easily detectable with the naked eye. I did not manipulate open pollination treatment plants, and all plants in the study remained accessible to pollinators for additional pollen transfer. After anthesis, flowers remain open for a period of 2-3 weeks. To determine any effects of plant size on reproduction, I also measured the leaf length and width of the largest leaf of all study plants that had not been eaten. I estimated total leaf area for each plant by calculating the area of an ellipse (length x width x π) and multiplying that value by three (for the three leaves of the plant).

I monitored fruit development monthly until fruits were mature. Each month prior to collection, I recorded the number of plants that had died back as a result of predation or unknown causes before fruits could mature. In June, I collected fruits and measured fruit diameter, counted seeds per fruit, and weighed the seeds with an analytical balance (Denver Instrument Company TL-104, Denver CO). I measured fruit diameter as the widest point between two carpel ridges (Appendix 3c) with digital calipers (Fisher Scientific, Pittsburgh, PA). To obtain mean seed mass per fruit, I massed seeds in ≤ 3 groups of 10. After measurements, I returned seeds to the forest.

I compared the probability of setting fruit among treatments with Chi-square tests. Plants that are more pollen-limited would be expected to have a higher fruit set and a greater number of seeds per fruit with the addition of supplemental pollen. If plants are more resource-limited, no difference in fruit set or seed number per fruit is expected between supplemental and open pollinated plants. To determine if plant size affected the probability of fruiting, I regressed the probability of fruit set against leaf area for all plants in a logistic regression. I regressed leaf area against the log of the number of seeds per fruit in a linear regression to determine the effect of plant size on seed number. I tested the effect of pollination treatment on the log-transformed number of seeds per fruit using ANCOVA with habitats as blocks, treatment as a fixed effect and leaf area as the covariate. I tested the association between fruit size and seed set using Pearson's correlation coefficient.

To determine if habitat type was affected the probability of setting fruit, I used a two-way model II contingency table (G-test). I used a one-way model I ANOVA to test if habitat type affected seed counts and fruit diameter. Seed mass data did not meet the assumptions for ANOVA and could not be transformed so I compared seed mass among habitats using a non-parametric Kruskal-Wallis test. Some fruits had already begun to drop seed before collection occurred. I omitted data from any fruits that dehisced before collection from any comparisons of seed counts, since I could not be sure that every seed had been collected.

Construction of stage-projection matrices

Due to the destructive processes required to determine age of *Trillium reliquum,* and because age cannot be determined without the presence of a taproot on the rhizome (Patrick 1973), I used stage-based projection models to calculate λ and project future population size. I used census data from 2003 and 2004 to determine transition probabilities based on a life cycle diagram (Figure 3.1) that summarized all possible transitions within and between stages for trilliums in sample plots. In the transition matrix (Table 3.1), **A**, each element, a_{ij}, represents a vital statistic for a particular stage class within the population. I calculated transition probabilities (P) by dividing the number of each transition type (forward, no-transition, or back-transition) in 2004 by the total number of individuals that were in that stage class in 2003 (Table 3.1). To calculate fecundity, I divided the number of seedlings found in a habitat in 2004 by the number of reproductives in that habitat in 2003. I pooled transition probabilities from the four sites in each habitat to construct transition matrices with 8-9 non-zero elements and a projection interval of one year (Appendix 7). To calculate λ and project population sizes I used the model:

$$\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t)$$

where $\mathbf{n}(t)$ is the population size at time t and $\mathbf{n}(t+1)$ is the population size one projection interval later. I calculated several population parameters using the model. The dominant eigenvalue, λ, determines the population growth rate , the right eigenvector, **w**, gives the stable age distribution, and the left eigenvector, **v**, represents the relative contribution of offspring an individual in a stage class will make to the population before death: its reproductive value (Caswell 2001). When $\lambda > 1$, population size is increasing, $\lambda < 1$ shows population in decline, and when $\lambda \approx 1$, the population is stable. I used the model to generate the sensitivities and elasticities of the matrices. Sensitivity predicts the effect of changes in any element in the matrix, **A**, on λ and is defined by the formula:

$$s_{ij} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_i}{\langle \mathbf{w}, \mathbf{v} \rangle}.$$

The elasticity of λ is defined by:

$$e_{ij} = \frac{\partial \log \lambda}{\partial \log a_{ij}}$$

and is the proportional response to proportional changes in transition probabilities or fecundities, or proportional sensitivity (Caswell 2001). Since survival and transition probabilities can be no greater than one, but fecundity can be, and is often, much greater than one, sensitivities represent an absolute effect on λ. Elasticity is a weighted sensitivity, with the sensitivity of λ to changes to all elements summing to one, making sensitivity of λ to one element proportional to all other elements. Sensitivities and elasticities have been used to determine the transitions having the highest impact on λ (Crouse et al. 1987, Kalisz and McPeek 1992, Horvitz and Schemske 1995, Knight 2004). I compared the sensitivities and elasticities of matrices among the four different habitats to determine the transitions to which λ was most sensitive.

RESULTS

Population size and structure

Overall, invasive vine habitats were associated with small populations of *Trillium reliquum*. Trillium density in the no-vine habitat was at least twice that of the other habitats in 2003 (Figure 3.2, $p<0.0001$ df=2 H=31.03) and 2004 (Figure 3.2, $p<0.0001$ df=2 H=31.36). Population density in the no-vine and honeysuckle habitats were not significantly different between the two sampling years, however *T. reliquum* density in the kudzu habitat decreased by nearly one-fifth (Figure 3.2, $p<0.001$ df=225). *Trillium reliquum* population stage structure differed between no-vine and invasive vine habitats in both years (Table 3.2, Figure 3.3, G-test $p<0.001$ df=9), except for 2004, when there was no difference in stage structure between no-vine and honeysuckle habitats (Table 3.2).

2003 Reproduction

The mean number of seeds (±SE) per fruit between habitats did not differ (Figure 3.4a). Mean seed mass (±SE) in invasive vine habitats was 7-13% higher than in no-vine habitat (Figure 3.4b; Honeysuckle: 0.0112g ±0.0002, n=159; Kudzu: 0.0120g ±0.0005, n=98; No-vine: 0.0105g ±0.0001, n=297; Kruskal-Wallis $P<0.001$ df=2). No significant difference in the mean eliaosome mass (±SE) per fruit was found. In over 250 man-hours of field work in trillium populations I observed just two suspected pollinators (beetles).

2004 Reproduction

In 2004, fruit and seed set in MBNA was low in all habitats, and pollen availability did not limit reproduction. On average, only 37% of flowering plants produced fruit and fruit set did not differ between the supplemental and open pollination treatments (χ^2=1.826, df=1, P=0.18). The mean number of seeds per fruit for both pollination treatments was 27.7 ± 1.46 (range of 7-70). There was no difference in mean number of seeds per fruit (Grand mean = 26.50 ± 1.27 seeds), fruit size (Grand mean = 13.02 ± 0.23mm), or mean seed mass per 10 seeds (Grand mean = 0.295 ± 0.005g) between pollination treatments (Table 3.3).

Parent plant size did not affect fruiting success (Logistic regression: χ^2= 2.31, df=1, $P>0.05$), but seed production did increase with plant size (Figure 3.5). The number of seeds was positively correlated with fruit diameter (r=0.74, $P<0.0001$). Leaf area of reproductive plants in kudzu habitat (518 ± 17.7 cm^2) was 14% greater than the leaf area of plants in natural habitat (455 ± 18.0 cm^2; F=3.66, df=2, 217, P=0.027), but leaf area of reproductives in the honeysuckle habitat (480 ± 11.7 cm^2) did not differ from that of the other habitats.

Fruiting success was independent of habitat type (Figure 3.6a, G=2.264, df=2, P=0.322). There was no difference in the number of seeds per fruit among habitats (Table 3.3, Figure 3.6b), however diameter of fruits in the no-vine habitat was 15% larger than the honeysuckle fruit diameter (Table 3.3, Figure 3.6c). Fruits produced in kudzu habitat did not differ in diameter from either the no-vine or honeysuckle habitat. Seeds produced in honeysuckle habitat had 14% greater mass than seeds in the kudzu habitat (Table 3.3, Figure 3.6d); mass of seeds from no-vine habitat did not differ from seed mass in other habitats.

Removal experiment

In the removal habitat, *Trillium reliquum* population density increased by 50% from 2003-2004 (Figure 3.7a, Wilcoxon Sign-Rank $p<0.001$). In 2004, there was a five-fold increase in recruitment (increased from 1 to 46 seedlings) and a 50% increase in the density of non-reproductives due to the appearance of 69 individuals that were in a below-ground dormant stage during the previous year's census (Figure 3.7b). Comparison of the population stage structure in 2003 and 2004 between the honeysuckle (Figure 3.3) and removal (Figure 3.7b) habitats showed that the stage structure differed in both years (Table 3.2, G-test, $p<0.0001$, df=3), with the greatest differences found in the proportions of reproductive and juvenile stages. In 2004, population stage structure differed between no-vine (Figure 3.3) and removal (Figure 3.7b) habitats (Table 3.2, $G=42.64$, $p<0.0001$, df=3), the greatest difference being in proportion of reproductives.

Projection matrix models

Projection matrix models showed that the no-vine and honeysuckle habitat populations were in stable condition and the kudzu and removal habitat populations would decline to near extinction in less than 25 years. Using the population growth rate calculated by dividing the 2004 population size by the 2003 population size, the removal population increased by 52% between 2003 and 2004, with $\lambda = 1.52$ (Table 3.4), whereas the no-vine and honeysuckle populations were nearly stable and the kudzu population decreased. Calculating the growth rate in this manner did allow the incorporation of data from shoots that spent 2003 in a dormant stage and then emerged in 2004; these formerly dormant shoots could not be incorporated in the projection matrix models. Projections of λ from the model (excluding dormant shoots) showed population growth in the no-vine habitat, stability in the honeysuckle population, and population declines in the kudzu and removal habitats (Table 3.4). The population projections indicate that after 25 years the kudzu habitat would be devoid of *Trillium reliquum*, having only ≈2 individuals remaining (Figure 3.8). Although $\lambda > 1$, population growth is slow in the honeysuckle habitat. After 25 years the population size in honeysuckle habitat is predicted to be only slightly more than twice its initial size. The no-vine site has such a high growth rate the population size quadruples in the first 10 years (Figure 3.8). The exponential growth of the no-vine population is not likely to occur because density-dependent effects like competition will eventually slow the rates of population growth. The values of $\lambda \geq 1$ suggest population stability rather than growth as populations may already be at or near their carrying capacity, making large increases in population size unlikely.

The stable stage distributions predicted with the matrix differed among all habitats (Figure 3.9). The four stages were nearly evenly represented in the no-vine population, the population that exhibited the strongest growth in the model. In the honeysuckle habitat, the proportion of non-reproductive individuals was three times greater than the next best represented stage, juvenile. In similar fashion, the stable distribution in the kudzu habitat was heavily adult dominated; over 95% of the stable stage distribution would be non-reproductive or reproductive three-leaved plants. In the removal habitat the juvenile stage was poorly represented, all other stages were 12-21 times higher in their representation in the stable stage distribution.

The reproductive value in three of four habitats showed that as *T. reliquum* individuals transition to more advanced stages their contribution to long term population growth increases (Figure 3.10). This was not the case in the removal habitat where the reproductive value of juveniles was comparable to the reproductive value of reproductives.

Lambda was most sensitive to changes in the transition probabilities of non-reproductives (Table 3.5). In fact, the single matrix element with the greatest impact on λ was the non-reproductive to reproductive transition, which had the highest sensitivity in all habitats (Table 3.5). Non-reproductive and reproductive stages had the greatest impact on λ based on the elasticities; however, the element with the greatest impact was no longer the non-reproductive to reproductive transition (Table 3.5). In the honeysuckle, kudzu and removal habitats, elasticity was highest for stasis of non-reproductives, whereas in the no-vine habitat the reproductive to reproductive transition had the highest elasticity and the non-reproductive to non-reproductive transition was second highest.

Since the sensitivities and elasticities of all models were in agreement, a closer look at the dynamics of the non-reproductive and reproductive stages was warranted. The proportion of non-reproductive plants in removal and honeysuckle habitat that retrogressed into the juvenile stage was 1.3 to 3 times higher than the proportion of back-transitions for non-reproductives in the no-vine habitat (Figure 3.11a). Non-reproductives in the kudzu habitat had the lowest proportion of retrogressions. In the no-vine habitat, none of the reproductive plants from 2003 back-transitioned but the proportion of back-transitions in the honeysuckle, kudzu and removal habitats ranged from 21-67% (Figure 3.11b). In invasive vine habitats non-reproductive plants had lower probabilities of forward or stasis transitions compared to no-vine habitat (G= 20.6, df=6, P<0.01). Reproductive stage plants in invasive vine habitats more 20-60% more likely to back transition than reproductives in no-vine habitat (Figure 3.11b, although there was no difference in the overall probability of survival (G= 5.62, df=3, P=0.13).

DISCUSSION

Population density, stage structure, and seed production

Invasive vine habitats are associated with reduced *Trillium reliquum* population sizes and smaller proportions of seedlings. Reduced population sizes may be the result of changes to population vital rates and may further impact population dynamics by altering density-dependent processes. Trillium population sizes may become small enough that the frequency of important interactions between trilliums and mutualists (pollinators and dispersers) is reduced because the population is not large enough to attract mutualists. This effect was seen in another perennial herb, *Nepeta cataria* (catnip); the number of out-crossed seeds produced was found to be highly dependent on the number of flowers in a patch (Sih and Baltus 1987). Small population sizes may also be more likely to be pushed to extinction by the catastrophic events such as stochastic weather events, disease outbreaks, or herbivore population increases.

Seed production in the MBNA populations of *Trillium reliquum* was not pollen limited in 2004, and was therefore likely to be limited by some other resource like light, water, or soil nutrients. Mean seed set in this species was 27.7 seeds per plant yet some flowers receiving supplemental pollen produced up to 70 seeds. Thus, it would seem that *T. reliquum* seed production in this population is far below its potential indicating microsite resource levels surrounding all plants may not be sufficient for high seed production. Research examining the seed:ovule ratio would provide a more comprehensive picture of *T. reliquum* seed output potential.

Fruit diameter and seed mass differed among habitats and seed count nearly differed among habitats (P=0.061, Table 3.3). These differences among habitats may further support the hypothesis that reproduction in *Trillium reliquum* is resource limited, and may suggest a mechanism responsible. Seed set and fruit size were lower while seed mass was greater in the honeysuckle habitat compared to the other habitats. This suggests two possibilities: 1) the habitats differ inherently in the availability of resources within the habitats, or 2) that competition for resources is more intense in the invasive vine habitats. Results from my research on community characteristics and abiotic differences between invaded and non-invaded habitats (Chapter 2) documented that soil fertility in honeysuckle habitat was lower than other habitats. Soil in the honeysuckle habitat may not support the same level of seed production as that of the other habitats. The larger seed size in the honeysuckle habitat may be the result of resource allocation issues in the plant. Kudzu and Japanese honeysuckle are both widely known as aggressive competitors that can suppress the growth of native plants (Wechsler 1977, Cain 1984, Allison 2003). Further research with more detailed analyses of available resources and the removal of invaders from trillium populations may help to elucidate the effects of invaders on trillium reproduction.

In this study, all habitats had low proportions of seedling stage plants in comparison with other life stages. The ratio of seedlings to reproductives (C:R) in no-vine habitat is higher than the kudzu or honeysuckle habitats in both years, when compared among habitats. This suggests that recruitment of *T. reliquum* is lower in invasive vine habitats. Low recruitment together with resource limited and low seed output may make it difficult for trillium populations in invasive vine habitats to sustain themselves through the generation of new individuals. Kudzu and honeysuckle have been shown to effectively decrease recruitment in other species (Leatherman 1955, Winberry and Jones 1973).

Removal experiment

The changes in *Trillium reliquum* population size and stage structure in the removal area suggest that it may not be abiotic differences between habitats affecting trillium population dynamics, but rather, honeysuckle is altering trillium population dynamics. The surprising 50% increase in the removal population size seen in 2004 was due to recruitment increasing from one to 46 seedlings and the appearance of 69 individuals that were in a below-ground dormant stage during the previous year's census. This suggests honeysuckle may somehow inhibit the recruitment of new trillium individuals and suppress the emergence of non-reproductive stage individuals. This suppression may drive trillium into an underground dormant state, remaining as an underground rhizome but producing no above-ground shoots. Below-ground summer dormancy has been seen in other trillium species (Hanzawa and Kalisz 1993, Knight 2004) but this is the first documented case of this for *Trillium reliquum*.

Projection matrix models

The matrix models further delineate how *Trillium reliquum* population dynamics in invasive vine habitats at MBNA were degraded. The models suggested that only the no-vine habitat has the potential to increase in population size over the next 25 years, assuming the population is not already at carrying capacity and environmental conditions remain consistent. The models also suggest that the invaders impact trillium population dynamics with different force. Projections suggest that if conditions remained stable trillium population size over a 25 year period in kudzu habitat would drastically decline, yet the trillium populations in honeysuckle habitat are more likely to remain stable in the same 25 year span. It is important that the models used are density-independent growth models. It is not likely that the no-vine habitat would experience 25 years of exponential growth as predicted by the model. Density-dependent processes like intra-specific population would begin to limit population size at some point.

The presence of honeysuckle in a habitat may be associated with a reduction in trillium carrying capacity. Further evidence for this hypothesis is seen when the results of the removal experiment are examined. When honeysuckle was removed, trillium population size increased by 50%. Therefore it would seem that removing honeysuckle from habitat may raise the carrying capacity of the trillium population. More research is needed to determine what mechanisms are acting in these interactions.

