Weeks, Sonia Cooper. Factors limiting growth in *Astragalus michauxii* (Sandhills milk-vetch) (under the direction of Theodore H. Shear).

The seed germination-inducing mechanism of *Astragalus michauxii* and effects of heat and smoke on germination were explored. Seed germination is limited by a hard outer coat, a tough inner coat and possible germination-inhibitory substances in the embryo. Ninety-five percent seed germination was accomplished artificially by scarification in sulfuric acid, followed by piercing of the inner coat, and then leaching. Heat treatment between 80°C and 86°C resulted in 20% imbibition ($p<.0001$), 2% germination ($p<.0031$). All ungerminated, imbibed seeds were killed. Smoke did not promote germination. Effects of height, age, stem number and inter-specific competition on over-winter survival were studied A younger and an older group of plants were raised under controlled conditions and distributed among two 20x10-meter plots at Fort Bragg Military Reservation, NC, in the summer of 2002. Diameter and distances of each *A. michauxii* plant from the nearest oak sapling, pine tree, pine seedling, and wiregrass cluster were measured and a competition index (CI) calculated as $\sum$ [diameter of each competitor / (distance)^2]. Fifty-nine percent of plants survived over-winter to the following spring. Logistic regression predicted height ($p<.0001$) and stem number ($p=.0004$) to increase over-winter survival. Effects of fire history and competition on vegetative and reproductive efforts and population persistence in *A. michauxii* were investigated among 48 sub-populations. Surveys of ground and canopy covers, growth and reproductive effort, along with fire history and population persistence data indicated that sub-populations with lower percentages of understory species covers other than pine, oak, and wiregrass were more likely to persist. Low regression coefficients for effects of competition and fire history indicate that a number of factors work together to impact

growth and reproduction. A population demographic model determined the population to be declining. Management conservation efforts can best be directed by 1) collection and artificial seed germination and transplanting of seedlings into existing sub- populations, and 2) experimental testing of the impacts of varying fire regimes on habitat quality, plant growth and reproduction, and population growth and persistence.

FACTORS LIMITING GROWTH IN *Astragalus michauxii* (SANDHILLS MILK-VETCH)

By

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In Memory of the late John Washington Cooper,

my daddy, my inspiration for this work, who passed on to me his love of nature and, by example, taught me to work and achieve the best …

In Memory of the late Ann Penella Crayton Pearson,

my granny, my source of unconditional love and support during some rough times...

In Memory of the late Sophie Dunbar,

my second mother, who impressed upon me her zeal for life...

To Joan W. Pearson,

my birth mother, the gentlest person I know, who never has a harsh word for anyone, an example to follow…

To Donna Jean Cooper Hayford

my stepmother, a friend to me, who raised me to work hard…

To Euphemia Reeves

my Godmother, who introduced me to the love of God

BIOGRAPHY

Sonia Audrey Cooper-Weeks was born on April 13th, 1957, in Bomi Hills, Liberia, West Africa, to John Washington Cooper and Joan Wesleyan Pearson. Her father held the position of Secretary of Agriculture of Liberia. His pride and joy was his five hundred acre farm in the tropical rainforest and he passed his love of nature on to his daughter. Although some of the land was planted in rubber, oil palm, rice, plantains, bananas, and various vegetables and citrus fruits, much of it was left untouched. Sonia's fondest childhood memories are of spending countless hours on weekends and holidays exploring in the forest with her brothers and sisters. Her father passed away suddenly when Sonia was thirteen, leaving her and the family devastated. Many years went by before she was able to recover from the loss.

Sonia was educated in Liberia until she left the country at the end of the 11th grade to attend the College du Leman, in Versoix, Switzerland. This was an opportunity to improve her knowledge of French, a foreign language taught throughout high school in Liberia. She returned to Liberia after graduating in May, 1974 and studied as an apprentice to a veterinarian; at the time her goal was to become a veterinarian. In July, 1975, she began her college education at the University of Liberia, but then transferred to the College of New Rochelle in New Rochelle, New York in January, 1976. During her time in New York, she spent weekends and holidays in Manhattan with her brother Jon and in Baltimore with her God mother Euphemia. Through the evangelical ministry of Euphemia, Sonia developed a personal relationship with God and made the decision to live her life for Christ.

Sonia earned her Bachelors in 1979, graduating with honors in chemistry and returning home. A military coup d'etat in April 1980 disrupted life in Liberia and marked

the beginning of years of political unrest and civil war. Sonia married her childhood friend James Weeks in July 1980 and accompanied him to Minneapolis where he had had been awarded a Fulbright Grant to study Physics at the University of Minnesota. Their first child, Israel, was born in 1981 and their second, Jared, in 1982. During their three year stay in Minneapolis, Sonia stayed at home and cared for their two children. Jimmie completed his Masters in 1983 and the family returned to Liberia where he taught Physics at the University of Liberian and at the College of West Africa. Sonia had their third son in Monrovia in 1984. She remained a stay-at-home mother to care for their three boys. The political climate in the country became progressively worse. In 1985, the family immigrated to North Carolina where Sonia's sister and her husband resided. In 1986, both Sonia and her husband were admitted into graduate programs at NCSU: Sonia in Organic Chemistry and Jimmie in Physics. In 1989, the birth of their fourth child, Arielle, delayed the completion of Sonia's program. She eventually completed her Masters in organic chemistry with a minor in toxicology in 1991.

Sonia began her professional career in 1991 as a laboratory chemist at Entropy, an air emissions company in Raleigh. Three years later, the company closed its laboratory. It was then that Sonia began her work as an educator, teaching chemistry at the North Carolina School of Science and Mathematics, a two-year residential state school for gifted students located in Durham. After three years, she began teaching physical science, and general and organic chemistry at Shaw University. It was during her time at Shaw that Sonia decided to return to school for a terminal degree. She remained at Shaw until she enrolled as a full-time doctoral student in the forestry program at NCSU in the fall of 2000.

For the past year, Sonia has taught environmental science at the School of Communication Arts in Raleigh. She particularly enjoys instructing in the NC Museum of Natural Science's outreach programs where she gets to be an educator and an ecologist, while learning about many wildlife species and their biology and behavior. She also enjoys miniature dachshunds, nature walks, traveling, biking, fishing and reading. Sonia has a passion for nature and considers science to be the discovery of God's many little miracles.