The stable stage distributions of the honeysuckle, kudzu and removal habitats all have lower proportions of seedling and juvenile stage plants than the no-vine habitat. Kudzu habitat has < 5% of all trillium individuals in the seedling and juvenile stages. This lends support to the hypothesis that recruitment is low in the invasive vine habitats.

Reproductive stage plants had the highest reproductive value in all habitats. As plants progress to older, larger stages the mortality rates decrease and they will be more likely to contribute a greater number of offspring into the population in the future; a pattern typical of most iteroparous perennials like trillium (Barbour et al. 1987).

The presence of invasive vines in a habitat results in the stagnation of trillium populations. Both sensitivity and elasticity analyses showed that λ was most sensitive to changes in the dynamics of non-reproductive and reproductive plants. Invaders affecting these life stages would create the greatest disturbances to population dynamics. I found that invasive vine habitats were associated with differences in transition and stasis probabilities of non-reproductive stage plants. All habitats with invasive vines had 25% or greater rates of regressing to previous stages whereas the no-vine habitat had zero plants regress. These are important points that demonstrate that invasive vines are essentially halting the forward progression of trillium populations. The emergence of non-seedling stage individuals from a dormant state and a larger proportion of forward transitions in the removal area compared to other invasive vine habitats further support this hypothesis.

Invasive vines do negatively affect *Trillium reliquum* population dynamics in MBNA, resulting in low population sizes and differing stage structure based on 2003-2004 environmental conditions. Populations are likely to continue to be adversely affected unless invaders are controlled. The results of the removal experiment have shown that trillium populations can recover from invader impacts if invaders are removed. The removal study has also shown that results of population models must be carefully interpreted in conjunction with results of empirical studies in order to best evaluate population dynamics. Calculating the population growth rate of the removal population resulted in two conflicting results, population growth using censuses and population decline with the model. This inconsistency is easily explained. The model uses transition probabilities from one year to the next to calculate λ. An assumption of this model is that environmental conditions remain constant. This assumption is met in the honeysuckle, kudzu, and no-vine habitats but is grossly violated in the removal experiment. After the first census in the removal habitat conditions were changed by removing honeysuckle. Hence, the model attempts to model two different environmental conditions for the removal habitat; something it cannot do. I was also unable to incorporate the non-reproductive individuals that emerged from dormancy into the model. These individuals could have potential been added into the model by adding a dormancy state into the matrix; however, it is nearly impossible to know the number of individuals in a dormant state in a given season and whether the non-emergence of a plant in the next season meant that individual was dead or dormant. Incorporating a dormant state would have put too many “black boxes” into the model and for this reason it was left out. The bright side for managers is that if dormancy does play a major role in population dynamics of *T. reliquum,* then the estimates of these models can be seen as conservative estimates.

I suggest that invasive vines have the greatest impact on two facets of trillium population dynamics: recruitment and adult stage transitioning. There are a variety of biotic interactions, including those with pollinators, seed disperser, and seed predators, that are not clearly understood in *T. reliquum*. This trillium species could benefit from studies aimed at determining how kudzu and honeysuckle interact with their surroundings and what aspect of *T. reliquum* biology is affected by these interactions. In conclusion, my results have provided a focal point which future research and management strategies should target: non-reproductive and reproductive transition dynamics. Research goals might include examining the relative growth rates of adult stage plants grown in the presence or absence of invaders. Increased knowledge of trillium physiology, in particular, what cues transition to different stages, may help to determine the mechanism behind invasive vine-associated changes in population dynamics. Managers should monitor populations and pay particular attention to transition rates of adult stage trillium, to assure that populations remain stable or progress and are not regressing.

Table 3.1. Transition matrix showing possible transitions for *Trillium reliquum*. In column and row headings C=seedling, J= juvenile, S= non-reproductive, R= reproductive. In the matrix P_{ij} represents the probability of an individual in stage *i* transitioning to stage *j* the next year. S represents the probability of an individual remaining in its present stage the next year. F represents the fecundity; in this paper F was calculated as the number of seedlings contributed to year *t+1* by reproductive plants in year *t*. Transitions that could not occur are shown as dashes.

Life Stage in year 2	**Life Stage in Year 1**			
	C	**J**	**S**	**R**
C	-	-	-	F
J	P_{cj}	P_{jj}	P_{sj}	-
S	-	P_{js}	P_{ss}	P_{rs}
R	-	-	P_{sr}	P_{rr}

Table 3.2. Pairwise comparisons of stage structures in different habitats by year. No-vine was not compared to removal in 2003 because removal habitat would not have been expected to resemble no-vine habitat.

	2003		**2004**		
Stage Distribution Comparison	**G**	**P**	**G**	**P**	**df**
No-Vine v. Kudzu	55.98	<0.0001	43.04	<0.0001	3
No-Vine v. Honeysuckle	18.28	<0.001	2.67	0.44	3
Removal v. Honeysuckle	48.15	<0.0001	40.06	<0.0001	3
No-Vine v. Removal	-	-	42.64	<0.0001	3

Table 3.3. Blocked ANCOVA results for seed number, fruit diameter, and mean seed mass.

Source	***df***	***SS***	***F***	***P***
Log # Seeds/Fruits ($r^2 = 0.17$, P=0.0314)				
Habitat	2	1.051	2.898	0.061
Treatment	1	0.202	1.113	0.295
Treatment x Habitat	2	0.104	0.285	0.753
Leaf Size	1	1.416	7.805	0.007
Error	74	13.424		
Fruit Diameter ($r^2= 0.28$, P<0.001)				
Habitat	2	52.620	8.219	0.001
Treatment	1	1.284	0.401	0.529
Treatment x Habitat	2	0.767	0.120	0.887
Leaf Size	1	43.735	13.662	<0.001
Error	72	230.480		
Seed Mass/10 seeds ($r^2= 0.24$, P=0.0021)				
Habitat	2	0.031	8.599	<0.001
Treatment	1	0.004	2.024	0.159
Treatment x Habitat	2	0.010	2.632	0.079
Leaf Size	1	0.004	2.376	0.128
Error	74	0.134		

Table 3.4. Population growth rates for study habitats. In the first column λ is the dominant eigenvector of a population transition matrix. In the second column, λ was calculated with empirical data from 2003 and 2004 using the formula: N_t/N_{t+1}.

	Population Growth Rate	
Habitat	**λ (model)**	**N_t/N_{t+1}**
No-vine	1.172	0.996
Kudzu	0.836	0.824
Honeysuckle	1.024	1.04
Removal	0.797	1.52

Table 3.5. Sensitivities and elasticities of all transitions in all habitats. c= seedling, j = juvenile, s= non-reproductive, r= reproductive

	Transition								
	c-j	**j-j**	**j-s**	**s-j**	**s-s**	**s-r**	**r-s**	**r-r**	**r-c**
Habitat	**Sensitivity**								
Honeysuckle	0.0739	0.2059	0.2377	0.5014	0.5788	2.2094	0.0375	0.1431	0.0317
Kudzu	0.0132	0.0130	0.0175	0.3977	0.5332	0.6283	0.3840	0.4524	0.0285
No-Vine	0.1828	0.1271	0.1693	0.2107	0.2807	1.0159	0.0000	0.5164	0.0444
Removal	0.0227	0.0018	0.0329	0.0316	0.5833	0.7236	0.3345	0.4149	0.0000
Habitat	**Elasticity**								
Honeysuckle	0.0722	0.0899	0.1160	0.0438	0.4384	0.0966	0.0244	0.0466	0.0722
Kudzu	0.0013	0.0061	0.0070	0.0057	0.4291	0.0984	0.0972	0.3539	0.0013
No-Vine	0.0758	0.0452	0.0819	0.0061	0.1989	0.0758	0.0000	0.4406	0.0758
Removal	0.0001	0.0001	0.0017	0.0017	0.4722	0.1095	0.1095	0.3055	0.0001

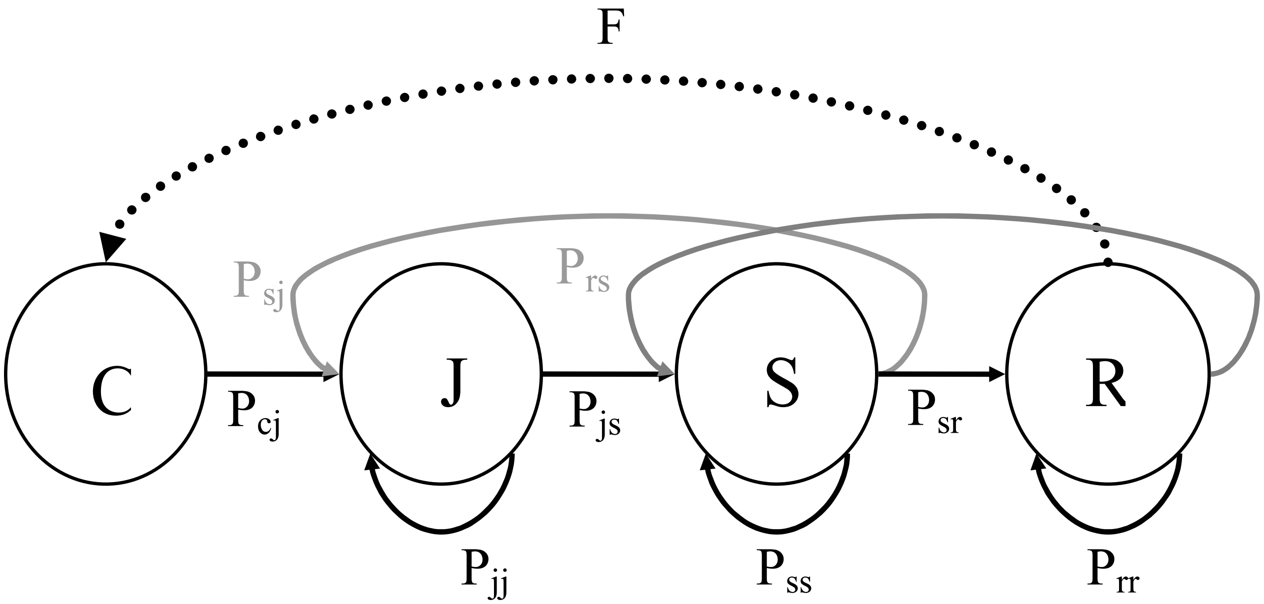


Figure 3.1. Stage-based life cycle graph for *Trillium reliquum*. C = seedling, J = juvenile, S = non-reproductive, and R = reproductive. P_{ij} represents the probability of an individual in stage *i* transitioning to stage *j* the next year. F represents the fecundity

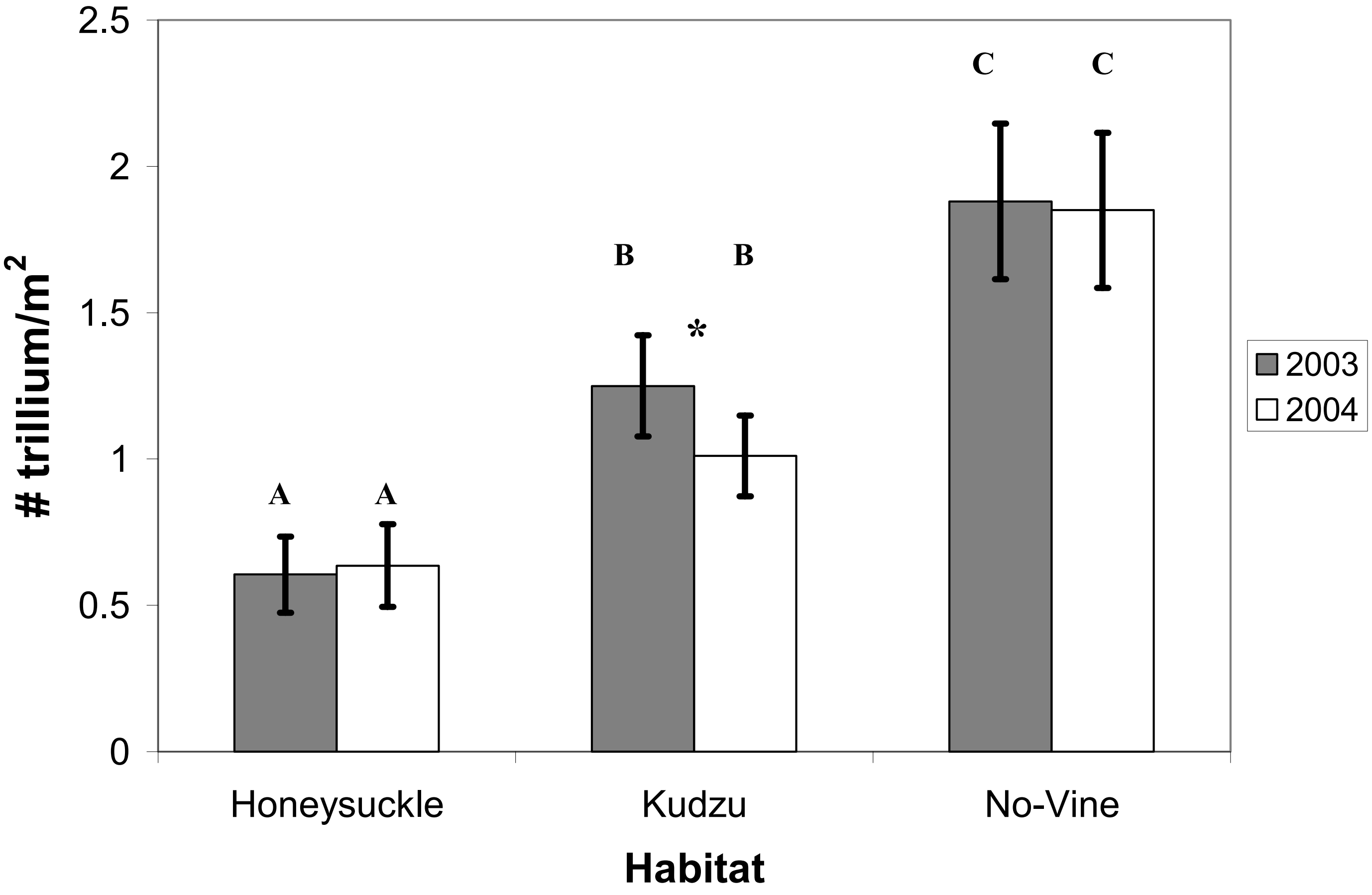


Figure 3.2. Mean density of *Trillium reliquum* in three habitats in two census years. Letters above bars denote differences between habitats with different letters within years. A asterisk above a pair of bars denotes a significant difference in population size between years within habitats.

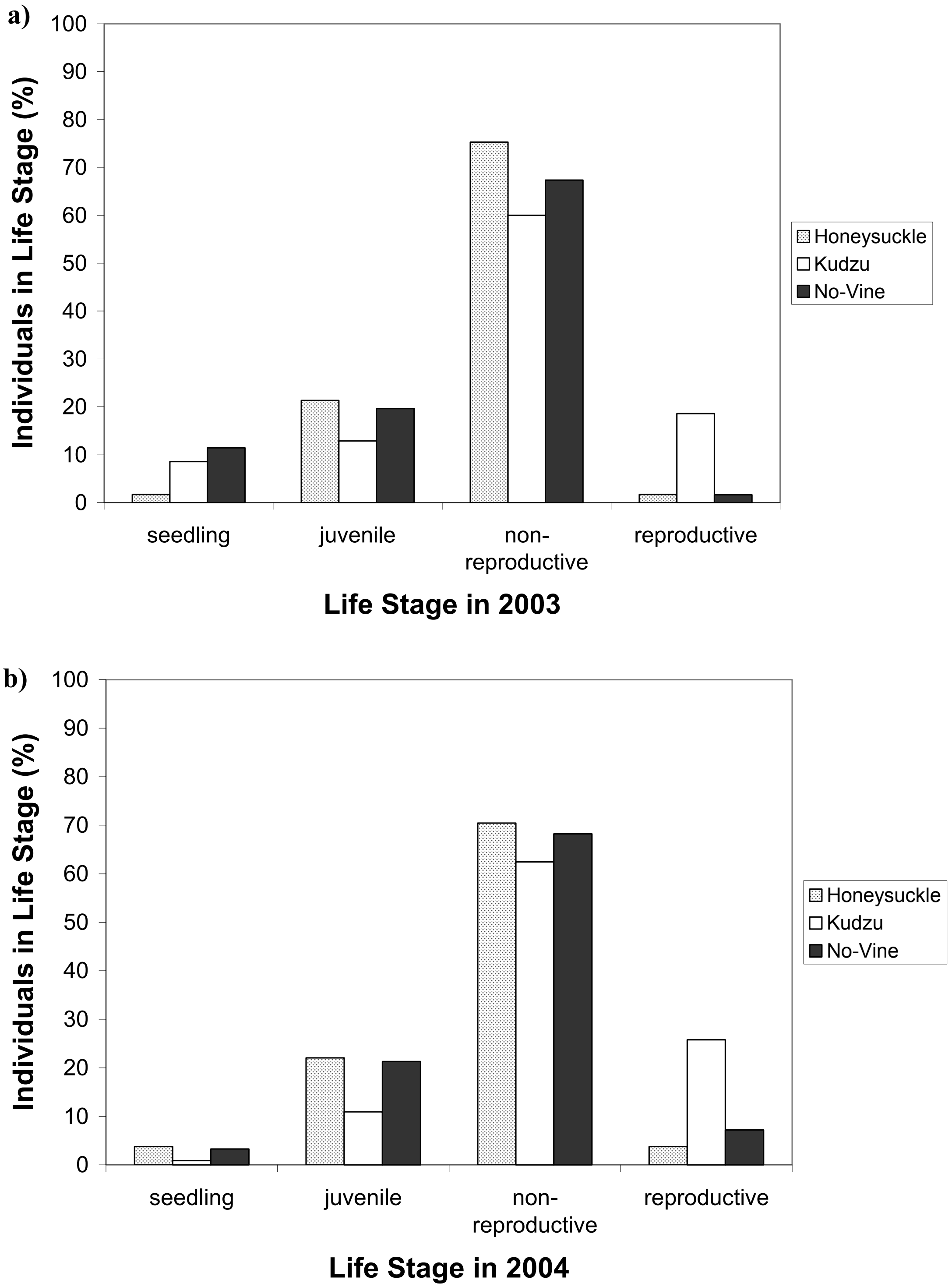


Figure 3.3. Stage structure of *Trillium reliquum* in three habitats over two census years.

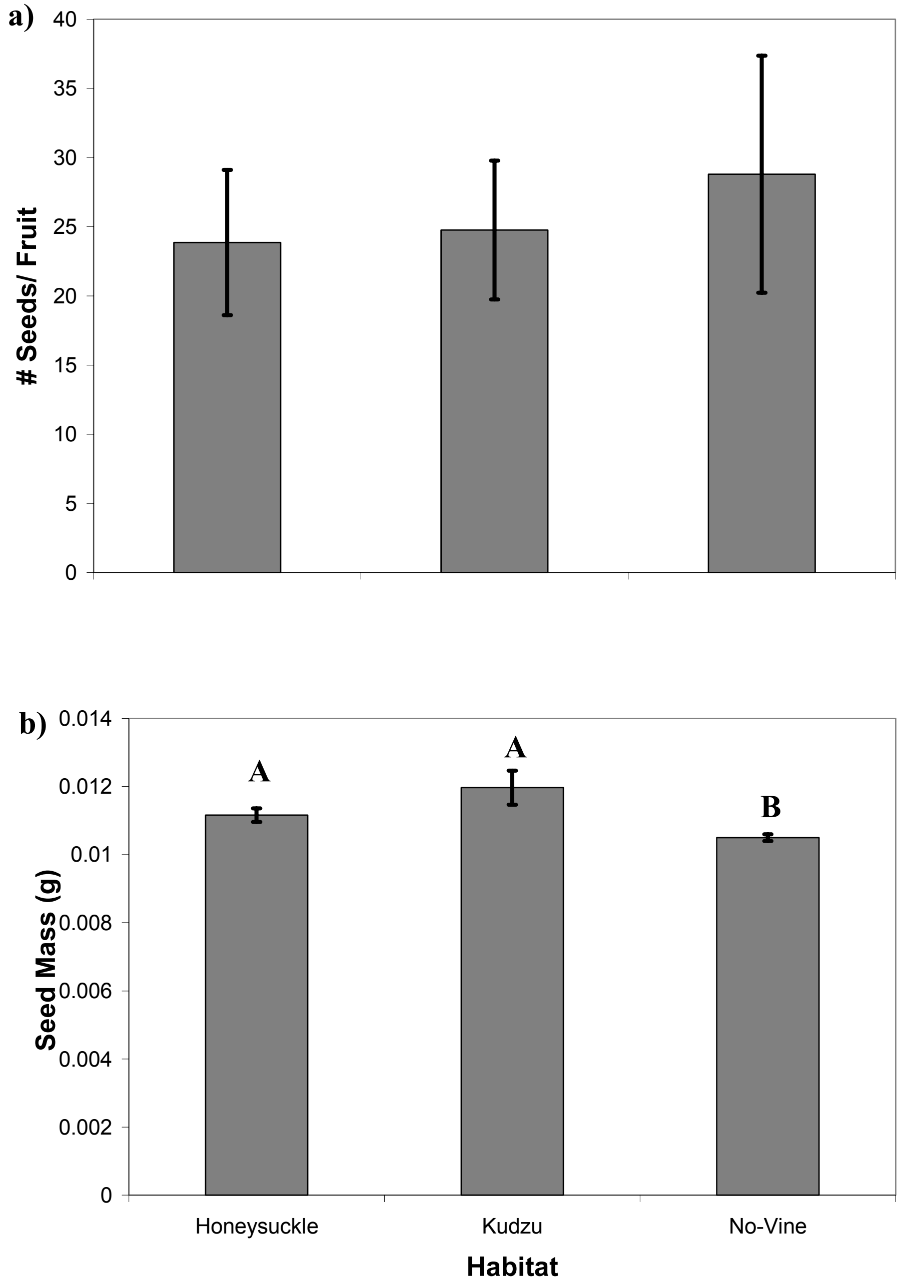


Figure 3.4. a) Seed counts per fruit and b) seed mass per fruit from fruits collected in 2003 in three different habitats. 3.7a) There was no difference in mean seed # (±SE) among habitats, while 3.7b) mean seed mass (±SE) was lower in the no-vine habitat compared to the kudzu and honeysuckle. Different letters above bars denote differences between habitats.

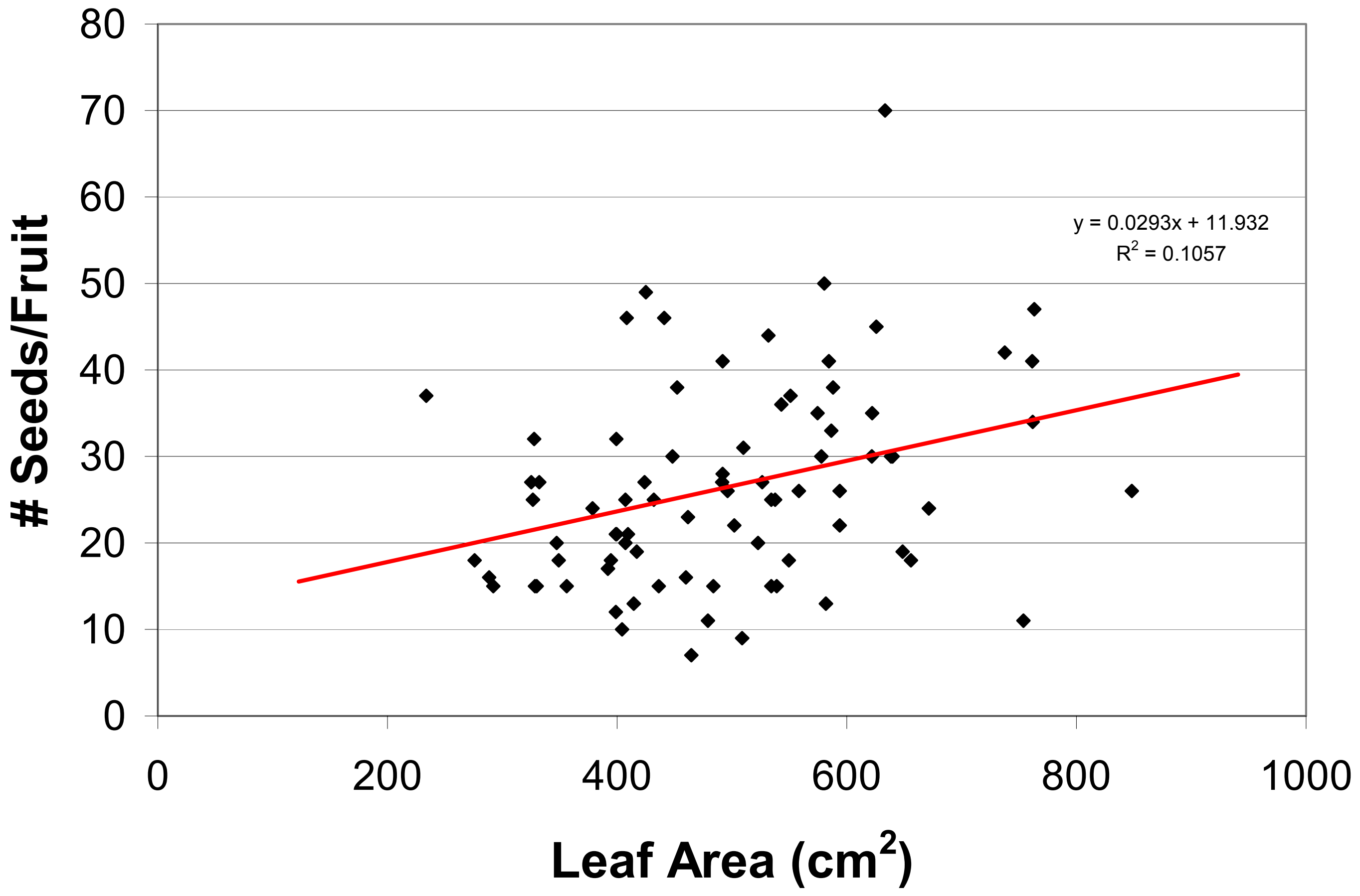


Figure 3.5. Linear regression of total leaf area (cm^2) versus seed # per fruit. P<0.001

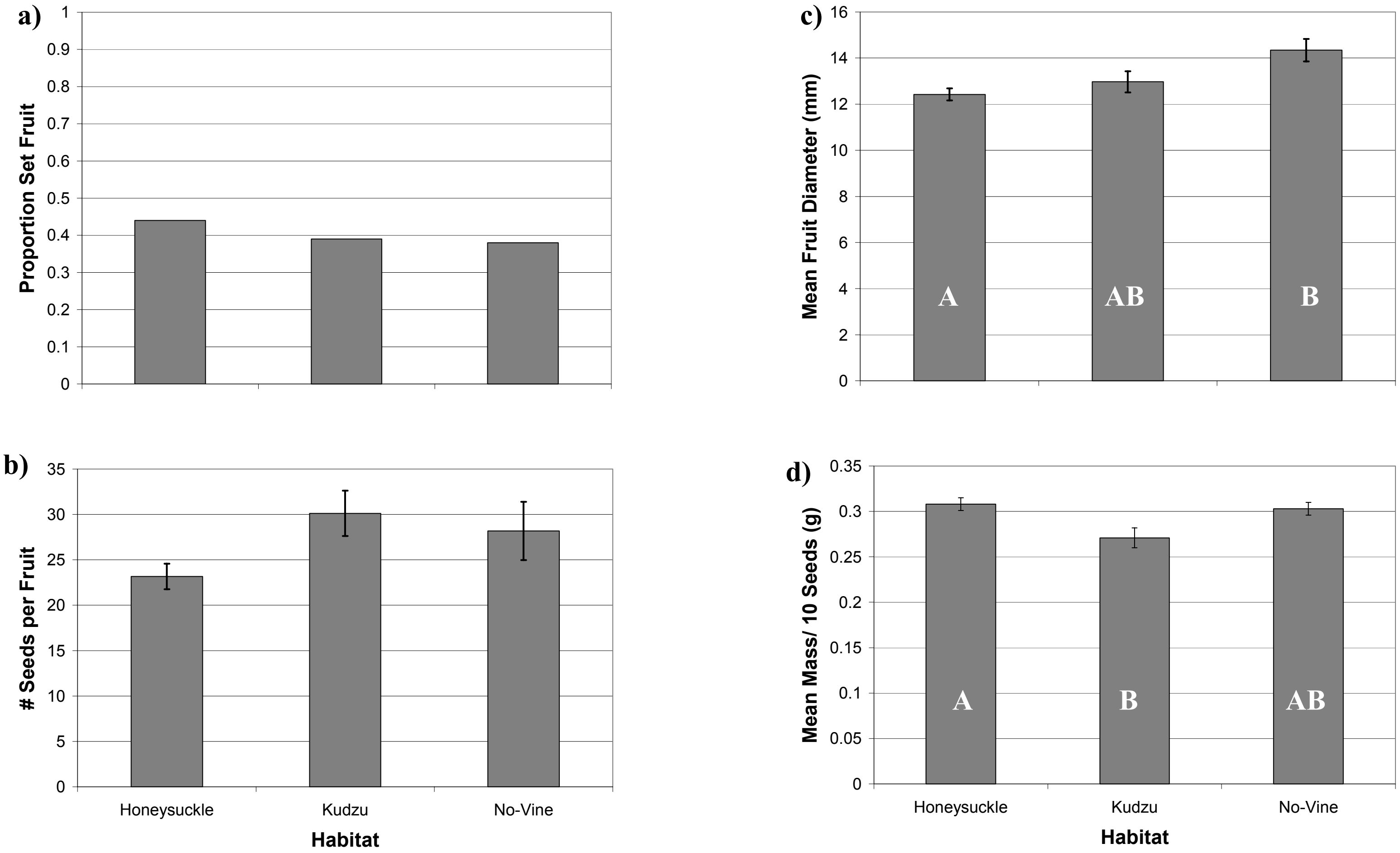


Figure 3.6. Comparison of fruiting success and fruit and seed characteristics among habitats. 4a) Proportion of reproductive flowers to set fruit, 4b) mean # seeds/fruit ±SE, 4c) mean fruit diameter (mm) ±SE, and 4d) mean seed mass (g) per 10 seeds ±SE. Means do not include data from autogamy and apomixis tests. Bars with different capital letters denote significant differences between bars.

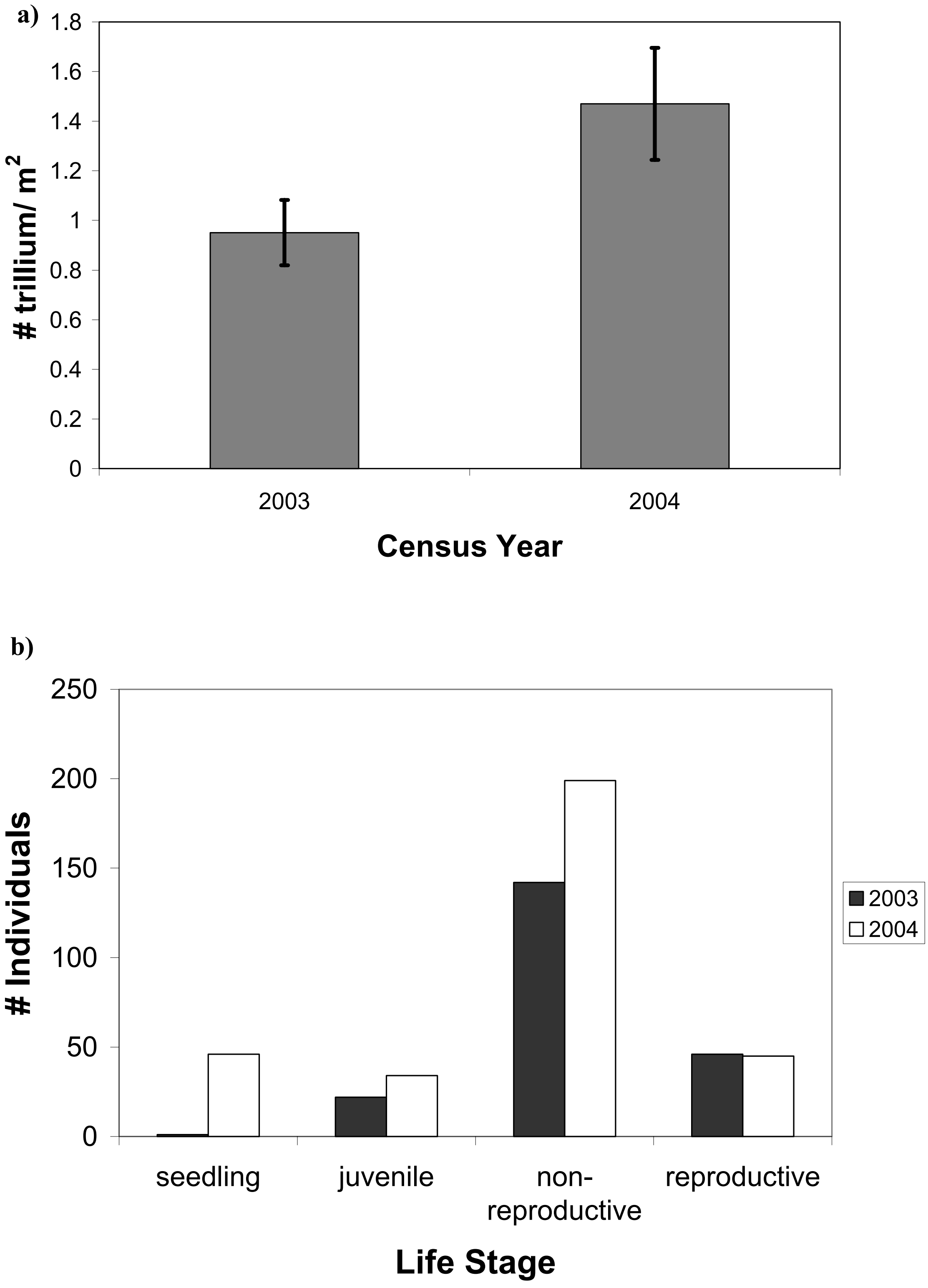


Figure 3.7. a) Population density (±SE) and b) life stage proportions in removal habitat pre- and post-treatment.