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TABLE OF CONTENTS

LIST OF TABLES ..... **VIII**

LIST OF FIGURES ..... **IX**

CHAPTER 1 ..... **1**

SEED GERMINATION REQUIREMENTS OF ASTRAGALUS MICHAUXII AND CONSEQUENT LIMITATIONS TO POPULATION GROWTH **1**

ABSTRACT 2
INTRODUCTION 3
MATERIAL AND METHODS 6
RESULTS & DISCUSSION 13
CONCLUSIONS 16
LITERATURE CITED 19

CHAPTER 2 ..... **27**

EFFECT OF AGE, HEIGHT, STEM NUMBER, AND COMPETITION ON PLANT SURVIVAL OF *A. MICHAUXII* OVER WINTER **27**

ABSTRACT 28
INTRODUCTION 29
MATERIAL & METHODS 31
RESULTS & DISCUSSION 34
CONCLUSIONS 36
LITERATURE CITED 37

CHAPTER 3 ..... **47**

EFFECTS OF FIRE AND COMPETITION ON VEGETATIVE GROWTH, REPRODUCTIVE EFFORT, AND POPULATION PERSISTENCE IN A. MICHAUXII **47**

ABSTRACT 48
INTRODUCTION 49
MATERIAL & METHODS 54
RESULTS AND DISCUSSION 58
CONCLUSIONS 61
LITERATURE CITED 62

CHAPTER 4 ..... **71**

A DEMOGRAPHIC MODEL FOR A. MICHAUXII SUB-POPULATIONS AT FORT BRAGG **71**

INTRODUCTION 72
MATERIAL & METHODS 75
RESULTS 79
CONCLUSIONS 81
LITERATURE CITED 84

APPENDICES **88**

APPENDIX A: SUPPLEMENTAL TABLE 89
APPENDIX B: SUPPLEMENTAL FIGURES 94
APPENDIX C: PRODUCTION OF PLANTS USED IN THE SURVIVAL EXPERIMENT 96

LIST OF TABLES

Table 1-1 Statistics of imbibition and germination with and without piercing and leaching for four treatments: no heat/no smoke, heat, heat and smoke, and smoke. 21
Table 1-2 Reduced analysis of variance models of the effect of heat on imbibition and germination in *A. michauxii* seeds. 22
Table 2-1 Descriptive statistics for the survival of *A. michauxii* plants on sites 1 and 2 over winter. 38
Table 2-2 Logistic Regression of survival over winter on height of *A. michauxii* plants (X^2 = Wald Chi-Square). 38
Table 3-1 Ground and canopy cover for 48 sub-populations of *A. michauxii* on Fort Bragg. 64
Table 3-2 Vegetative and reproductive effort of *A. michauxii*. 64
Table 3-3 Correlation matrix of 11 parameters for 88 *A. michauxii* plants. 65
Table 3-4. Correlation matrix (r, probability of p>t) of 10 parameters for 49 plots within 48 *Astragalus michauxii* sub-populations surveyed. 66
Table 4.1 Probabilities of life cycle parameters of *A. michauxii* in 2003 and the value of λ when parameters are maximized. 85
Table 4-2 Sensitivities and elasticities for the life cycle of *A. michauxii* sub-populations. 85
Table A-1 Average monthly recorded readings in ° C from 1971-2000 from Fayetteville Pope Air Force Base in Cumberland County, NC. 89
Table A-2 Survey data taken in 1991-93 and 1998-99 for all *A. michauxii* sites with sub-populations recorded by Element Occurrence Number (EONUMBER), on Fort Bragg, including area, density, and burn history of each sub-population. 90

LIST OF FIGURES

Figure 1-1 Typical legume seed depicting outer and inner seed coats of *A. michauxii*..... 23
Figure 1-2 Scheme of germination procedures for seeds of *A. michauxii.*..... 24
Figure 1-3 *A. michauxii* seed depicting outer and inner seed coats. 25
Figure 1-4 Cumulative percent germination of *A. michauxii* seeds after scarification, piercing and leaching..... 26
Figure 2-1 Half-sib *A. michauxii* seedlings at 52 and 60 days, showing variation in size. 39
Figure 2.2 Mean heights of plants in younger (52-70 days) and older (108-256 days) cohorts of *A. michauxii* plants at time of planting in the field..... 40
Figure 2-3 Mature (218 days old) *A. michauxii* plants from one maternal line..... 41
Figure 2-4 *A. michauxii* plant showing a short and bushy structure. 42
Figure 2-5 Mature (217 days old) *A. michauxii* plants from the 30°/26°C treatment have a more robust size than plants in other treatments. 43
Figure 2-6 *A. michauxii* plants under short day conditions at 10ºC/5ºC showing root structure..... 44
Figure 2-7 *A. michauxii* inflorescence showing formed pods. 45
Figure 2-8 *A. michauxii* flowers after anthesis showing anthers with pollen grains surrounding the stigma. 46
Figure 3-1 *A. michauxii* in its habitat with long-leaf pine, shrub oak, and wiregrass. 67
Figure 3-2 Element occurrences of *A. michauxii* on Fort Bragg and Camp MacKall Military Reservations in North Carolina. 68
Figure 3-3 *A. michauxii* flower with banner, wings and keel..... 69
Figure 3-4 Graph of the effect of % other species cover on heights of 88 *A. michauxii* plants in 48 sub-populations at Fort Bragg. 70
Figure 3-5 Graph of the effect of % fire frequency on heights of numbers of flowers on 88 *A. michauxii* plants in 48 sub-populations at Fort Bragg. 70
Figure 3-6 Graph of the effect of % oak cover on the number of A. michauxii plants in 49 4-m2 plots in 48 sub-populations at Fort Bragg. 70
Figure B-1 Plot layout of Site 1 showing spatial relationships between *A. michauxii* and competing species. Asmi stands for *A. michauxii* 94
Figure B-2 Plot layout of Site 2 showing spatial relationships between *A. michauxii* and competing species. Asmi stands for *A. michauxii*. 95

CHAPTER 1

Seed germination requirements of *Astragalus michauxii* and consequent limitations to population growth

ABSTRACT

The germination-inducing mechanism of *Astragalus michauxii* (Kuntze) Hermann seeds and germination-imposed limitations to population growth in long-leaf/wiregrass habitats at Fort Bragg Military Reservation in North Carolina were explored. Germination of *A. michauxii* seeds is limited by the presence of a hard outer coat, a tough inner coat and possible germination inhibitory substances present in the embryo. Maximum germination of seeds was accomplished artificially by scarifying, followed by piercing, and then leaching. 95% of seeds had viable embryos. Heat treatments, between 80°C and 86°C, accounted for 93% and 60% of the variation in imbibition ($p<.0001$) and germination ($p=.0031$) respectively. However, imbibition (20%) and germination (2%) were low indicating a limitation to germination in natural sub-populations. In addition, heat killed the imbibed seeds that did not germinate. In natural systems, fire facilitates removal of the strophiolar plug in the outer coat, allowing imbibition, but may also result in seed mortality. After imbibition, seeds may need to undergo long-term exposure to chemicals in the soil to allow penetration of the inner coat and leaching of inhibitory chemicals. Smoke did not significantly increase germination. This may be because fire-related chemical conditions of the soil in natural habitats over time could not be duplicated *in vivo*. Also, the outer coat is impenetrable to smoke. The seed bank averaged 12 seeds per plant and may not be adequate for population persistence. In the absence of fire, physical barriers to germination, in combination with unsuitable environmental conditions, can potentially limit recruitment into sub-populations and subsequently limit population growth. Future research can be done by sowing seeds into burned and unburned sites to observe effects of heat and smoke on germination in natural populations.