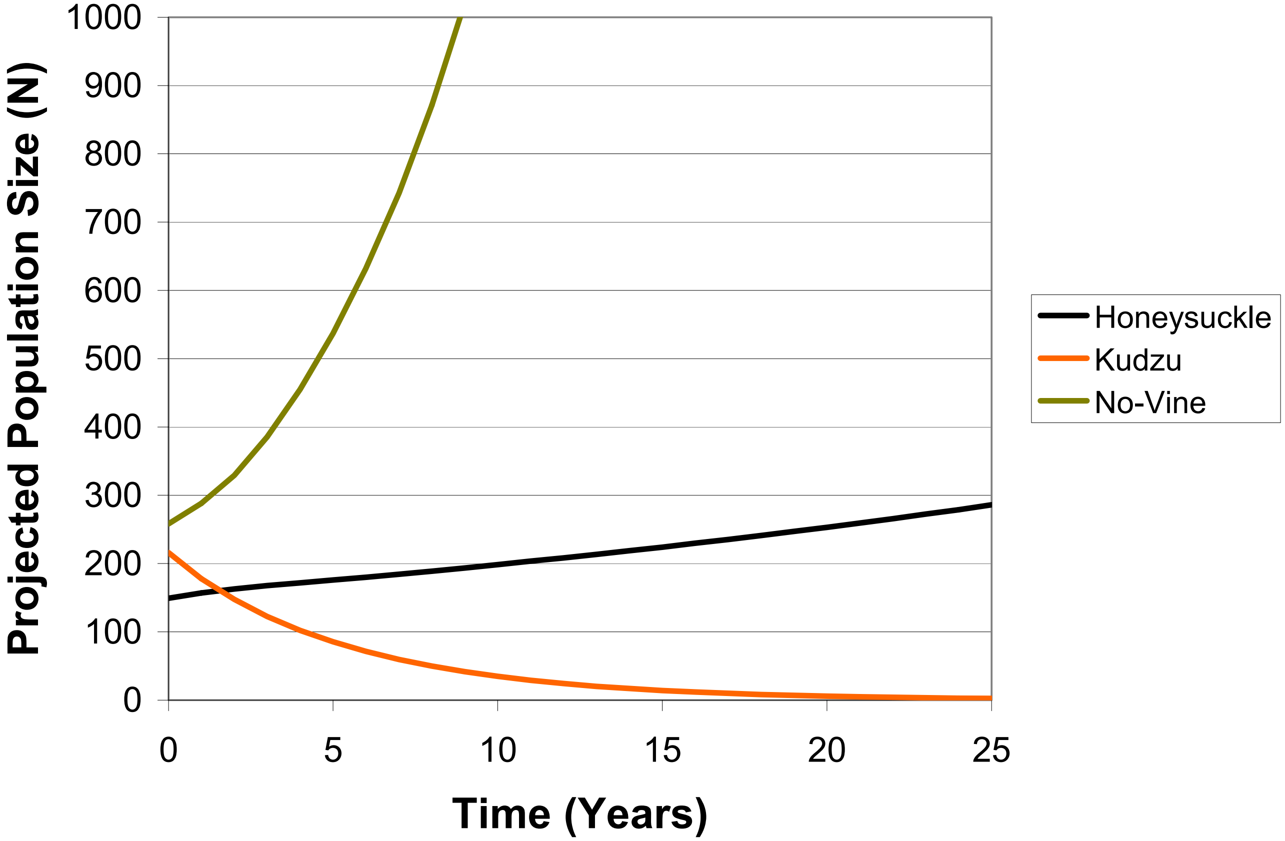


Figure 3.8. Projected *Trillium reliquum* population size in 25 years in honeysuckle, kudzu, and no-vine habitats. Projections are based on 2003 and 2004 census data, excluding new non-seedling plants that emerged after spending 2003 growing season in an underground dormant stage.

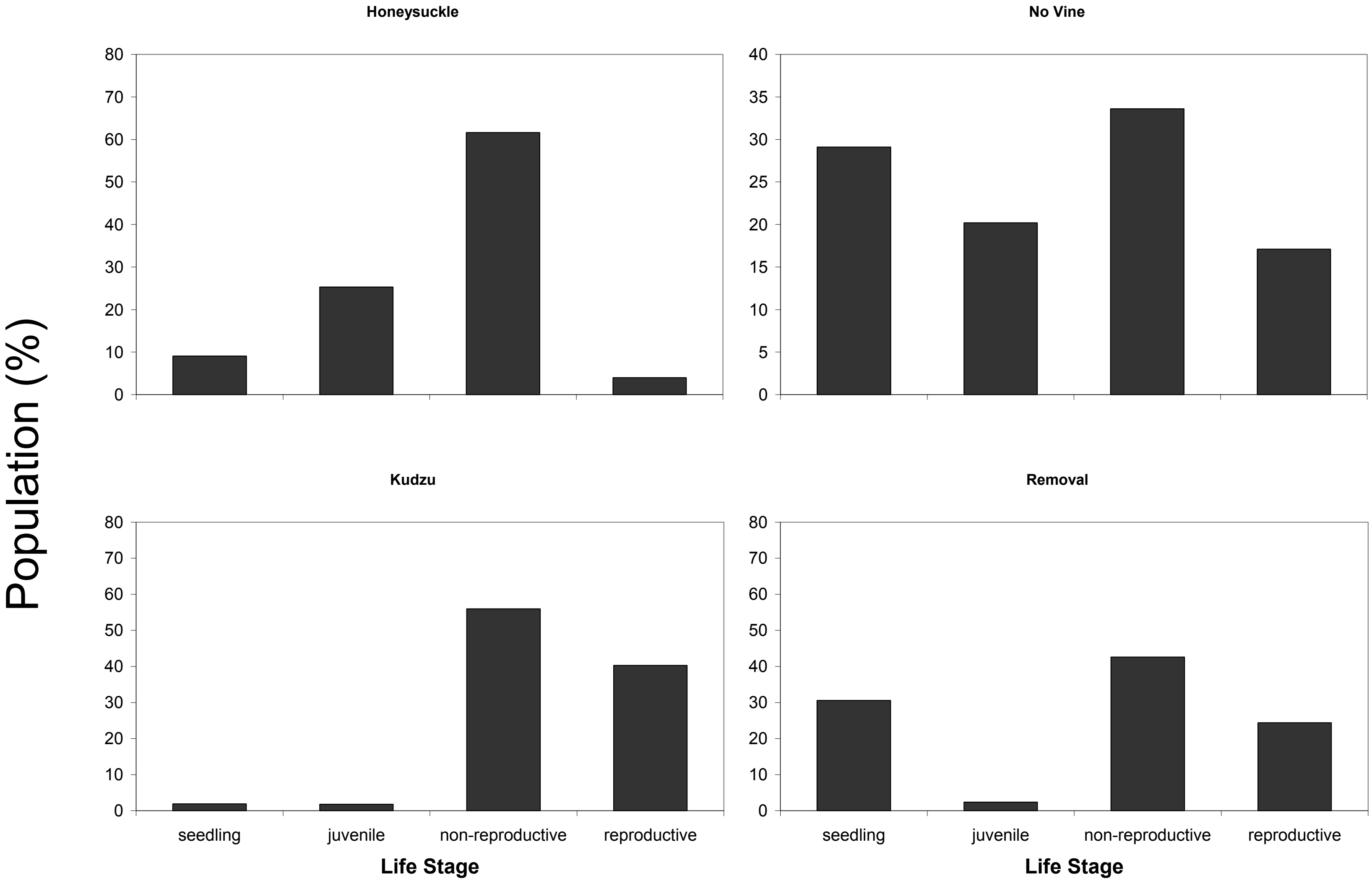


Figure 3.9. Stable stage distributions predicted by the population projection matrix model by habitat.

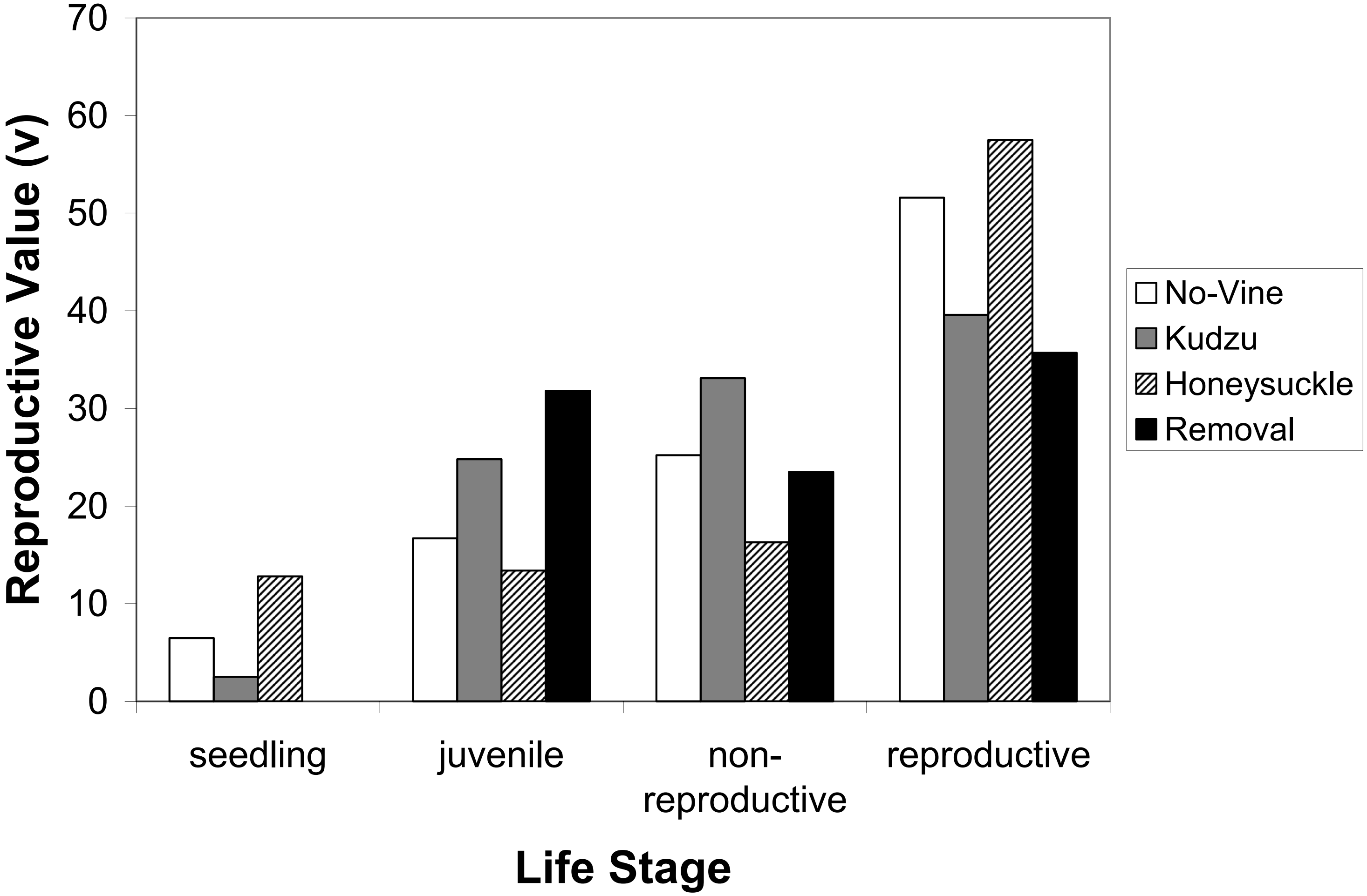


Figure 3.10. Reproductive value of *Trillium reliquum* life stages. Reproductive value, calculated as the left eigenvector of the matrix model, represents the current value of offspring produced by individuals currently in that stage class to the future of the population.

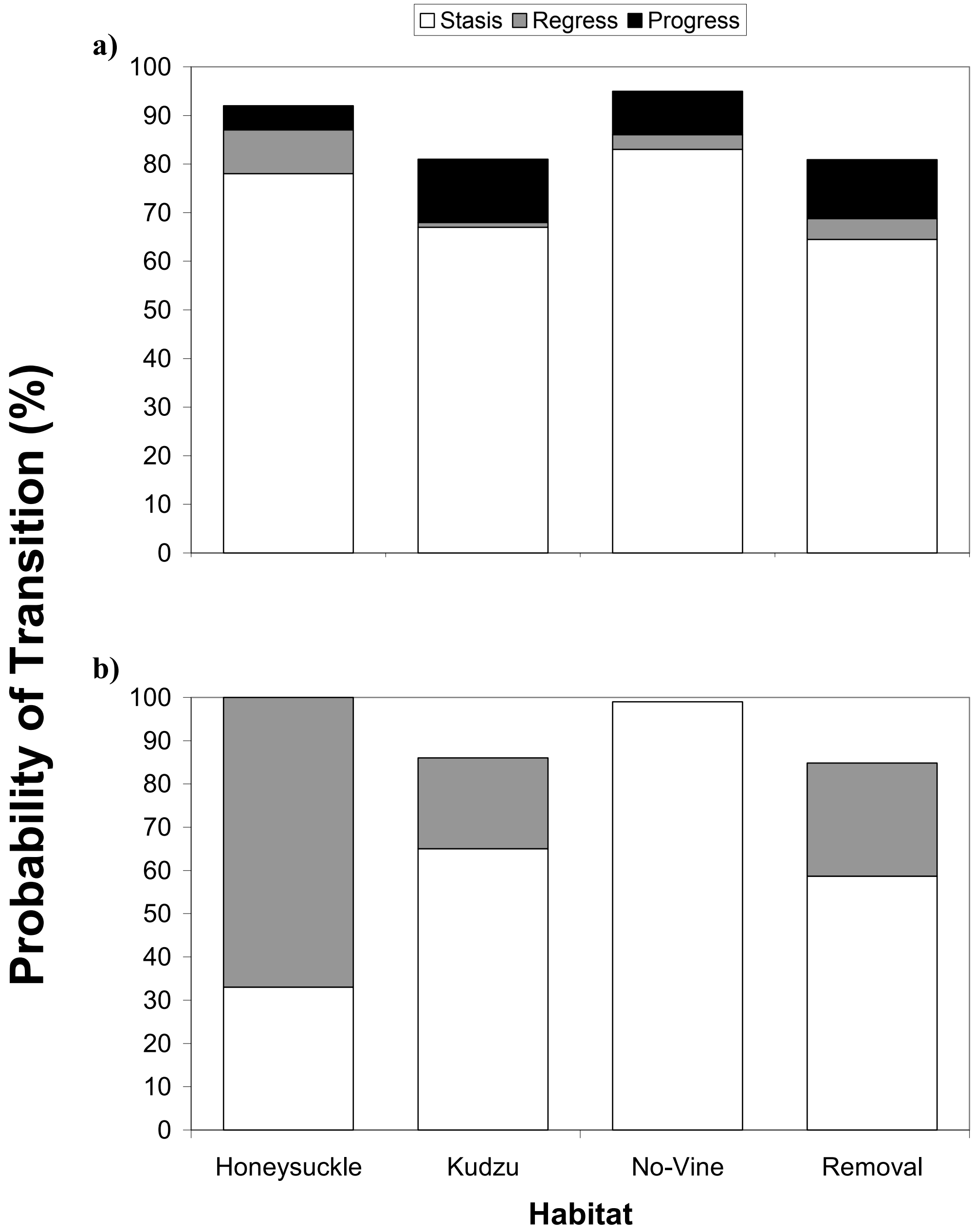


Figure 3.11. Transition probabilities of *Trillium reliquum* life stages with the greatest impacts on λ. a) Non-reproductive or b) reproductive individuals that re-emerged in 2004 could either have progresses to the next stage (except reproductives), regress to the previous stage, or remain in the stage they were in 2003.

CHAPTER IV

Implications for Management

Kudzu and Japanese honeysuckle may play important roles in shaping plant community structure and determining the population dynamics of the endangered *Trillium reliquum* at MBNA. My research has shown that these two invasive vines are associated with decreased species richness and increased total understory cover. These differences may lead to changes in *T. reliquum* population dynamics. Invasive vine habitats were associated with low trillium population sizes, declining populations in kudzu habitat and stabile populations in honeysuckle habitat. In addition, fruit and seed production in *T. reliquum* was found to be resource-limited. The differences in community structure associated with invasive vines may further compound resource-limitation by increasing competition for resources due to increased understory cover in habitats with invaders.

Conclusions

Results of the honeysuckle removal experiment support the findings of the descriptive study, and identify community and population level impacts of invasive vines. The removal of honeysuckle resulted in a decrease in overall cover, but understory cover of native plants was unaffected. Release of trillium populations from honeysuckle pressure resulted in a population increase of 52% after only one winter dormancy period, suggesting that honeysuckle may be suppressing the emergence of trillium. This suppression may further reduce population growth by preventing plants from accumulating the carbohydrate stores needed to progress to larger life stages. The presence of honeysuckle may result in a continuous drain on carbohydrate resources stalling population growth.

The mechanisms behind kudzu impacts may be less subtle than those of honeysuckle. I suggest that kudzu may impact populations by changing the physical structure, such as vertical stand structure, canopy and understory cover, and creating a disturbance regime to which the local woodland herbs are not adapted. Canopy cover in kudzu habitat was significantly lower, and this may allow greater intensities of light to reach the understory. Its rapid growth rate and dense foliage make kudzu the dominant canopy plant in these open areas by mid-April. This may affect trillium populations in that kudzu creates an insulating blanket over the trillium population that traps in higher heat levels created by the more intense light in kudzu habitat. *Trillium reliquum*, adapted to life in a forest understory, likely cannot take this increased heat, and dry up before they can set fruit. More research is needed to determine microclimate differences between kudzu and forested habitat that may support this hypothesis.

Sustaining trillium populations

My research has shown that when invasive vines are not present, *Trillium reliquum* populations are large and stable. This growth occurs even with low fruit set and recruitment rates suggested by my results. Demographic modeling suggests that management efforts should focus on first conserving the non-reproductive and reproductive stages of this species, as changes in the survival and transition rates of these stages should have the greatest impact on population growth. Steps should be taken to continue to monitor this and other population of *T. reliquum*. Population monitoring must be done in a way that the year to year fates of individual plants can be assessed.

High proportions of back-transitions may be used as a red flag to signal that more intensive management action may need to be taken. Also, the results of monitoring efforts from multiple populations can be used in metapopulation analyses to provide a more robust estimate of *T. reliquum* population dynamics.

Steps must also be taken to eliminate or control the spread of invaders. This study has shown that honeysuckle control with an herbicide can effectively eliminate the invader and did allow the *Trillium reliquum* population to rebound in only one year. Elimination or control of kudzu may not be so simple because kudzu is difficult to control (Zidac and Backman 1996). If it is too costly and difficult to remove kudzu, I suggest that the focus be placed on protecting existing trees along the kudzu habitat edge and improving the recruitment rates of trees within the kudzu habitat. Saving edge trees and improving growth of new trees in the kudzu area should have two positive outcomes for trillium. First, kudzu is not shade tolerant; therefore if it is prevented from altering vertical stand structure it may reduce spread to other trillium populations. Second, increases in canopy cover in the kudzu habitat may reduce light intensity enough so that trillium do not desiccate before they can set fruit.