INTRODUCTION

Astragalus michauxii (Kuntze) Hermann is an erect perennial legume occurring in open understory habitats of xeric to dry-mesic, nutrient poor, fire-dependent long-leaf pine (*Pinus palustris* Miller) / wiregrass (*Aristida stricta* Michaux) communities of the southeastern Atlantic coastal plain (Schafale and Weakley 1990). In fire-dependant communities, frequent fires maintain the open understory habitat necessary for *A. michauxii* and other species (Schafale and Weakley 1990). Fire has the physical effect of optimizing the environment for seed germination, seedling emergence, and growth.

Seeds of many species, stored in the seed bed, are adapted to germinate only in response to physical and chemical cues generated by fire (Keeley 1994; Keeley and Fotheringham 1998, Bell *et al*. 1993). Physical cues indicate that the habitat has been optimized and include cracking or desiccation of the hard coat (Jeffrey *et al*. 1988; Bell *et al*. 1993), direct heat stimulation of the embryo (Musil and DeWitt 1991), or loosening of cells in the areas of the hilum or strophiolar plug (Bell *et al*. 1993). Smoke is a chemical signal associated with fire which, when released, may serve as another means by which a plant ensures germination only when environmental conditions are most favorable. Smoke may be a key factor in a species' response to fire in fire-dependent ecosystems. It has been shown to break seed dormancy in species from many plant communities, possibly due to the chemical ethylene (Brown *et al*. 1994; Keeley and Fotheringham 1998; Dixon *et al*. 1995), but it is unknown whether smoke promotes germination or seedling establishment in legumes (Van Staden *et al*. 2000).

Poor seed germination and subsequent seedling establishment are problems common to legumes (Miklas *et al*. 1987). Most legumes, including species of *Astragalus*, are known

to have 'hardseeds' which do not readily imbibe water (Miklas *et al*. 1987; Van Staden *et al*. 1989) due to a hard outer coat (figure 1-1). Some species also have a tough inner coat and, possibly, inhibitory substances which may prevent germination after imbibition (Van Staden *et al*.). Scarification allows water to enter the seed through the hard outer coat. Splitting or removal of the inner coat, followed by leaching, allows chemicals that stimulate germination to enter or inhibitors to leave the embryo (Dixon *et al*. 1995). Seeds of *A. tennesseensis* have been successfully germinated in vitro through scarification, splitting, or removal of the inner coat and leaching (Baskin and Quarterman 1969). Scarification of the outer hard coat followed by leaching led to germination of seeds of three varieties of *A. lentiginosus* (Ziemkiewicz and Cronin 1981).

Although there is general information about the phenology, habitat, and range of *A. michauxii* (TNC 1993), there is a broad lack of knowledge pertaining to its reproductive ecology, in particular, limitations placed on population growth by seed germination. The number of stems in a population is directly linked to population persistence in a suite of rare species (Gray 2001). Since germination is the first step leading to recruitment and subsequent increases in stem count, it is important that those factors affecting germination be identified. I examined factors likely to affect germination, including: 1) the germination mechanism characterizing the species, 2) the role of heat and smoke, 3) seed viability, and 4) adequacy of the seed bank. Information about the factors limiting germination and consequent recruitment into sub-populations should prove useful for guiding management decisions concerning conservation of the species.

In order to begin to understand limitations exerted on population growth by seed germination, I attempt to answer the following questions:

1) What are the roles of the inner and outer seed coats in imbibition and germination of this species?
2) Can seeds of *A. michauxii* be artificially germinated?
3) Do heat and smoke promote germination?
4) Is the plant producing live seeds and is there an adequate seed bank?

MATERIAL AND METHODS

Seed Source

Approximately 1200 *A. michauxii* seeds were collected from 14 maternal plants in four different subpopulations on Fort Bragg. A second set of about 1800 seeds was collected from the plants produced by the first set of seeds and subsequently reintroduced into the natural subpopulations at Fort Bragg. To collect seeds, selected inflorescences were bagged before the pods dehisced. Each bag was sewn from nylon netting and secured around an entire inflorescence using fabric-coated wire ties. No more than 25% of the inflorescences with pods were bagged on each plant to ensure that an adequate supply of seeds was left in the field. After the pods dehisced, the seeds were collected, mixed, and stored in brown paper envelopes at room temperature until use.

General Germination Procedures

Procedures used for the artificial germination of *A. michauxii* seeds were modifications of the method of Baskin and Quarterman (1969) used to germinate seeds of *A. tennesseensis*. Germination temperature ranges of 26°C/22°C and 30°C/20 °C day/night were chosen because they fall within the range of the average recorded monthly temperatures (Appendix A-1) in the natural habitat of the species during spring and summer. Germination is defined as emergence of the radical through the outer seed coat. Seeds were considered healthy if they showed no signs of microbial infection and did not appear concave or flat. During the course of all experiments, infected seeds were promptly removed along with the surrounding mm of upper blotter layer to limit infection of neighboring seeds. A schematic outline of all germination procedures is given in Figure 1-2.

Impermeability of Outer Seed Coat

One set of 100 seeds was placed on water-saturated blotters in each of two Petri dishes (Figure 1-2, procedure 1). The Petri dishes were placed in either a 26°C/22°C or a 30°C/20°C day/night alternating temperature germinator. Blotters were kept saturated with deionized water, and seeds were observed every 24 hours for 72 hours for possible imbibition. A preliminary test was conducted on a few seeds to determine the effectiveness of puncturing seeds with a dissecting needle.

Acid Scarification of Outer Seed Coat

The same two sets of 100 seeds were scarified by soaking in concentrated sulfuric acid for twenty minutes. The scarified seeds were rinsed well with deionized water, using cheesecloth to facilitate rinsing, placed on separate water-saturated blotters and returned to the original germinators. Observations for signs of imbibition were made every 24 hours for a 72-hour period.

Piercing of Inner Seed Coat

Two seeds were sectioned and examined under a dissecting scope, verifying the presence of the inner coat (Figure 1-3). After the 72-hour period following scarification, both the outer and the inner seed coats of those scarified seeds that had not germinated were pierced with a dissecting needle to a depth just inside the inner coat, so as not to risk damage to the embryo. The seeds were returned to their respective germinators and observed once every 24 hours for a 72-hour period.

Effect of Leaching

After seventy-two hours following seed coat piercing, the pierced seeds that had not germinated were leached with deionized water, placed on fresh blotters, and returned to the germinators. The seeds were observed every 24 hours for 72 hours.

A Procedure for Optimizing Germination

The preceding procedure of scarifying, followed by piercing, and then leaching was repeated, with the time between treatments shortened to decrease the time to germination (Figure 1-2, procedure 2). Three hundred seeds were scarified in concentrated sulfuric acid for 20 minutes. The seeds were then rinsed well with deionized water, placed on water-saturated blotters, and put in a 30°/26°C germinator. Twenty-four hours following scarification, both the inner and outer seed coats of the remaining ungerminated seeds were pierced using a dissecting needle and the seeds were returned to the germinator. I waited 24 hours after scarification to pierce, so that all the viable seeds had imbibed; non-imbided seeds are extremely difficult to pierce. Twenty-four hours after piercing, the remaining ungerminated seeds were leached by flushing with deionized water, placed on fresh blotters, and returned to the germinator. The numbers of seeds imbibed, germinated, and microbe-infested were recorded every 24 hours.

Determination of a Heating Procedure Suitable for Imbibition

To evaluate the effect of heat on germination under controlled conditions, a procedure for heating resulting in the compromising of the outer seed coat had to be determined (Figure 1-2, procedures 3 & 4). A forced air draft oven was the heat source in all experiments requiring heat treatment. A temperature of 80°C was used because this is the approximate soil temperature at 2 cm below the soil surface during a moderate intensity fire

(Auld and O'Connell 1991). Soil used in all experiments was collected from the sites of sub-populations. The seeds were germinated at 30º/26ºC. Methods used in treatments 1 and 2 were modifications of procedure used by Read *et al*. (2000) and Lloyd *et al*. (2000). Treatment 1 failed to induce imbibition; therefore Treatment 2 was used as a more severe treatment to destroy the integrity of the outer coat.

Treatment 1: Seeds Placed in Cool Soil and Then Heated

Two 43 x 31 x 6.4 cm rectangular aluminum trays were filled to 3 cm with soil. Two nylon mesh bags, each containing 50 seeds, were positioned approximately 2 cm below the soil surface. Thermocouples were placed in the bags and the trays were placed in the oven pre-heated to 120ºC. The soil temperature was allowed to increase to at least 80ºC but no more than 86ºC, at which time the trays were removed from the oven.

Treatment 2: Seeds in Pre-Heated Soil and Then Re-Heated

Two trays were prepared in the same way as in Treatment 1, however this time the soil was first heated to 80ºC, and then the seeds were placed in the soil. Then the trays were returned to the oven, and the temperature was allowed to return to at least 80ºC but no more than 86ºC.

The heated seeds from both treatments were allowed to cool to room temperature, and then placed on water-saturated blotters in Petri dishes in the germinator. The seeds were observed after 24 hours for signs of imbibing.

Effect of Heat and Smoke

The following treatments were applied to test the effects of heat followed by exposure to smoke chemicals on imbibition and germination of *A. michauxii* seeds under controlled conditions (Figure 1-2, procedure 4).

1) no heat and no smoke with 3 replicates of 50 seeds each

2) heat with 3 replicates of 50 seeds each

3) smoke with 3 replicates of 50 seeds each

4) heat and smoke with 3 replicates of 50 seeds each

For treatments 1 and 3 with no heat, the aluminum trays containing the seeds were kept at room temperature. The seeds of treatments 2 and 4 were first heated using the same method in Treatment 2 in the prior determination of heating effects. Following treatment, the seeds were positioned in Petri dishes on water-saturated blotters. All dishes were placed in the germinator. For treatments 1 and 2, blotters were kept moist with deionized water. For the smoke treatments (3 and 4), blotters were kept moist with a 50:50 liquid smoke[1]: water solution (Dixon *et al*. 1995). The seeds were observed every 24 hours for two weeks for signs of imbibition and germination. Ten randomly selected seeds were examined under a dissecting microscope for cracks in the seed coat.

Seed Viability after Heating

To determine if the embryos survived heating, the seeds were treated as above with heat and smoke chemicals, followed by piercing and leaching, with the following sample sizes and replications (Figure 1-2, procedure 5):

1) no heat and no smoke (control) with 3 replicates of 100 seeds each,

2) heat with 4 replicates of 150 seeds each,

3) smoke with 3 replicates of 100 seeds each, and

4) heat and smoke with 4 replicates of 150 seeds each.

[1] Liquid smoke is produced by the controlled combustion of fresh and dry plant material. The resulting smoke is condensed to form liquid smoke.

I had a limited number of seeds to work with; therefore I used smaller sample sizes with the no heat treatments. This should not have created any significant bias because almost all of these seeds were unable to imbibe water and germinate in the absence of heat (or scarification) due to the impenetrable seed coat.

The seeds were placed on blotters in Petri dishes and then put in the germinators. Twenty-four hours later, the numbers of imbibed and germinated seeds were counted. Then, both the outer and the inner seed coats of the ungerminated imbibed seeds were pierced with a dissecting needle. After piercing, the seeds were returned to the germinator. Twenty-four hours later, they were leached with deionized water, placed on fresh blotters, and returned to the germinator. The total of imbibed seeds, a cumulative total of germinated seeds, and the total number of dead seeds were recorded daily. Each day, seeds that germinated were removed and placed in another dish to facilitate tracking of seed imbibition and germination.

This experiment was terminated after twelve days due to rampant microbial infection. The scarified seeds had all germinated within 5 days and did not have as much microbial infestation.

Determination of the Size of the Seed Bank

Ten mature *A. michauxii* plants at two sites on Fort Bragg were chosen for seed bank evaluation (Figure 1-2, procedure 6). The criteria for choosing plants were that they were in flower and/or fruit, and they appeared to have flowered the previous year, as indicated by the persistence of the previous year's inflorescences. I removed the top 3 cm of soil from the outer 0.25 m ring of a 0.50 m radius ring surrounding each plant. A tenth of the soil collected was sampled for seeds. A coarse metal sifter was used initially to remove the large debris. Then a series of smaller-holed sifters were sequentially used until the size that did

not allow *A. michauxii* seeds to pass through was determined. In this way, I visually searched for seeds.

All vascular nomenclature of species in this document follows Radford (1964).

Data Analyses

The effects of heat and smoke on seed germination were analyzed using linear analysis of variance (ANOVA) models. These analyses were performed with the Statistical Analysis System (SAS 1999-2001) software.

RESULTS & DISCUSSION

Impermeability of Outer Seed Coat

Untreated seeds did not imbibe water or germinate. Seeds were considered imbibed when they appeared swollen and puffy. The impenetrable outer coat must have prevented the seed from absorbing water. I could not pierce the hard outer coat of the unimbibed seeds with a dissecting needle, as the tiny seed would slide out from under the needle.

Acid Scarification of Outer Coat

When the hard outer coats of *A. michauxii* seeds were scarified in concentrated sulfuric acid, they absorbed water readily but only a few seeds germinated within 72 hours. This suggested that an inner impermeable seed coat was present and was preventing water from reaching the embryo. The few seeds that germinated after scarification and prior to piercing may have done so because both the outer and the inner coats of these few seeds were compromised during scarification.

Piercing of the Inner Coat

The presence of an inner coat, verified by sectioning seeds, indicated that the inner coat had to be penetrated to promote the germination. However, the introduction of a passage way through the inner seed coat following scarification resulted in minimal germination after 72 hours. Although a passageway had been created, germination of most seeds was still inhibited.