Finally, my research has shown that invasive vines are associated with community-level and population-level impacts on *Trillium reliquum* and also impact its reproduction. More research is needed to determine if community-level impacts are responsible for differences in *T. reliquum* population dynamics or if the invaders directly interact with this endangered species. This research needs to be specifically focused on the non-reproductive and reproductive life stages. The results of my research and future research of this system may not only benefit the endangered populations of *T. reliquum*, but may also benefit all rare plant species by providing broader insight into the interactions that occur between rare and invasive plants.

LITERATURE CITED

Allison, J. R. 2003. Georgia's Alien UFO's. Tipularia **18**:20-23.

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.

Baer, S. G., J. M. Blair, S. L. Collins, and A. K. Knapp. 2004. Plant community responses to resource availability and heterogeneity during restoration. Oecologia **139**:617-629.

Barbour, M. G., J. H. Burk, and W. D. Pitts. 1987. Terrestrial Plant Ecology, 2nd edition. Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.

Barrett, S. C. H., and K. Helenurm. 1987. The reproductive biology of boreal forest herbs. I. Breeding systems and pollination. Canadian Journal of Botany **65**:2036-2046.

Belote, R. T., J. F. Weltzin, and R. J. Norby. 2004. Response of an understory plant community to elevated CO^2 depends on differential responses of dominant invasive species and is mediated by soil water availability. The New Phytologist **161**:827-835.

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

Bockelmann, A.-C., and R. Neuhaus. 1999. Competitive exclusion of *Elymus athericus* from a high-stress habitat in a European salt marsh. The Journal of Ecology **87**:503-519.

Bond, W., and P. Slingsby. 1984. Collapse of an ant-plant mutualism: The Argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. Ecology **65**:1031-1037.

Boyette, C. D., H. L. Walker, and H. K. Abbas. 2002. Biological control of kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. Biocontrol Science and Technology **12**:75-82.

Brower, J. E., J. H. Zar, and C. N. vonEnde. 1998. Field and laboratory methods for general ecology, 4th edition. WCB/McGraw-Hill, New York.

Byers, D. L., and T. R. Meagher. 1997. A comparison of demographic characteristics in a rare and a common species of *Eupatorium*. Ecological Applications **7**:519-530.

Cain, M. D. 1984. Japanese honeysuckle and associated ground cover inhibit establishment and growth on pine seedlings in all-aged stands. Pages 300-304 *in* E. Shoulders, editor. Third Biennial Southern Silviculture Research Conference. U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Atlanta, GA.

Callaway, R. M., and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science **290**:521-523.

Calvo, R. N., and C. C. Horvitz. 1990. Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. The American Naturalist **136**:499-516.

Carson, W. P., and S. T. A. Pickett. 1990. Role of resources and disturbance in the organization of an old-field community. Ecology **71**:226-238.

Carter, G. A., and A. H. Teramura. 1988. Nonsummer stomatal conductance for the invasive vines kudzu and Japanese honeysuckle. Canadian Journal of Botany **66**:2392-2395.

Case, F. W., Jr., and R. B. Case. 1997. Trilliums. Timber Press, Inc., Portland.

Caswell, H. 1982. Stable population structure and reproductive value for populations with complex life cycles. Ecology **63**:1223-1231.

Caswell, H. 2001. Matrix population models: construction, analysis, and interpretation, 2nd edition. Sinauer Associates, Inc., Sunderland, MA.

Cortes, E. 1999. A stochastic stage-based population model of the sandbar shark in the western north atlantic. American Fisheries Society Symposium **23**:115-136.

Crouse, D. T., L. B. Crowder, and H. Caswell. 1987. A stage-based population model for loggerhead sea turtles and implications for conservation. Ecology **68**:1412-1423.

D'Antonio, C. D., and L. A. Meyerson. 2002. Exotic plant species as problems and solutions in ecological restoration: a sythesis. Restoration Ecology **10**:703-713.

D'Antonio, C. M. 1993. Mechanisms controlling invasion of coastal plant communities by the alien succulent *Carpobrotus edulis*. Ecology **74**:83-95.

Davis, M. A., and K. Thompson. 2000. Eight ways to be a colonizer; two ways to be an invader: a proposed nomenclature scheme for invasio ecology. Bulletin of the Ecological Society of America:226-230.

Dillenburg, L. R., D. F. Whigham, A. H. Teramura, and I. N. Forseth. 1993. Effects of below- and aboveground competition from the vines *Lonicera japonica* and *Parthenocissus quinquefolia* on the growth of the tree host *Liquidambar styraciflua*. Oecologia **93**:48-54.

Dukes, J. S. 2001. Biodiversity and invasibility in grassland microcosms. Oecologia **126**:563-568.

Duncan, W. H., and M. B. Duncan. 1988. Trees of the Southeastern United States. University of Georgia Press, Athens, Georgia.

Duncan, W. H., and M. B. Duncan. 1999. Wildflowers of the Eastern United States. University of Georgia Press, Athens, Georgia.

Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen & Co. LTD, London.

Equihua, M., and M. B. Usher. 1993. Impact of carpets on the invasive moss *Campylopus introflexus* on *Calluna vulgaris* regeneration. The Journal of Ecology **81**:359-365.

Ewe, S. M., and d. L. Sternberg. 2002. Seasonal water-use by the invasive exotic, *Schinus terebinthifolius*, in native and disturbed communities. Oecologia **133**:441-448.

Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. Proceedings of the National Academy of Sciences **100**:8916-8920.

Fiedler, P. L., and J. J. Ahouse. 1992. Hierarchies of cause: toward an understanding of rarity in vascular plant species. Pages 23-47 *in* P. L. Fiedler and S. K. Jain, editors. Conservation biology: the theory and practice of nature conservation, preservation, and management. Routledge, Chapman & Hall, Inc., New York.

Flecker, A. S., and C. R. Townsend. 1994. Community-wide consequences of trout introduction in New Zealand streams. Ecological Applications **4**:798-807.

Forseth, I. N., and A. H. Teramura. 1987. Field photosynthesis, microclimate and water relations of an exotic temperate liana, *Pueraria lobata*, kudzu. Oecologia **71**:262-267.

Freeman, J. D. 1975. Revision of the *Trillium* subgenus Pyllantherum (Liliaceae). Brittonia **27**:1-62.

Fujita, K., K. Matsumoto, G. K. Ofusu-Budu, and S. Ogata. 1993. Effect of shading on growth and dinitrogen fixation of kudzu and tropical pasture legumes. Soil Science and Plant Nutrition **39**:43-54.

Gill, R. A., and I. C. Burke. 1999. Ecosystem consequences of plant life form changes at three sites in the semiarid United States. Oecologia **121**:551-563.

Gordon, D. R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. Ecological Applications **8**:975-989.

Grosholz, E. D. 1996. Contrasting rates of spread for introduced species in terrestrial and marine systems. Ecology **77**:1680-1686.

Hager, H. A. 2004. Competitive effect versus competitive response of invasive and native wetland plant species. Oecologia **139**:140-149.

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.

Holmes, P. M., and R. M. Cowling. 1997. The effects of invasion by Acacia saligna on the guild structure and regeneration capabilities of South African fynbos shrublands. Pages 317-332 *in* Journal of Applied Ecology. Blackwell Publishing Limited.

Horvitz, C. C., and D. W. Schemske. 1995. Spatiotemporal variation in demographic transitions of a tropical understory herb: projection matrix analysis. Ecological Monographs **65**:155-192.

Huenneke, L. F., and J. K. Thomson. 1995. Potential interference between a threatened endemic thistle and an invasive nonnative plant. Conservation Biology **9**:416-425.

Irwin, R. E. 2000. Morphological variation and female reproductive success in two sympatric Trillium species: evidence for phenotypic selection in Trillium erectum and Trillium grandiflorum (Liliaceae). American Journal of Botany **87**:205-214.

Irwin, R. E. 2001. Field allozyme studies investigating optimal mating success in two sympatric spring-ephemeral plants, *Trillium erectum* and *T. grandiflorum*. Heredity **87**:178-189.

Jeffrey, E. C., and E. J. Haertl. 1939. Apomixis in *Trillium*. Cellule **48**:79-88.

Jewett, D. K., C. J. Jiang, K. O. Britton, J. H. Sun, and J. Tang. 2003. Characterizing specimens of kudzu and related taxa with RAPDs. Castanea **68**:254-260.

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., F. M. Hanzawa, S. J. Tonsor, D. A. Theide, and S. Voigt. 1999. Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. Ecology **80**:2620-2634.

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

Kalisz, S., J. D. Nason, F. M. Hanzawa, and S. J. Tonsor. 2001. Spatial population genetic structure in *Trillium grandiflorum:* the roles of dispersal, mating, history, and selection. Evolution **55**:1560-1568.

Kawano, S., M. Ohara, and F. H. Utech. 1986. Life history studies on the genus *Trillium* (Liliaceae) II. Reproductive biology and survivorship of four eastern North American species. Plant Species Biology **1**:47-58.

Kaye, T. N., and D. A. Pyke. 2003. The effect of stochastic technique on estimates of population viability from transition matrix models. Ecology **84**:1464-1476.

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

Kidd, K. A., and D. B. Orr. 2001. Comparative feeding and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) on kudzu and soybean foliage. Annals of the Entomological Society of America **94**:219-225.

Knight, T. M. 2003. Effects of herbivory and its timing across populations of *Trillium grandiflorum* (Liliaceae). American Journal of Botany **90**:1207-1214.

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

Knight, T. M. 2004. The effects of herbivory and pollen limitation on a declining population of *Trillium grandiflorum*. Ecological Applications **in press**.

Kourtev, P. S., J. G. Ehrenfeld, and W. Z. Huang. 1998. Effects of exotic plant species on soil properties in hardwood forests of New Jersey. Water, Air, and Soil Pollution **105**:493-501.

Ladine, T. A., and J. Robert E. Kissell. 1994. Escape behavior of Virginia opossums. American Midland Naturalist **132**:234-238.

Leatherman, A. D. 1955. Ecological life-history of *Lonicera japonica* Thunb. Dissertation. University of Tennessee, Knoxville, Tennessee.

Mack, M. C., and C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology and Evolution **13**:195-198.

Menges, E. S. 1992. Stochastic modeling of extinction in plant populations. Pages 253-275 *in* P. L. Fiedler and S. K. Jain, editors. Conservation biology: the theory and practice of nature conservation, preservation, and management. Routledge, Chapman & Hall, Inc., New York.

Miller, K. E., and D. L. Gorchov. 2004. The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. Oecologia **139**:359-375.

Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences **98**:5446-5451.

Mooney, H. A., and J. A. Drake. 1986. Ecology of biological invasions of North America and Hawaii. Springer-Verlag, New York.

Myster, R. W., and S. T. A. Pickett. 1992. Dynamics of associations between plants in ten old fields during 31 years of succession. Journal of Ecology **80**:291-302.

Nesom, G. L., and J. C. L. Duke. 1985. Biology of *Trillium nivale* (Lilliaceae). Canadian Journal of Botany **63**:7-14.

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.

Olson, B. E., and R. T. Wallander. 2002. Effects of invasive forb litter on seed germination, seedling growth and survival. Basic and Applied Ecology **3**:309-317.

Oostermeijer, J. G. B., M. L. Brugman, E. R. DeBoer, and H. C. M. DenNijs. 1996. Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. Journal of Ecology **84**:153-166.

Pappert, R. A., J. L. Hamrick, and L. A. Donovan. 2000. Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. American Journal of Botany **87**:1240-1245.

Parker, I. M. 1997. Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. Ecology **78**:1457-1470.

Parker, I. M., and K. A. Haubensak. 2002. Comparative pollinator limitation of two non-native shrubs: do mutualisms influence invasions? Oecologia **130**:250-258.

Patrick, T. S. 1973. Observations on the life history of *Trillium grandiflorum* (Michaux) Salisbury. Master of Science. Cornell University, Ithaca, NY.

Patrick, T. S., J. R. Allison, and G. A. Krakow. 1995. Protected plants of Georgia. Georgia Department of Natural Resources, Social Circle, GA.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience **50**:53-65.

Porcher, R. D., and D. A. Rayner. 2001. A guide to the Wildflowers of South Carolina. University of South Carolina Press, Columbia, SC.

Porter, S. D., and D. A. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts athropod community. Ecology **71**:2095-2106.

Rabinowitz, D., S. Cairns, and T. Dillion. 1986. Seven forms of rarity and their frequency in the flora of the British isles. Pages 182-204 *in* M. E. Soule, editor. Conservation biology: the science of scarcity and diversity.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill.

Robertson, D. J., M. C. Robertson, and T. Tague. 1994. Colonization dynamics of four exotic plants in a northern Piedmont natural area. Bulletin of the Torrey Botanical Club **121**:107-118.

Routhier, M.-C., and L. Lapointe. 2002. Impact of tree leaf phenology on growth rates and reproduction in the spring flowering species *Trillium erectum* (Liliaceae). American Journal of Botany **89**:500-505.

Sage, T. L., S. R. Griffin, V. Pontieri, P. Drobac, W. W. Cole, and S. C. H. Barrett. 2001. Stigmatic self-incompatibility and mating patterns in *Trillium grandiflorum* and *Trillium erectum* (Melanthiaceae). Annals of Botany **88**:829-841.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics **32**:305-332.

Sasek, T. W., and B. R. Strain. 1989. Effects of carbon dioxide enrichment on the expansion and size of kudzu (*Pueraria lobata*) leaves. Weed Science **37**:23-28.

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

Scheu, S., and D. Parkinson. 1994. Effects of invasion of an aspen forest (Canada) by *Dendrobaena octaedra* (Lubricidae) on plant growth. Ecology **75**:2348-2361.

Schierenbeck, K. A., J. L. Hamrick, and R. N. Mack. 1995. Comparison of allozyme variability in a native and introduced species of *Lonicera*. Heredity **75**:1-9.

Schierenbeck, K. A., and J. D. Marshall. 1993. Seasonal and diurnal patterns of photosynthetic gas exchange for *Lonicera sempervirens* and *L. japonica* (Caprifoliaceae). American Journal of Botany **80**:1292-1299.

Schnitzer, S. A., J. W. Dalling, and W. P. Carson. 2000. The impact of lianas on tree regeneration in tropical forest canopy gaps: evidence for an alternative pathway of gap-phase regeneration. Journal of Ecology **88**:655-666.

Schweitzer, J. A., and K. C. Larson. 1999. Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. Journal of the Torrey Botanical Society **126**:15-23.

Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. Trends in Ecology and Evolution **17**:170-176.

Sih, A., and M.-S. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology **68**:1679-1690.

Slezak, W. F. 1976. *Lonicera japonica* Thunb., an aggressive introduced species in a mature forest ecosystem. Master of Science. Rutgers University, New Brunswick.

Smith, B. H., P. D. Forman, and A. E. Boyd. 1989. Spatial patterns of seed dispersal and predation of two myrmecochorous forest herbs. Ecology **70**:1649-1656.

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

Stevens, M. H. H., and W. P. Carson. 2002. Resource quantity, not resource heterogeneity, maintains plant diversity. Ecology Letters **5**:420-426.

Susko, D. J., J. P. Mueller, and J. F. Spears. 2001. An evaluation of methods for breaking seed dormancy in kudzu (*Pueraria lobata*). Canadian Journal of Botany **79**:197-203.

Tickner, D. P., P. G. Angold, A. M. Gurnell, and J. O. Mountford. 2001. Riparian plant invasions: hydrogeomorphological control and ecological impacts. Progress in Physical Geography **25**:22-52.

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

Trammell, M. A., and J. L. Butler. 1995. Effects of exotic plants on native ungulate use of habitat. Journal of Wildlife Management **59**:808-816.

Tsugawa, H., and R. Kayama. 1985. Studies on population structure of kudzu vine (*Pueraria lobata* Ohwi) VI. The structure of overwintering aboveground parts of individual plants which constitute a natural kudzu population. Journal of Japanese Grassland Science **31**:167-176.

U.S. Fish and Wildlife Service. 1988. Endangered and threatened wildlife and plants; Determination of endangered status for the relict trillium. Federal Register **53**:10879-10884.

U.S. Fish and Wildlife Service. 1990. Relict trillium recovery plan. U.S. Fish and Wildlife Service, Atlanta, GA.

Vellend, M., J. A. Myers, S. Gardescu, and P. L. Marks. 2003. Dispersal of *Trillium* seeds by deer: implications for long-distance migration of forest herbs. Ecology **84**:1067-1072.

Vitousek, P. M., and L. R. Walker. 1989. Biological invasion by *Myrica faya* in Hawaii: plant demography, nitrogen fixation, ecosystem effects. Ecological Monographs **59**:247-265.

Wechsler, N. R. 1977. Growth and physiological characteristics of kudzu, *Pueraria lobata* (Willd.) Ohwi, in relation to its competetive success. Master of Science. University of Georgia, Athens, GA.

Werner, P. A., and H. Caswell. 1977. Population growth rates and age versus stage-distribution models for teasel (*Dipsacus sylvestris* Huds.). Ecology **58**:1103-1111.

Westbrooks, R. 1998. Invasive plants, changing the landscape of America: Fact book. Federal Interagency Committee for the Management of Noxious and Exotic Weeds, Washington D.C.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience **48**:607-915.

Williams, J. D., and G. K. Meffe. 1998. Nonindigenous species. Pages 117-129 *in* M. J. Mac, P.A. Opler, C.E. Puckett Haecker, and P.D. Doran, editor. Status and Trends of the Nation's Biological Resources. U.S. Department of the Interior, U.S. Geological Survey, Reston, VA.