Effect of Leaching

Scarifying the outer seed coat, then piercing the inner seed coat, and finally leaching the seed resulted in 95% germination within 72 hours. This indicated that the embryos were alive. The seeds still needed to be leached after compromising of the outer coat and

penetration of the inner coat, presumably to flush out germination inhibitors from the embryo and promote germination.

A Procedure for Optimizing Germination

Seed viability was high with minimal seed mortality (Figure 1-4). Scarification in concentrated sulfuric acid resulted in 100% imbibition of viable seeds within 24 hours. Four percent of seeds had germinated within 24 hours of scarification, and an additional 5% within 24 hours of piercing. Eighty-eight percent of the seeds germinated within 24 hours of leaching, and 100% germination of viable seeds was attained within 72 hours. Ninety-five percent of all seeds germinated, indicating that very few seeds were not viable (did not have live embryos). Although this procedure resulted in high and efficient germination rates, it is unknown whether other combinations of scarification, piercing, and leaching would have resulted in better germination rates.

Determination of a Heating Procedure Suitable for Imbibition

Seeds placed in the cool soil followed by slow heating to 80ºC did not imbibe water, whereas 15% of seeds placed in pre-heated soil did.

Effect of heat and smoke

Two percent of heated seeds germinated. Seeds that were not heated did not imbibe or germinate (Tables 1-1, 1-2). The heat apparently compromised the impenetrable outer coat of approximately 20% of the seeds, and these seeds imbibed. There were no cracks in the seed coat evident in these heated seeds. However, there were dark spots in the area of the hilum, most likely due to acid damage; so it appeared that the strophiolar plugs in the seed coats were disrupted by heat (Xiaojie *et al.*1999).

Liquid smoke did not affect imbibition or germination. In natural systems, only a small percentage of seeds in the soil might imbibe and germinate in response to fire. A combination of other processes working together are likely required for imbibition and these processes may have varying effects on individual seeds and may take a long time to occur. These natural conditions were unknown and could not be replicated under controlled conditions.

Did the Embryos Survive Heating?

In the second heat and smoke trial (with piercing and leaching), only 4-7% of the heated seed germinated, although 20-22% had actually imbibed (Table 1-1). In the prior investigations of the effects of scarification, essentially any seed that imbided also germinated after piercing and leaching. So it appears that the heat killed a large percentage of those seeds that imbibed. The large number of microbial infected imbibed seeds may have resulted from the seeds being dead. It is not known if the other unimbibed seeds (the majority of the seeds) were also killed by heat. So heating slightly promoted the short-term germination rate of otherwise untreated seeds, but at the cost of killing the remaining seeds.

The Seed Bank

The seed bank averaged twelve seeds per plant. Approximately 343 seeds were produced per plant (page 6). Insects were observed on pods that showed obvious bite marks, so herbivory may account for the low number of seeds stored in the soil. Dispersal of seeds from the site by insects, such as ants, or small mammals may reduce the seed bank surrounding the plant.

CONCLUSIONS

The seed anatomy and the mechanism of germination in *Astragalus michauxii* are similar to those of other *Astragalus* species. Seeds of *A. michauxii* are 'hardseeds' and will not readily imbibe and germinate due to the hard outer coat, tough inner coat, and the possible presence of germinating inhibitors (Miklas *et al*. 1987; Van Staden *et al*. 1989). Typically, the inner seed coats of *Astragalus* species are either removed or split to promote artificial germination. In this study, piercing the seed coat led to germination within a relatively short period and obviated the more laborious removal or splitting of seed coats. Germination frequency was high, 95%, and was complete within 72 hours of piercing. In seeds of *A. tennesseenis*, Baskin and Quartermann (1969) observed 100% imbibition within 6 to 8 hours after scarification, but only 3% germination by 3 weeks. Furthermore, the needle rarely damaged the embryo or cotyledons, as later observed by seedlings grown from these seeds (Chapter 2). This system should work well with most hard-coated seeds, especially those that are small in size. Conservation agencies should find this procedure useful for propagation of rare plants when a large number of seedlings are needed.

Heat increased imbibition and germination of otherwise untreated seeds. Although the percent of heat-treated seeds that germinated was low, 2%, germination rates may be higher in natural populations than experience fire. While heat removes the strophiolar plug and allows imbibition, seeds in the soil are exposed to many factors, which, over time, are likely to compromise the seed coats. Penetration of the outer and inner seed coats and subsequent expulsion of inhibitory chemicals may be accomplished by microbial action, weathering, or perhaps exposure to ethylene or other smoke-related chemicals in the soil.

Piercing and leaching of imbibed seeds after heat and smoke treatments had no effect on germination rates. This was an indication that heat killed the imbibed seeds. When a fire passes over the seed bank, some seeds may die from over-heating, while germination is promoted in those seeds that survive. Also, soil temperatures after a moderate fire may be less than 80º-86ºC, resulting in lower mortality and higher germination percentages of imbibed seeds than obtained in this study.

Smoke was not effective in stimulating germination. Smoke chemicals may not have penetrated the outer coat. Even if it penetrated, the embryos may not have been dormant (Hartmann *et al*. 2002). Additionally, the seeds may require certain sequential and perhaps additive stimuli from smoke chemicals in the soil over an extended period of time (De Lange and Boucher 1990). This species may require long-term exposure to the soil environment, followed by brief smoke treatment to induce germination (Keeley and Fotheringham 1998). The procedure involving heating seeds is an alternate means of plant propagation available to conservation biologists. Although the germination yield is only 2%, this may be enough.

At best, only 4% of the seeds produced were found in the seed bank near the plants. Twelve seeds per plant may not be a substantial enough seed bank to be a dependable source of new recruits into the population.

Although the *A. michauxii* sub-populations at Fort Bragg are producing a substantial number of seeds with live embryos, higher seed survival rates and a subsequent larger seed bank may be needed to compensate for slow germination and seedling mortality, and increase the chances of population growth. Further studies need to be done on the effects of fire on seed germination in the field in order to determine if fire induces germination in

natural systems. In addition, the actual temperatures experienced in soils during fires and the degree of subsequent seed mortality can be determined.

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Table 1-1 Statistics of imbibition and germination with and without piercing and leaching for four treatments: no heat/no smoke, heat, heat and smoke, and smoke.

	TREATMENT	MEAN	MIN	MAX	SD
Trial 1 - Without Piercing And Leaching					
% Imbibition	No heat/no smoke	0	0	0	0
% Imbibition	Heat	20	13	25	6
% Imbibition	Heat and Smoke	20	18	22	2
% Imbibition	Smoke	0	0	0	0
Trial 2 - With Piercing And Leaching					
% Imbibition	No heat/no smoke	0	0	0	0
% Imbibition	Heat	20	11	36	11
% Imbibition	Heat + smoke	22	13	35	9
% Imbibition	Smoke	0	0	0	0
Trial 1 - Without Piercing And Leaching					
% Germination	No heat/no smoke	0	0	0	0
% Germination	Heat	2	1	3	1
% Germination	Heat and Smoke	2	1	4	2
% Germination	Smoke	0	0	0	0
Trial 2 - With Piercing And Leaching					
% Germination	No heat/no smoke	0	0	0	0
% Germination	Heat	7	0	14	1
% Germination	Heat + smoke	4	1	9	4
% Germination	Smoke	0	0	0	0

Table 1-2 Reduced analysis of variance models of the effect of heat on imbibition and germination in *A. michauxii* seeds.