Winberry, J. J., and D. M. Jones. 1973. Rise and decline of the "miracle vine" kudzu in the southern landscape. Southeastern Geographer **13**:60-70.

Woods, K. D. 1993. Effects of invasion by *Lonicera tatarica* L. on herbs and tree seedlings in four New England forests. American Midland Naturalist **130**:62-74.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics **25**:443-466.

Yamashita, N., A. Ishida, H. Kushima, and N. Tanaka. 2000. Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. Oecologia **125**:412-419.

Zidac, N. K., and P. J. Backman. 1996. Biological control of Kudzu (*Pueraria lobata*) with the plant pathogen *Psuedomonas syringae* pv. *phaseolicola*. Weed Science **44**:645-649.

APPENDICES

Appendix 1. *Trillium reliquum* Natural History

Trillium reliquum Freeman was first described by John Freeman (1975) in a revision of the genus *Trillium*. In Freeman's original description he used the specific epithet "reliquum", which means relict, to describe the disjunct populations of the species that may have been remnants of a once more widely distributed species (Freeman 1975). Indeed, *T. reliquum* is one of two species of trillium listed as federally endangered by the Endangered Species Act (Case and Case 1997). Although some data are available about the reproductive biology and ecology of this species (see Patrick et al. 1995), no formal studies have been published on these aspects of *T. reliquum* biology.

Loss of habitat and encroachment from two invasive vines, kudzu (*Pueraria montana*) and Japanese honeysuckle (*Lonicera japonica*), are considered to be major threats to the survival of *Trillium reliquum* (U.S. Fish and Wildlife Service 1990). Invasive plants can negatively affect native plants via competitive exclusion for resources (Gordon 1998, Bockelmann and Neuhaus 1999) and pollinators (Parker and Haubensak 2002). It is not likely that invasive vines compete with *T. reliquum* for pollinators because its flowering phenology does not overlap with that of kudzu or Japanese honeysuckle.

Invasive vines may affect *Trillium reliquum* in other ways beside direct competition for pollinators. Invasion may result in reduced population density of the rare plant which may decrease attractiveness to pollinators, thus, a reduction in pollination occurs. Pollen availability may also be affected by invaders that alter plant community structure in a manner by which competition with another native plant for pollinators increases. Trillium species are thought to be pollinated by animals (insects) (Case and Case 1997), but the specific pollinators of *T reliquum* are not known.

Trillium species are varied in their breeding systems, however, the breeding system of *T. reliquum* is not currently known. Irwin found that *T. erectum* and *T. grandiflorum* had greater reproductive success when flowers were cross-pollinated, but that both species were self-compatible (Irwin 2000, 2001), whereas other studies have shown *T. erectum* and *T. grandiflorum* to be self-incompatible (Kalisz et al. 1999, Sage et al. 2001, Knight 2003). Self-compatibility was also seen in *T. undulatum* (Barrett and Helenurm 1987) and *T. nivale* (Nesom and Duke 1985). Several authors have also suggested that apomixis occurs in some trillium species (Jeffrey and Haertl 1939, Nesom and Duke 1985, Barrett and Helenurm 1987). Self-compatibility and apomixis may provide early flowering species reproductive assurance at times when pollinator density is low or unpredictable.

Understanding the biotic interactions and abiotic factors that govern the reproductive ecology of *Trillium reliquum* is essential for the longevity of this species (U.S. Fish and Wildlife Service 1990). Here I report the results of tests to determine the breeding system of *T. reliquum.* In addition, I provide a description of the life history of a *T. reliquum* population in the southeastern U.S.

METHODS

I conducted my study at Montezuma Bluffs Natural Area (MBNA) (N32°20’ W84°1’) in Macon County, GA. The 202 hectare natural area lies along the east bank of the Flint River and is characterized by limestone outcroppings and a mixture of beech-magnolia hardwood and coniferous forests growing on steep, moist slopes. Montezuma

Bluffs Natural Area encompasses large populations of *Trillium reliquum* in habitats with varying degrees of kudzu and honeysuckle encroachment. At this site *T. reliquum* grows in sympatry with the spotted trillium, *T. maculatum*. I censused *Trillium reliquum* populations in three pre-existing habitats within MBNA during 2003 and 2004. I followed the fates of a subset of individuals in the MBNA population to examine the impacts of invasive vine species on trillium population dynamics and the local plant community (Chapter 1).

Breeding System

I used four experimental pollination treatments to determine the breeding system of *Trillium reliquum.* On March 12, 2004 I arbitrarily selected and randomly assigned 30 reproductive plants in no-vine habitat to receive either a self-pollination (autogamy) treatment or an apomictic treatment until there were 15 plants of each treatment. I administered the pollination treatments on March 17, 2004 prior to anthesis. For the self-pollination treatment, I bagged flowers with a nylon mesh bag to prevent pollinators from entering the flower and did not manipulate pollen transfer. For the apomictic treatment, I removed anthers from the flower (emasculated) and bagged the flower to prevent pollinators from entering the flower. In addition to the autogamy and apomictic treatments, another 321 flowers outside of the demography study area were randomly assigned to receive either supplemental pollen or open pollination treatments to determine if pollen or resources limit fruit and seed production and if seed production is greater when flowers receive outcross pollen (see Chapter 3).

I administered supplemental and open pollination treatments between March 16 and March 20, 2004 after the first signs of anthesis. To provide supplemental pollen, I collected anthers from flowering individuals not selected for this study and deposited their pollen on the stigmas of selected plants by rubbing the anther over the stigma until the entire receptive surface was saturated with pollen. *Trillium reliquum* reproductive parts are relatively large, so pollen saturation was easily detectable with the naked eye. I did not manipulate open pollination treatment plants, and all plants in the study remained accessible to pollinators for additional pollen transfer.

Fruit and seed development in the apomictic treatment would provide evidence of apomixis, the development of ovules into seeds in the absence of fertilization. Likewise, fruit and seed development in the autogamy treatment would provide further evidence for apomixis and potential self-compatibility. This treatment could not serve as a definitive test for compatibility of self-pollen as I did not directly transfer self pollen to the stigmas before excluding natural pollinators. Development of fruit and seed in the supplemental pollen treatment would indicate outcrossing in this species.

I monitored the maturation of fruits monthly until fruits were mature. Each month prior to collection, I recorded the number of plants that had died back as a result of predation or unknown causes before fruits reached maturity. In June, I collected fruits and measured fruit diameter, counted seeds per fruit, and massed the seeds with an analytical balance (Denver Instrument Company TL-104, Denver CO). I measured fruit diameter as the widest point between two carpel ridges (Appendix 2) with digital calipers (Fisher Scientific, Pittsburgh, PA). To obtain mean seed mass per fruit, I massed seeds in ≤ 3 groups of 10. After measurements, I returned seeds to the forest for natural dispersal.

I compared the probability of setting fruit among treatments with a Chi-square test to determine if *T. reliquum* is more successful when cross- versus self-pollinated. I compared seed set, fruit diameter, and mean seed mass among all pollination treatments (open, supplemental, autogamy, and apomictic) using one-way ANOVA. Seed count data were log transformed to meet test assumptions; fruit diameter and seed mass data fit normality and homoscedasticity assumptions for these tests. When there was a significant difference, I compared each pair of means using Student's t-test. Some fruits had already begun to drop seed before collection occurred. I omitted data from any fruits that dehisced before collection from any comparisons of seed counts, since I could not be sure that every seed had been collected. I used Pop Tools version 2.5.9 (2003), an Excel spreadsheet add-in, to perform Chi-square tests. All other statistical analyses were performed with JMP IN 5.1 (SAS Institute, 2005, Cary NC).

RESULTS and DISCUSSION

Life history

Trillium reliquum is a spring ephemeral perennial species; its average life span is not known. *T. reliquum* overwinters as an underground rhizome that puts out new shoots beginning in late February. The production of new individuals in *Trillium reliquum* occurs primarily from seed via sexual reproduction. Although multiple shoots can arise from a single rhizome, this type of reproduction is rare in *T. reliquum* and occurs in <4% of the surveyed population at MBNA. These findings are in agreement with studies of other trillium species (Nesom and Duke 1985, Kawano et al. 1986, Hanzawa and Kalisz 1993).

The flowering period for this population begins in mid-March and lasts for 2-3 weeks depending on weather conditions. In >250 hours of field work I observed only two candidate pollinators that were probably Coleopteran or Hemipteran species. Petal color of *Trillium reliquum* flower is variable, ranging from a deep reddish-purple (most common) to a clear yellow-green form (rare). At approximately the time of pollen dehiscal the flowers produce a musty odor like the smell of sweaty gym socks. Flower scent was not easily detectable more than 50cm from a flower, however. Fruits mature from the time of pollination until late June when the fruits drop off from the whorl of leaves and release seeds. Seeds are a yellow or copper-yellow color. A variety of sizes and species of ants (Hymenoptera) were found in and around fruits feeding on the elaiosomes attached to the seeds. Ants are known to disperse other species of trilliums.

Although not previously documented, *Trillium reliquum* has a life cycle similar to other *Trillium* species (see Patrick 1973, Kawano et al. 1986, Jules 1998). *Trillium reliquum* individuals go through four distinct morphological stages in their lifetime. An individual spends its first season as a seedling with a single cotyledon, and emerges the next season as a juvenile with one true leaf. Once the rhizome accumulates enough photosynthate, the individual transitions into a three-leaf non-reproductive stage followed by a three-leaf reproductive (flowering) stage. The leaves are mottled with three distinct shades of green, and a silvery stripe down the leaf mid-vein. Reproductive plants produce a sessile flower with three sepals, three petals, six anthers, and three fused carpels. This species is most easily identified by its distinctive beaked anthers (Appendix 2, Freeman 1975, Patrick et al. 1995). In other trillium species there can be several years between transitions (Case and Case 1997); the mean number of years between transitions for this species is not known. Individuals that experience physical damage or other stressful conditions may also back-transition to an earlier stage. In addition to the four above-ground stages, *T. reliquum* may also remain in a dormant stage with no above-ground shoots during the growing season, similar to *T. grandiflorum* (Hanzawa and Kalisz 1993, Knight 2004).

Trillium reliquum **Breeding System**

Fruit and seed were produced by flowers in all four treatments. Five of the autogamous treatment flowers did set fruit, and four of the apomictic treatment flowers produced fruits. On average, only 37% of flowering plants produced fruit (Figure A.1) and fruit set did not differ among treatments (Table A.1). This suggests that *T. reliquum* produces fruit and seed equally well using either outcross or (potential) self pollen and via apomixis. These findings agree with other field-based studies of pollen compatibility in other trilliums (Nesom and Duke 1985, Irwin 2000, Sage et al. 2001). More research is needed to determine if the breeding system patterns exhibited by *T. reliquum* at MBNA are representative of the species as a whole.

The mean number of seeds per fruit for all pollination treatments was 27.7 ± 1.46 (range of 7-70). Number of seeds per fruit and mean seed mass did not differ among all pollination treatments (Table A.2, Figure A.2). Fruits from plants receiving either of the bagged treatments were 16-35% larger than fruits that received open or supplemental pollination treatments (Table A.2, Figure A.2). The number of seeds was positively correlated with fruit diameter ($r=0.74$, $P<0.0001$).

Compared to published studies of fruiting success in other trillium species, seed production of *Trillium reliquum* seemed below average. Irwin (2000) reported fruiting success rates in *T. erectum* and *T. grandiflorum* nearly double (85-90% for cross pollinated, 62-47% for open pollinated respectively) the rates found in this study. Although fruiting success was low, the mean number of seeds produced per plant was comparable to mean seed values for three other trillium species reported by Kawano et al. (1986). In 2004, the number of seeds per fruit was consistent with 2003 data on the number of seeds per fruit for this species (see Chapter 3).

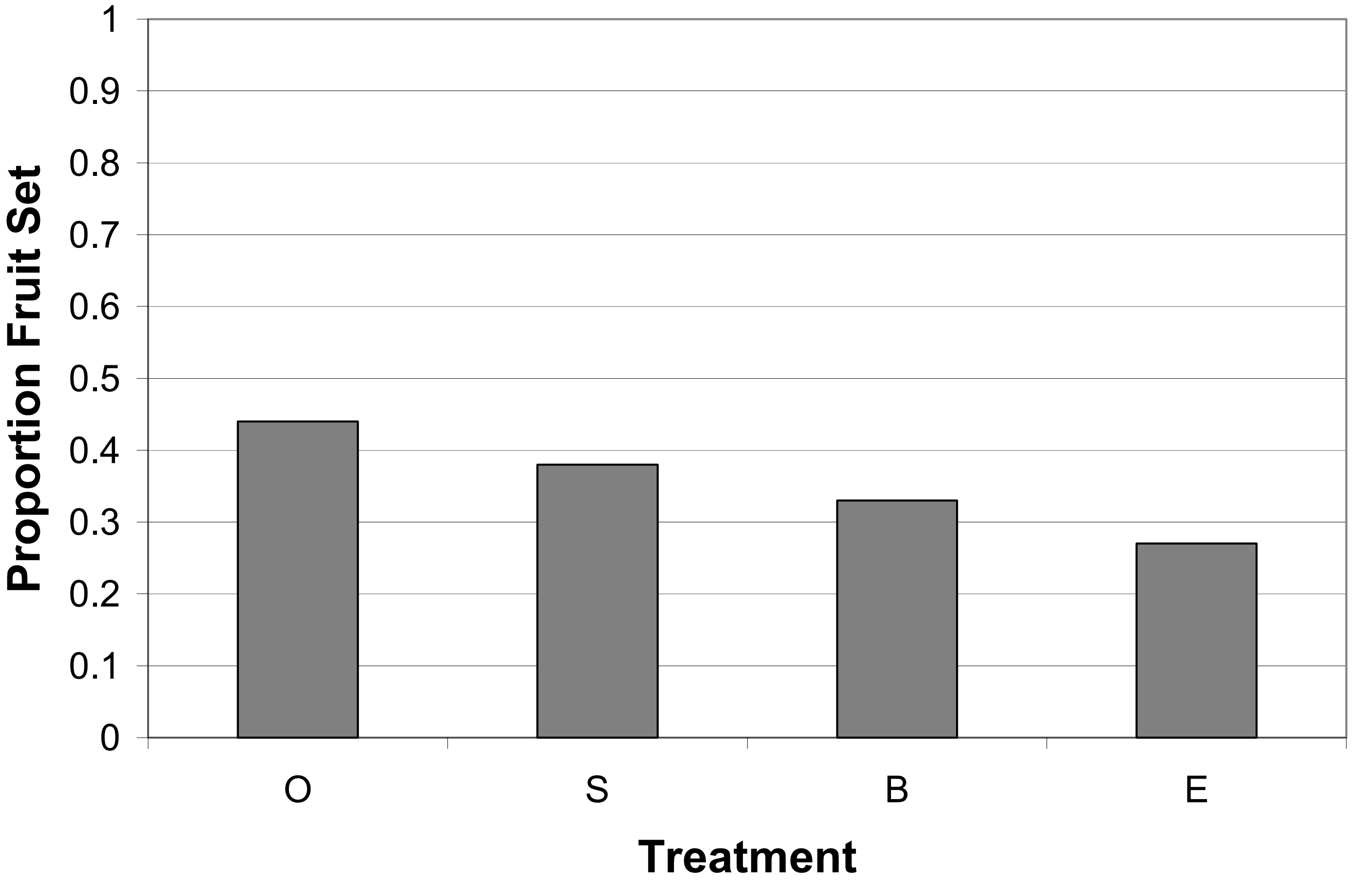
In summary, *Trillium reliquum* had proportionally lower fruit set but similar seed production/fruit in comparison with reproductive output values found in the literature for other trillium species. Future research should examine the breeding system of other *T. relquum* populations to determine if the populations in this study are representative of other *T. reliquum* populations. If the reproductive output from 2004 is the norm for this species and recruitment rates are equally low, this may help to explain why *T. reliquum* is restricted to disjunct populations located across its once more widespread range.

Table A.1. Comparisons of fruiting success among pollination treatments using Chi-square tests.

Comparison	**$\chi 2$**	**df**	**P**
Open v. Supplemental	1.826	1	0.177
Supplemental v. Autogamous	1.971	1	0.16
Autogamous v. Apomixis	2.104	1	0.147

Table A.2. One-way ANOVA showing effects of pollination treatment on seed number, fruit diameter, and mean seed mass.

Variable	**df**	**MS**	**F**	**P**
# Seeds per Fruit	3, 84	0.513	2.44	0.07
Fruit Diameter	3, 81	18.49	4.28	0.007
Mean Seed Mass/ 10 Seeds	3, 84	0.003	1.53	0.214



.