		% Imbibition				% Germination			
Trial 1									
Source	DF	SS	MS	F	p>F	SS	MS	F	p>F
Model	1	1200	1200	139.5	<0.0001	12	12	15	0.0031
Error	10	86	8.6			8	0.8		
Corrected Total	11	1286				20			
R-Square		0.93				0.60			
Trial 2									
Model	1	1469	367	25.6	0.0003	101	101	11.9	0.0048
Error	12	689	57			102	2.1		
Corrected Total	13	2159				203	8.5		
R-Square		0.68				0.50			

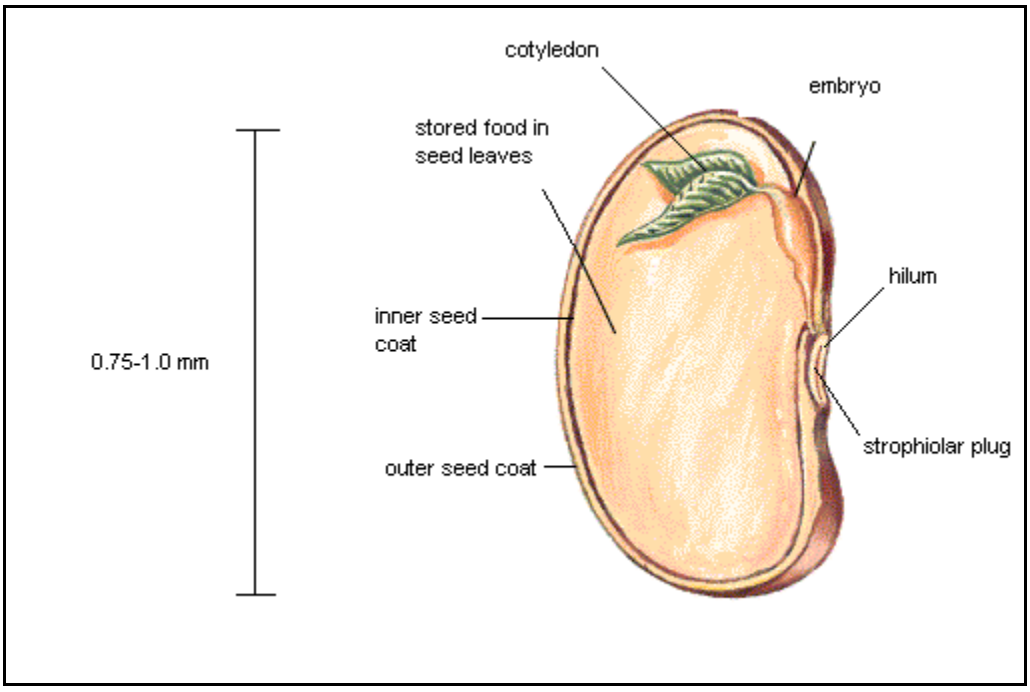

Figure 1-1 Typical legume seed depicting outer and inner seed coats of *A. michauxii*.