Figure A.1. The proportion of flowers that produced fruits after different pollination treatments. Sample sized for pollination treatments were O = 165, S = 161, B = 15, and E = 15. O = open pollinated, S = supplemental pollen, B = bagged and unmanipulated (autogamy test), E = emasculated and bagged (apomixis test)

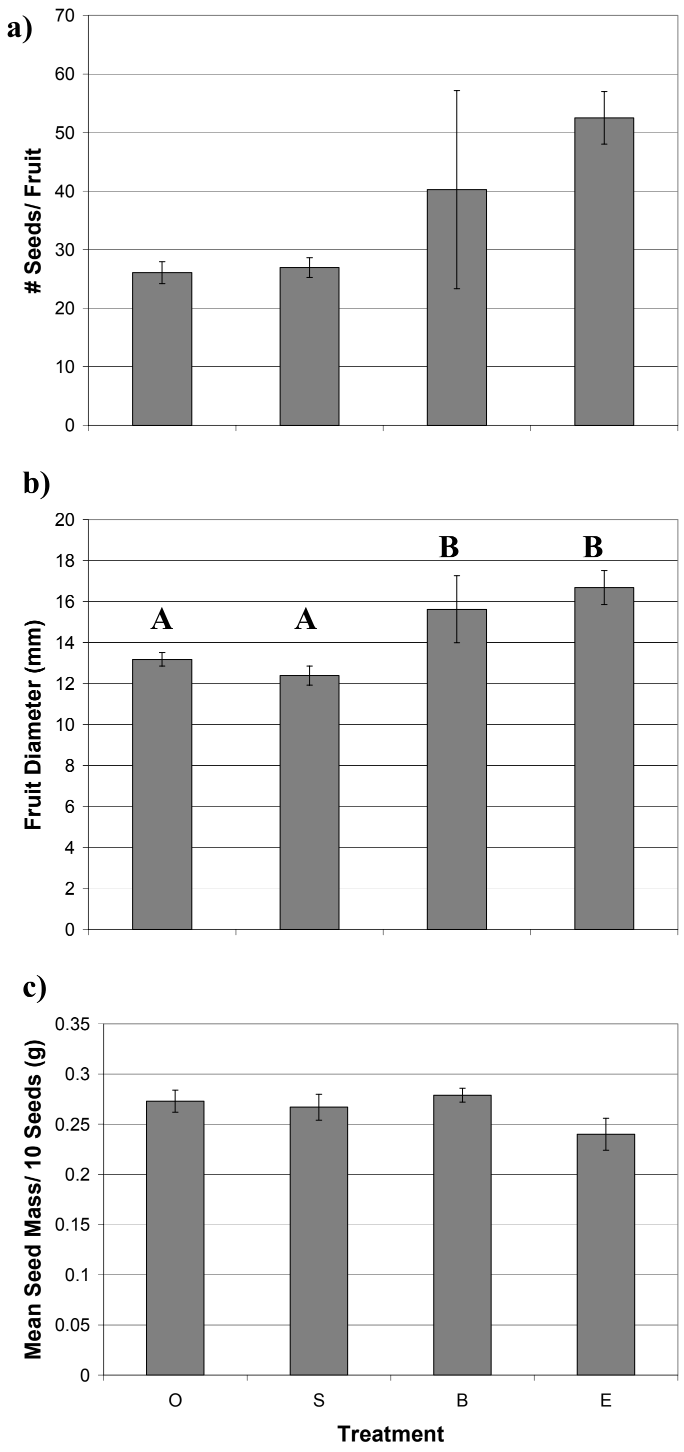
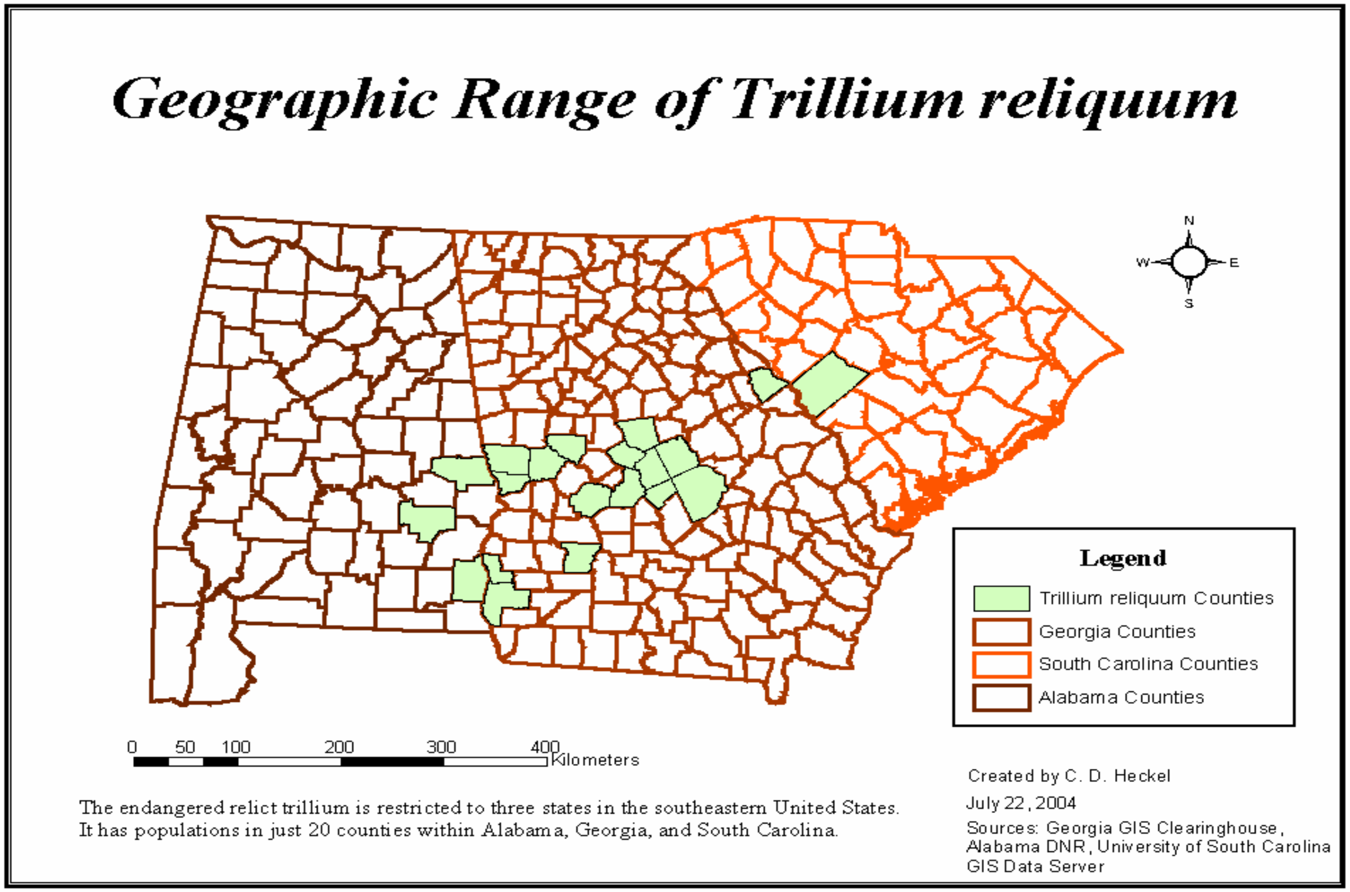


Figure A.2. Comparison of fruit and seed characteristics among pollination treatments. 3a) mean # seeds/fruit ±SE, 3b) mean fruit diameter (mm) ±SE, and 3c) mean seed mass (g) per 10 seeds ±SE. O = open pollinated, S = supplemental pollen, B = bagged and unmanipulated (autogamy test), E = emasculated and bagged (apomixis test). Capital letters above bars denote significant differences between bars with different letters.

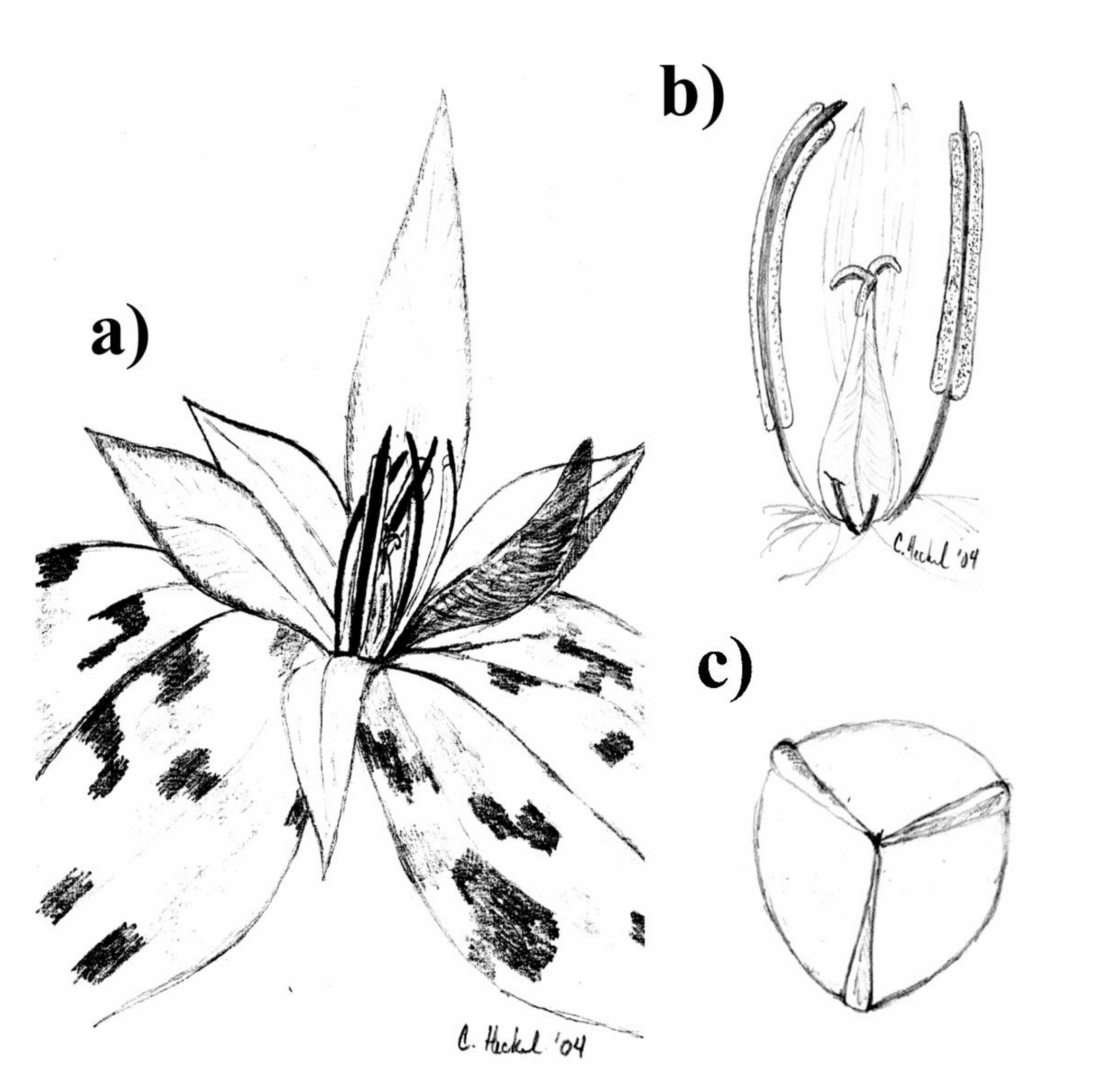


Geographic Range of *Trillium reliquum*

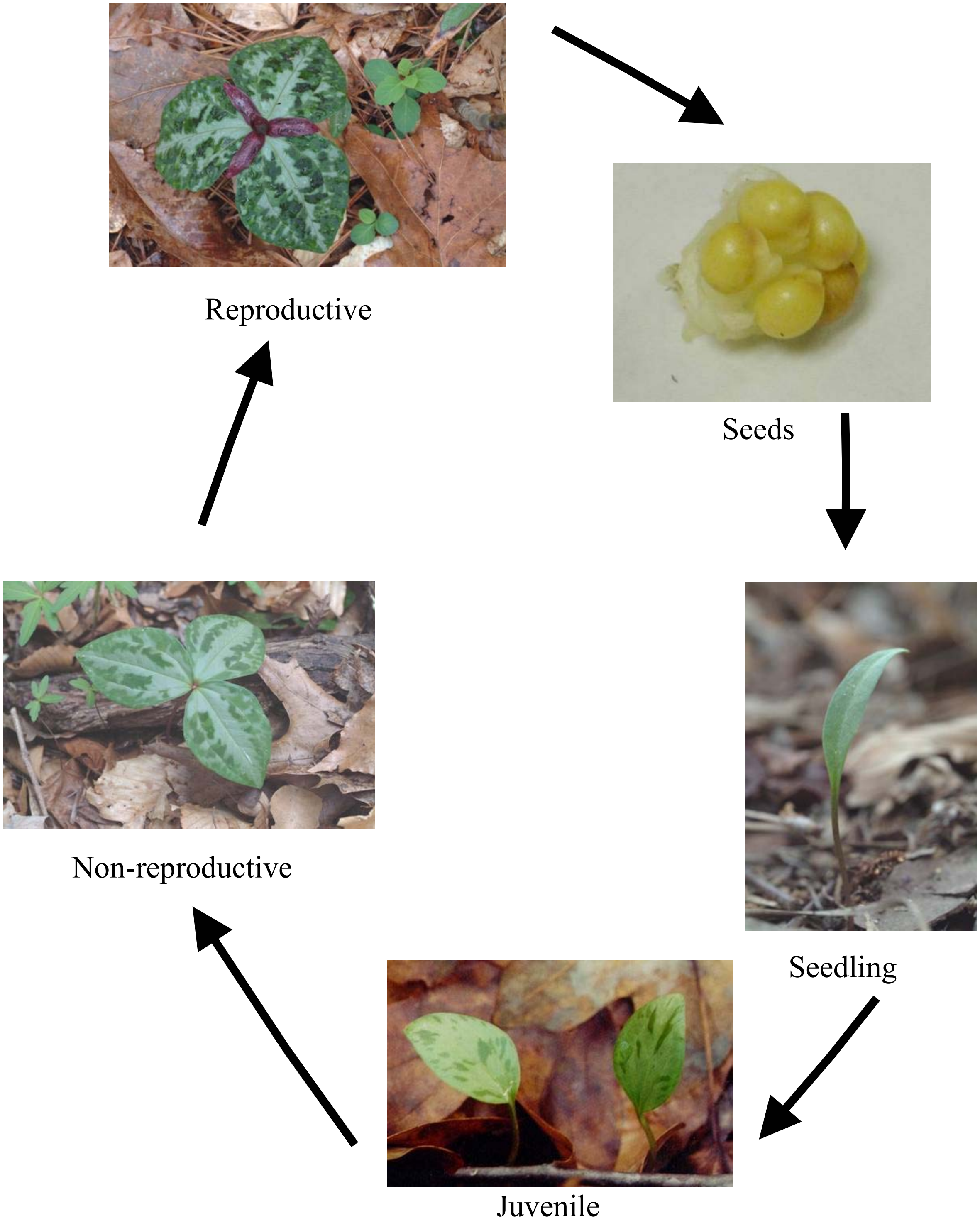
The endangered relict trillium is restricted to three states in the southeastern United States. It has populations in just 20 counties within Alabama, Georgia, and South Carolina.

Created by C. D. Heckel
July 22, 2004
Sources: Georgia GIS Clearinghouse, Alabama DNR, University of South Carolina GIS Data Server

Appendix 2. The geographic range of *Trillium reliquum* in North America. There are only 21 known populations of *T. reliquum* ranging through Alabama, Georgia, and South Carolina.



Appendix 3. ***Trillium reliquum* flower and fruit.** a) *Trillium reliquum* flower, b) reproductive parts and c) mature fruit. This species can be distinguished by the beaks located at the tips of the large anthers surrounding the stigma.



Appendix 4. The life cycle of *Trillium reliquum*.

Appendix 5. Research completed at Montezuma Bluff Natural Area. Data collection began in February 2003 and was completed in June 2004. The table illustrates the timing of specific research objectives and their occurrence relative to phenological phenomena of the forest canopy and *Trillium reliquum.*

Date	January	February	March	April	May	June	July	Aug - Dec
Trillium Phenology	Winter dormancy	Above ground parts emerge	Emergence Flowers open/ pollination	Last days of pollination/ fruit initiation and growth	Fruit growth and maturation	Fruit dehiscence	Above ground parts die back and plant overwinters as underground rhizome	
Forest Canopy Phenology	Winter dormancy		Mid-march canopy leaf out and closure	Closed canopy				Late fall leaves drop/ begin winter dormancy
Year 1 Work Schedule	Scout research area	Set-up sites Begin trillium census	Complete census Begin community measurements Apply herbicide in removal habitat	Complete community measurements Assess seasonal longevity	Monitor fruit development	Collect fruits and count seeds per fruit	Data analysis	Assess herbicide efficiency (Nov)
Year 2 Work Schedule	Mass yr 1 seeds and eliaosomes		Trillium census Ceptometer readings Collect voucher specimens	Community measurements Ceptometer readings Forest basal area Collect soil samples	Ceptometer readings Mid-story sampling Fruit initiation assessment	Collect and measure fruits and seeds		

Appendix 6. GPS Coordinates of Demography Plots

Habitat	Site	Plot	Longitude	Latitude
Honeysuckle	H1	1	N 32.33513	W084.02728
Honeysuckle	H1	2	N 32.33506	W084.02743
Honeysuckle	H1	3	N 32.33500	W084.02749
Honeysuckle	H1	4	N 32.33506	W084.02743
Honeysuckle	H1	5	N 32.33496	W084.02744
Honeysuckle	H1	6	N 32.33488	W084.02752
Honeysuckle	H1	7	N 32.33499	W084.02744
Honeysuckle	H1	8	N 32.33509	W084.02748
Honeysuckle	H1	9	N 32.33510	W084.02747
Honeysuckle	H1	10	N 32.33511	W084.02749
Honeysuckle	H1	11	N 32.33576	W084.02800
Honeysuckle	H1	12	N 32.33489	W084.02759
Honeysuckle	H1	13	N 32.33484	W084.02756
Honeysuckle	H1	14	N 32.33498	W084.02758
Honeysuckle	H1	15	N 32.33497	W084.02762
Honeysuckle	H1	16	N 32.33498	W084.02758
Honeysuckle	H1	17	N 32.33502	W084.02762
Honeysuckle	H1	18	N 32.33501	W084.02768
Honeysuckle	H1	19	N 32.33510	W084.02761
Honeysuckle	H1	20	N 32.33507	W084.02764
Honeysuckle	H2	1	N 32.33488	W084.02741
Honeysuckle	H2	2	N 32.33485	W084.02746
Honeysuckle	H2	3	N 32.33483	W084.02752
Honeysuckle	H2	4	N 32.33486	W084.02750
Honeysuckle	H2	5	N 32.33491	W084.02739
Honeysuckle	H2	6	N 32.33486	W084.02747
Honeysuckle	H2	7	N 32.33488	W084.02749
Honeysuckle	H2	8	N 32.33496	W084.02754
Honeysuckle	H2	9	N 32.33491	W084.02731
Honeysuckle	H2	10	N 32.33501	W084.02751
Honeysuckle	H2	11	N 32.33498	W084.02750
Honeysuckle	H2	12	N 32.33497	W084.02749
Honeysuckle	H2	13	N 32.33498	W084.02751
Honeysuckle	H2	14	N 32.33476	W084.02753
Honeysuckle	H2	15	N 32.33480	W084.02764
Honeysuckle	H2	16	N 32.33482	W084.02766
Honeysuckle	H2	17	N 32.33486	W084.02762
Honeysuckle	H2	18	N 32.33487	W084.02761
Honeysuckle	H2	19	N 32.33495	W084.02755
Honeysuckle	H2	20	N 32.33490	W084.02764
Honeysuckle	H3	1	N 32.33468	W084.02725
Honeysuckle	H3	2	N 32.33471	W084.02730
Honeysuckle	H3	3	N 32.33481	W084.02748
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
Honeysuckle	H3	4	N 32.33471	W084.02732