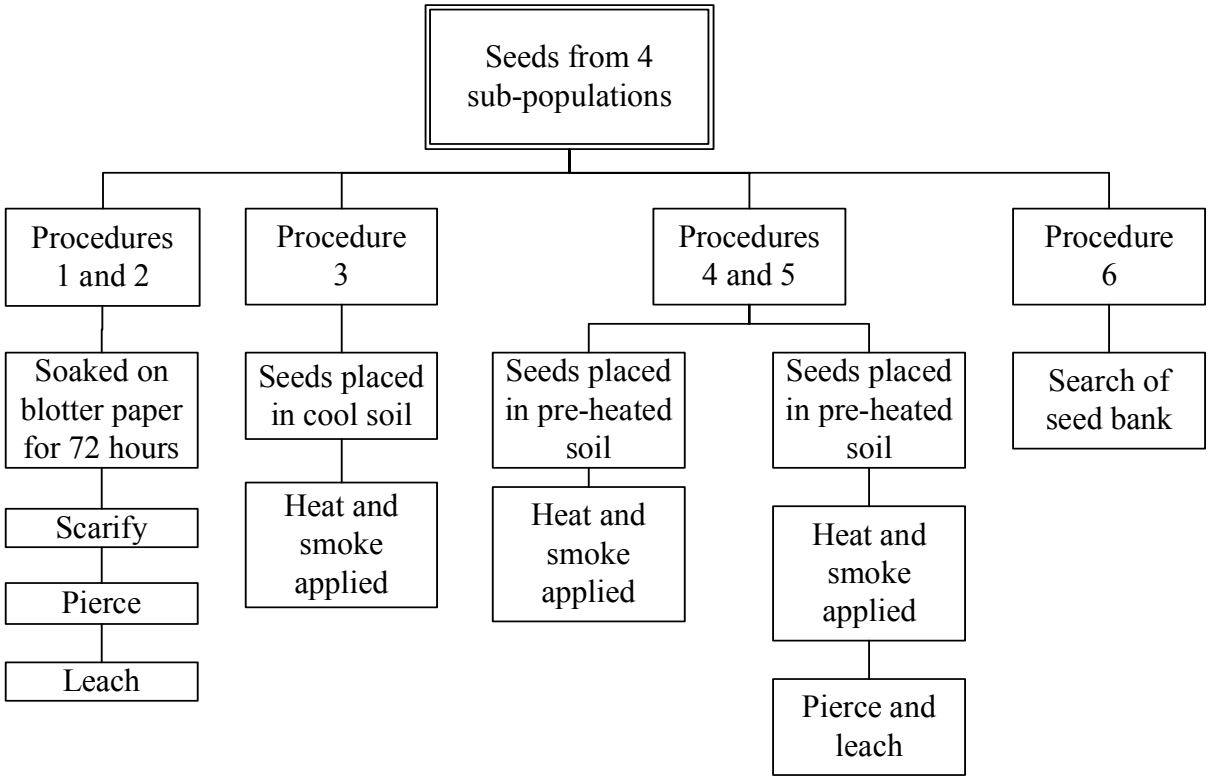

Figure 1-2 Scheme of germination procedures for seeds of *A. michauxii.*

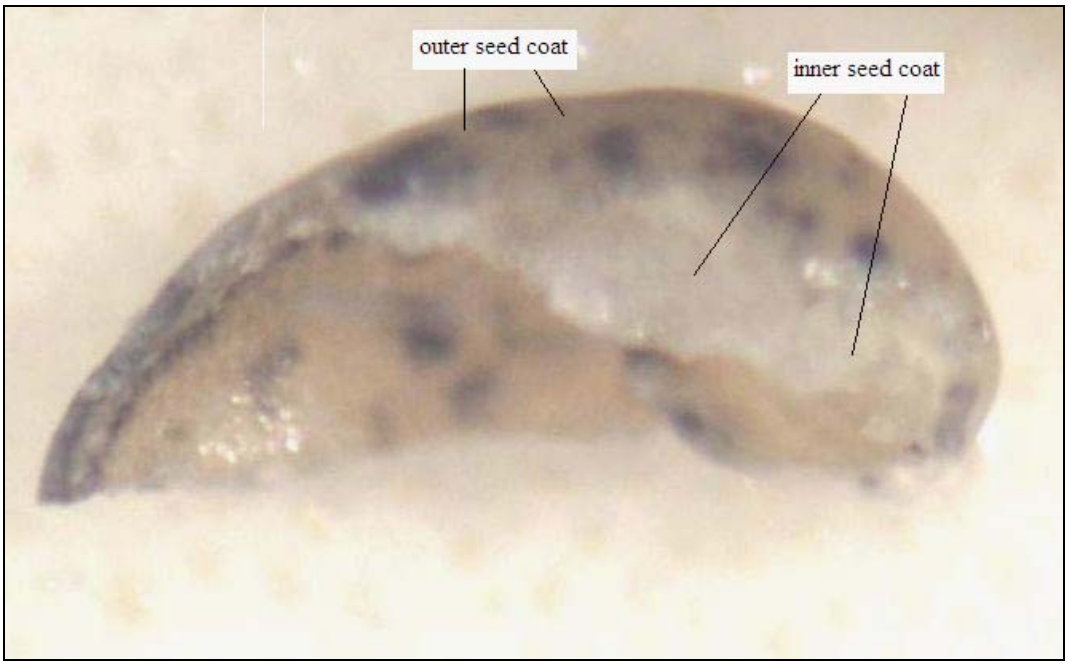

Figure 1-3 *A. michauxii* seed depicting outer and inner seed coats.

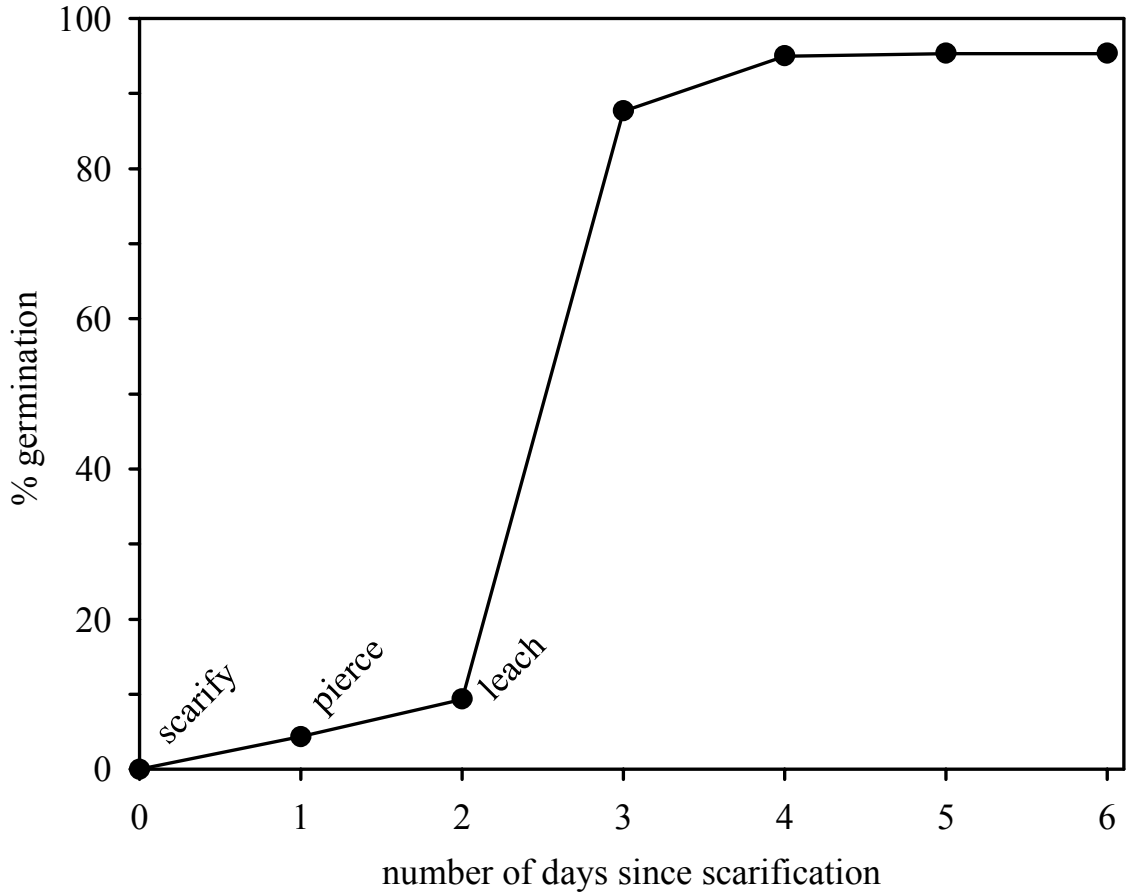

Figure 1-4 Cumulative percent germination of *A. michauxii* seeds after scarification, piercing and leaching.

CHAPTER 2

Effect of age, height, stem number, and competition on plant survival of *Astragalus michauxii* over winter

ABSTRACT

I investigated factors influencing over-winter survival rates in *Astragalus michauxii* (Kuntze) Hermann sub-populations on Fort Bragg Military Reservation, North Carolina. I began by growing plants under the controlled conditions in a phytotron, starting with previously germinated seeds. In July of 2002, a 20x10-meter plot was measured on each of two *A. michauxii* sites and two sets of plants, between the ages of 52-70 days and 108 - 256 days, distributed among them. Height, stem number, and age from time of germination for each individual plant were recorded when they were planted. Distances of each *A. michauxii* individual from the nearest oak saplings, pine trees, wiregrass clusters, and pine seedlings within the plots were measured. The diameter of each competing plant was measured, and the total competition experienced by each *A. michauxii* plant calculated as: competition index = $\sum$ [diameter of each competitor / (distance)^2]. In May of 2003, survival data were collected from both sites. Fifty-nine percent of plants survived over-winter. Logistic regression predicted over-winter survival to be positively affected by plant height ($p<.0001$) and number of stems ($p=.0004$). Sub-populations with larger numbers of tall individuals having multiple stems are more likely to persist. Age and interspecific competition were not significant.

INTRODUCTION

Astragalus michauxii (Kuntz) Hermann (Sandhills milk-vetch) is a rare and threatened perennial legume (USFWS 1990, TNC 1993) occurring in open understory habitats of nutrient poor, fire-dependent, long-leaf pine (*Pinus palustris* Miller) /wiregrass (*Aristida stricta* Michaux) communities of the southeastern Atlantic coastal plains (Schafale and Weakley 1990). The largest *A. michauxii* population is located in the North Carolina Sandhills, on the federally owned Fort Bragg Military Reservation.