Honeysuckle	H3	5	N 32.33486	W084.02747
Honeysuckle	H3	6	N 32.33472	W084.02755
Honeysuckle	H3	7	N 32.33462	W084.02750
Honeysuckle	H3	8	N 32.33479	W084.02755
Honeysuckle	H3	9	N 32.33475	W084.02757
Honeysuckle	H3	10	N 32.33475	W084.02758
Honeysuckle	H3	11	N 32.33474	W084.02760
Honeysuckle	H3	12	N 32.33477	W084.02762
Honeysuckle	H3	13	N 32.33482	W084.02759
Honeysuckle	H3	14	N 32.33479	W084.02757
Honeysuckle	H3	15	N 32.33440	W084.02709
Honeysuckle	H3	16	N 32.33479	W084.02761
Honeysuckle	H3	17	N 32.33479	W084.02755
Honeysuckle	H3	18	N 32.33476	W084.02755
Honeysuckle	H3	19	N 32.33477	W084.02760
Honeysuckle	H3	20	N 32.33477	W084.02762
Honeysuckle	H3	21	N 32.33487	W084.02749
Honeysuckle	H4	1	N 32.33498	W084.02755
Honeysuckle	H4	2	N 32.33508	W084.02766
Honeysuckle	H4	3	N 32.33511	W084.02765
Honeysuckle	H4	4	N 32.33505	W084.02777
Honeysuckle	H4	5	N 32.33513	W084.02771
Honeysuckle	H4	6	N 32.33502	W084.02776
Honeysuckle	H4	7	N 32.33501	W084.02772
Honeysuckle	H4	8	N 32.33503	W084.02778
Honeysuckle	H4	9	N 32.33504	W084.02775
Honeysuckle	H4	10	N 32.33503	W084.02776
Honeysuckle	H4	11	N 32.33501	W084.02772
Honeysuckle	H4	12	N 32.33493	W084.02764
Honeysuckle	H4	13	N 32.33499	W084.02772
Honeysuckle	H4	14	N 32.33496	W084.02761
Honeysuckle	H4	15	N 32.33502	W084.02765
Honeysuckle	H4	16	N 32.33496	W084.02770
Honeysuckle	H4	17	N 32.33503	W084.02814
Honeysuckle	H4	18	N 32.33495	W084.02777
Honeysuckle	H4	19	N 32.33502	W084.02785
Honeysuckle	H4	20	N 32.33498	W084.02766
Honeysuckle	H4	21	N 32.33502	W084.02765
Kudzu	K2	1	N 32.33948	W084.02894
Kudzu	K2	2	N 32.33950	W084.02895
Kudzu	K2	3	N 32.33952	W084.02894
Kudzu	K2	4	N 32.33952	W084.02895
Kudzu	K2	5	N 32.33951	W084.02896
Kudzu	K2	6	N 32.33946	W084.02895
Kudzu	K2	7	N 32.33965	W084.02893
Kudzu	K2	8	N 32.33962	W084.02892
Kudzu	K2	9	N 32.33976	W084.02891
Kudzu	K2	10	N 32.33974	W084.02894
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
Kudzu	K2	11	N 32.33967	W084.02895

Kudzu	K2	12	N 32.33968	W084.02898
Kudzu	K2	13	N 32.33968	W084.02899
Kudzu	K2	14	N 32.33969	W084.02898
Kudzu	K2	15	N 32.33968	W084.02900
Kudzu	K2	16	N 32.33965	W084.02898
Kudzu	K2	17	N 32.33971	W084.02904
Kudzu	K2	18	N 32.33970	W084.02906
Kudzu	K2	19	N 32.33972	W084.02906
Kudzu	K2	20	N 32.33973	W084.02908
Kudzu	K2	21	N 32.33966	W084.02904
Kudzu	K3	1	N 32.33968	W084.02931
Kudzu	K3	2	N 32.33968	W084.02927
Kudzu	K3	3	N 32.33966	W084.02922
Kudzu	K3	4	N 32.33966	W084.02925
Kudzu	K3	5	N 32.33966	W084.02925
Kudzu	K3	6	N 32.33964	W084.02926
Kudzu	K3	7	N 32.33961	W084.02927
Kudzu	K3	8	N 32.33960	W084.02930
Kudzu	K3	9	N 32.33962	W084.02929
Kudzu	K3	10	N 32.33959	W084.02927
Kudzu	K3	11	N 32.33966	W084.02931
Kudzu	K3	12	N 32.33969	W084.02938
Kudzu	K3	13	N 32.33969	W084.02938
Kudzu	K3	14	N 32.33965	W084.02942
Kudzu	K3	15	N 32.33959	W084.02945
Kudzu	K3	16	N 32.33962	W084.02954
Kudzu	K3	17	N 32.33959	W084.02954
Kudzu	K3	18	N 32.33958	W084.02949
Kudzu	K3	19	N 32.33958	W084.02948
Kudzu	K3	20	N 32.33959	W084.02947
Kudzu	K4	1	N 32.33958	W084.02918
Kudzu	K4	2	N 32.33953	W084.02921
Kudzu	K4	3	N 32.33953	W084.02926
Kudzu	K4	4	N 32.33952	W084.02926
Kudzu	K4	5	N 32.33956	W084.02930
Kudzu	K4	6	N 32.33958	W084.02930
Kudzu	K4	7	N 32.33954	W084.02931
Kudzu	K4	8	N 32.33954	W084.02932
Kudzu	K4	9	N 32.33953	W084.02937
Kudzu	K4	10	N 32.33951	W084.02937
Kudzu	K4	11	N 32.33938	W084.02928
Kudzu	K4	12	N 32.33940	W084.02929
Kudzu	K4	13	N 32.33943	W084.02928
Kudzu	K4	14	N 32.33946	W084.02927
Kudzu	K4	15	N 32.33946	W084.02932
Kudzu	K4	16	N 32.33957	W084.02926
Kudzu	K4	17	N 32.33951	W084.02934
Kudzu	K4	18	N 32.33954	W084.02932
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
Kudzu	K4	19	N 32.33954	W084.02930

Kudzu	K4	20	N 32.33958	W084.02933
No-Vine	N1	1	N 32.33960	W084.02844
No-Vine	N1	2	N 32.33960	W084.02843
No-Vine	N1	3	N 32.33955	W084.02844
No-Vine	N1	4	N 32.33952	W084.02844
No-Vine	N1	5	N 32.33947	W084.02844
No-Vine	N1	6	N 32.33947	W084.02844
No-Vine	N1	7	N 32.33950	W084.02847
No-Vine	N1	8	N 32.33950	W084.02850
No-Vine	N1	9	N 32.33947	W084.02853
No-Vine	N1	10	N 32.33947	W084.02852
No-Vine	N1	11	N 32.33950	W084.02852
No-Vine	N1	12	N 32.33948	W084.02854
No-Vine	N1	13	N 32.33947	W084.02856
No-Vine	N1	14	N 32.33948	W084.02858
No-Vine	N1	15	N 32.33950	W084.02860
No-Vine	N1	16	N 32.33950	W084.02859
No-Vine	N1	17	N 32.33945	W084.02860
No-Vine	N1	18	N 32.33944	W084.02863
No-Vine	N1	19	N 32.33940	W084.02865
No-Vine	N1	20	N 32.33945	W084.02861
No-Vine	N2	1	N 32.33956	W084.02834
No-Vine	N2	2	N 32.33951	W084.02831
No-Vine	N2	3	N 32.33958	W084.02837
No-Vine	N2	4	N 32.33959	W084.02839
No-Vine	N2	5	N 32.33958	W084.02841
No-Vine	N2	6	N 32.33962	W084.02838
No-Vine	N2	7	N 32.33970	W084.02839
No-Vine	N2	8	N 32.33959	W084.02839
No-Vine	N2	9	N 32.33970	W084.02834
No-Vine	N2	10	N 32.33972	W084.02834
No-Vine	N2	11	N 32.33974	W084.02824
No-Vine	N2	12	N 32.33975	W084.02824
No-Vine	N2	13	N 32.33975	W084.02821
No-Vine	N2	14	N 32.33970	W084.02825
No-Vine	N2	15	N 32.33974	W084.02841
No-Vine	N2	16	N 32.33973	W084.02839
No-Vine	N2	17	N 32.33972	W084.02850
No-Vine	N2	18	N 32.33968	W084.02852
No-Vine	N2	19	N 32.33965	W084.02851
No-Vine	N2	20	N 32.33962	W084.02844
No-Vine	N3	1	N 32.33940	W084.02810
No-Vine	N3	2	N 32.33940	W084.02811
No-Vine	N3	3	N 32.33935	W084.02827
No-Vine	N3	4	N 32.33939	W084.02813
No-Vine	N3	5	N 32.33944	W084.02819
No-Vine	N3	6	N 32.33939	W084.02816
No-Vine	N3	7	N 32.33937	W084.02824
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
No-Vine	N3	8	N 32.33943	W084.02815

No-Vine	N3	9	N 32.33943	W084.02810
No-Vine	N3	10	N 32.33936	W084.02808
No-Vine	N3	11	N 32.33938	W084.02808
No-Vine	N3	12	N 32.33939	W084.02795
No-Vine	N3	13	N 32.33935	W084.02802
No-Vine	N3	14	N 32.33940	W084.02812
No-Vine	N3	15	N 32.33929	W084.02816
No-Vine	N3	16	N 32.33943	W084.02809
No-Vine	N3	17	N 32.33933	W084.02815
No-Vine	N3	18	N 32.33946	W084.02814
No-Vine	N3	19	N 32.33950	W084.02807
No-Vine	N3	20	N 32.33956	W084.02813
No-Vine	N4	1	N 32.33961	W084.02791
No-Vine	N4	2	N 32.33960	W084.02790
No-Vine	N4	3	N 32.33960	W084.02788
No-Vine	N4	4	N 32.33961	W084.02784
No-Vine	N4	5	N 32.33965	W084.02791
No-Vine	N4	6	N 32.33966	W084.02792
No-Vine	N4	7	N 32.33966	W084.02793
No-Vine	N4	8	N 32.33965	W084.02792
No-Vine	N4	9	N 32.33979	W084.02778
No-Vine	N4	10	N 32.33961	W084.02786
No-Vine	N4	11	N 32.33959	W084.02788
No-Vine	N4	12	N 32.33957	W084.02791
No-Vine	N4	13	N 32.33956	W084.02794
No-Vine	N4	14	N 32.33954	W084.02794
No-Vine	N4	15	N 32.33953	W084.02793
No-Vine	N4	16	N 32.33948	W084.02785
No-Vine	N4	17	N 32.33946	W084.02774
No-Vine	N4	18	N 32.33950	W084.02770
No-Vine	N4	19	N 32.33942	W084.02768
No-Vine	N4	20	N 32.33948	W084.02771
Removal	R1	1	N 32.32816	W084.02844
Removal	R1	2	N 32.32811	W084.02844
Removal	R1	3	N 32.32824	W084.02851
Removal	R1	4	N 32.32816	W084.02843
Removal	R1	5	N 32.32813	W084.02841
Removal	R1	6	N 32.32812	W084.02841
Removal	R1	7	N 32.32805	W084.02835
Removal	R1	8	N 32.32808	W084.02834
Removal	R1	9	N 32.32805	W084.02830
Removal	R1	10	N 32.32805	W084.02833
Removal	R1	11	N 32.32805	W084.02841
Removal	R1	12	N 32.32797	W084.02841
Removal	R1	13	N 32.32803	W084.02841
Removal	R1	14	N 32.32804	W084.02846
Removal	R1	15	N 32.32805	W084.02847
Removal	R1	16	N 32.32804	W084.02844
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
Removal	R1	17	N 32.32802	W084.02847

Removal	R1	18	N 32.32801	W084.02849
Removal	R1	19	N 32.32801	W084.02850
Removal	R1	20	N 32.32802	W084.02850
Removal	R1	21	N 32.32804	W084.02845
Removal	R2	1	N 32.32807	W084.02819
Removal	R2	2	N 32.32804	W084.02830
Removal	R2	3	N 32.32807	W084.02830
Removal	R2	4	N 32.32810	W084.02827
Removal	R2	5	N 32.32808	W084.02827
Removal	R2	6	N 32.32821	W084.02834
Removal	R2	7	N 32.32813	W084.02829
Removal	R2	8	N 32.32812	W084.02837
Removal	R2	9	N 32.32807	W084.02837
Removal	R2	10	N 32.32806	W084.02824
Removal	R2	11	N 32.32807	W084.02824
Removal	R2	12	N 32.32824	W084.02823
Removal	R2	13	N 32.32817	W084.02824
Removal	R2	14	N 32.32792	W084.02823
Removal	R2	15	N 32.32783	W084.02827
Removal	R2	16	N 32.32776	W084.02835
Removal	R2	17	N 32.32789	W084.02831
Removal	R2	18	N 32.32799	W084.02823
Removal	R2	19	N 32.32800	W084.02824
Removal	R2	20	N 32.32801	W084.02827
Removal	R3	1	N 32.32820	W084.02841
Removal	R3	2	N 32.32822	W084.02838
Removal	R3	3	N 32.32818	W084.02835
Removal	R3	4	N 32.32821	W084.02851
Removal	R3	5	N 32.32818	W084.02852
Removal	R3	6	N 32.32814	W084.02856
Removal	R3	7	N 32.32824	W084.02845
Removal	R3	8	N 32.32835	W084.02834
Removal	R3	9	N 32.32832	W084.02836
Removal	R3	10	N 32.32822	W084.02843
Removal	R3	11	N 32.32820	W084.02838
Removal	R3	12	N 32.32818	W084.02841
Removal	R3	13	N 32.32817	W084.02842
Removal	R3	14	N 32.32822	W084.02838
Removal	R3	15	N 32.32818	W084.02841
Removal	R3	16	N 32.32817	W084.02850
Removal	R3	17	N 32.32819	W084.02849
Removal	R3	18	N 32.32813	W084.02851
Removal	R3	19	N 32.32818	W084.02844
Removal	R3	20	N 32.32816	W084.02846
Removal	R4	1	N 32.32826	W084.02828
Removal	R4	2	N 32.32827	W084.02827
Removal	R4	3	N 32.32827	W084.02825
Removal	R4	4	N 32.32826	W084.02834
Habitat	**Site**	**Plot**	**Longitude**	**Latitude**
Removal	R4	5	N 32.32831	W084.02837

Removal	R4	6	N 32.32794	W084.02819
Removal	R4	7	N 32.32799	W084.02819
Removal	R4	8	N 32.32809	W084.02806
Removal	R4	9	N 32.32811	W084.02812
Removal	R4	10	N 32.32818	W084.02829
Removal	R4	11	N 32.32821	W084.02828
Removal	R4	12	N 32.32819	W084.02830
Removal	R4	13	N 32.32805	W084.02847
Removal	R4	14	N 32.32804	W084.02847
Removal	R4	15	N 32.32800	W084.02842
Removal	R4	16	N 32.32813	W084.02841
Removal	R4	17	N 32.32812	W084.02841
Removal	R4	18	N 32.32812	W084.02837
Removal	R4	19	N 32.32814	W084.02837
Removal	R4	20	N 32.32812	W084.02836

Appendix 7. Transition Matrices of All Habitats

Honeysuckle

	C	**J**	**S**	**R**
C	0.0000	0.0000	0.0000	2.3333
J	0.9999	0.4474	0.0896	0.0000
S	0.0000	0.5000	0.7761	0.6667
R	0.0000	0.0000	0.0448	0.3333

$\lambda =$ 1.02466

Kudzu

	C	**J**	**S**	**R**
C	0.0000	0.0000	0.0000	0.0385
J	0.0833	0.3889	0.0119	0.0000
S	0.0000	0.3333	0.6726	0.2115
R	0.0000	0.0000	0.1310	0.6539

$\lambda =$ 0.83583

No-Vine

	C	**J**	**S**	**R**
C	0.0000	0.0000	0.0000	2.0000
J	0.4857	0.4167	0.0340	0.0000
S	0.0000	0.5667	0.8301	0.0000
R	0.0000	0.0000	0.0874	0.9999

$\lambda =$ 1.17184

Removal

	C	**J**	**S**	**R**
C	0.0000	0.0000	0.0000	1.0000
J	0.0001	0.0409	0.0426	0.0000
S	0.0000	0.0409	0.6454	0.2609
R	0.0000	0.0000	0.1206	0.5870

$\lambda =$ 0.79731