In 1993, there were 88 *A. michauxii* sub-populations at Fort Bragg, with a mean stem number of 6.6 per sub-population (Appendix A). In 1999, there were 87 sub-populations, with a mean stem number of 10.7. Of these 87, 69 had persisted from 1993 and 18 had colonized since 1993. Nineteen of the 88 sub-populations present in 1993 were not found in 1999. Sub-populations with larger stem numbers were more likely to persist from 1993 to 1999. It is unknown whether these 19 represent local extirpations, or if the plants do not produce shoots during certain years, or are not detectable after fire.

Although the number of sub-populations remained stable between 1993 and 1999, stem counts within sub-populations are small. In general, stem number is closely related to local population persistence (Gray 2001), so low stem number in *A. michauxii* is a concern. Increasing stem number is important for population viability and persistence because it decreases inbreeding depression (Hiers *et al*. 2000). Also, in the event of stochastic environmental or demographic disturbance, small populations may not recover (Walker and Peet 1983) and so are at risk of extirpation. Although there is as yet no evidence linking population persistence of *A. michauxii* to stem number, these sub-populations may be at risk

of extirpation. It is therefore important to understand those environmental factors and plant characteristics that are correlated with *A. michauxii* over-winter survival.

A. michauxii is a perennial which blooms from the beginning of May to late June. Fruits are set in early June and seeds are dispersed from July to October by apical dehiscing of the pods. At the first killing frost, the above-ground stems die. In the spring, new stems emerge. Pods sometimes persist on dead stems until the next growing season. A plant going into dormancy has a root crown that persists through dormancy (Schafale and Weakley 1990, TNC 1993). In the spring, the size of the new emergent plant is partially determined by the size of the root structure.

Mortality is a major determinant of population size and local extinction. Winter survival may depend on plant vigor going into the dormant season, which, in turn, may be determined by environment and competitive ability. Competition, both interspecific and intraspecific, can be a major determining factor in the size of plants as well as the growth of a population. A plant must compete successfully with other plants for light, water, space, and soil nutrients if it is to avoid density-dependent mortality. The age of an individual may also influence survival. Younger plants are not as robust and may experience a higher rate of mortality than more established plants.

The general purpose of this study was to investigate the role of plant vigor and competition on survival from one year to the next. I addressed the following specific question:

Is over-winter survival affected by plant age, height, stem number, and interspecific competition?

MATERIAL & METHODS

Experimental Plant Material

I raised *A. michauxii* plants from seeds originally collected at Fort Bragg. The plants were grown in the controlled environment of the phytotron as described in Appendix C. The mature plants were placed under varying temperatures and photoperiod conditions to observe the effects on root structure, growth, flowering, and pod formation. Two groups of plants, between the ages of 52 and 70 days and 108 and 256 days, were selected for the survival study. Variation in ages represents differences in time of germination. Both juvenile and mature plants were included because mortality rates tend to be higher with juveniles than with mature plants.

Selection of Study Sites

Two Fort Bragg *A. michauxii* sites were chosen for study. These sites were chosen because they 1) contained large areas of unoccupied area adjacent to clusters of *A. michauxii* plants, and 2) were not scheduled to be burned during the year of the study. Plot 1 was established in a clearing adjacent to a cluster of *A. michauxii* plants on Site 1. Plot 2 was established on Site 2, in a clearing unoccupied by *A. michauxii*, and approximately 20 meters apart from any *A. michauxii* plants.

Planting

A 20 m x 10 m study plot was established on each of the two study sites in July of 2002. Areas chosen for the study plots were sparsely vegetated, and I did not remove any vegetation. Ninety-one plants, between the ages of 108 and 256 days old and 1.2 to 104 cm tall, made up the older cohort. Forty-two plants of ages 52, 60, and 70 days old, and 1.8 to 8.3 cm made up the younger cohort. At Site 1, twenty-four of the younger cohort (all 52-

day-old) and 47 plants of the older cohort (of varying ages) were planted in the plot. At Site 2, eighteen 60- and 70-day-old plants and 44 older plants, also of varying ages were planted in the plot. All *A. michauxii* individuals were marked using numbered metal tags. The plants were watered initially at planting, but were not watered again for the duration of the experiment because plants in natural habitats depend on rainfall for water.

Initial Measurements

At the time of planting, the following plant measurements were taken:

1) Number of stems arising from each *A. michauxii* underground crown.
2) Height of the tallest stem from ground level to the tip of the apical meristem.
3) Distances from each *A. michauxii* individual to the nearest oak sapling (*Quercus* L. spp.), wiregrass cluster (*Aristida stricta*), pine tree (*Pinus palustris*), and pine seedling (*Pinus palustris*) within 10 m. Sometimes these competitors were outside the plot. Vascular plant nomenclature follows Radford (1964).
4) Diameter of each competitor identified in (3) using calipers as follows: Pine trees were measured at a height of approximately 1.3 m. Wiregrass clusters and pine seedlings at the base. The diameter of each oak sapling was estimated by taking the diameter of a single stem and multiplying by the total number of stems. Frequent fires in this long-leaf pine community kill the apical meristem of oak saplings, resulting in multiple lateral buds formation and branching.

Data collection after winter

The plants were surveyed in the spring of 2003 for over-winter survival, and later for flowering and fruiting. The locations of each plant of every species in the plots were mapped (Appendix B).

Calculations

A competition Index (CI) was calculated for each *A. michauxii* individual as the sum of the diameter of each competitor divided by its squared distance from the *A. michauxii* individual: $CI = \sum [\text{diameter} / (\text{distance})^2]$.

Data analyses

T-tests were used to determine differences in mean vegetative characteristics and competition indices between those plants that survived and those that did not. Logistic regression was used to investigate relationships between plant survival and vegetative characteristics (age, number of stems, height) and competition index (oak sapling, pine tree, wiregrass cluster, and pine seedling). The data from both sites were combined into one data set for analysis. All statistical analyses were performed with the Statistical Analysis System (SAS 1999-2001) software.

RESULTS & DISCUSSION

Experimental Plant Material

Features of Younger Plants

Growth of seedlings was slow and half-siblings showed wide variation in survival and growth rate (Figure 2-1). Seedling height varied widely from 1.8 to 8.3 cm at 52, 60, and 70 days (Figure 2-2). These plants were not well established, had not developed a crown root, and had only one stem. Most of the variation was in plant height and number of branches growing from the single stem.

Features of Older Plants

Although they developed from seeds that were quite uniform in size, mature half-siblings of the same age varied in height, stem number, and structure when grown under similar conditions (Table 2-1). Some plants developed erect and ascending stems (Figure 2-3) while others developed short and bushy structures (Figure 2-4). Plant structure also varied as a result of treatment conditions. Plants placed under 30°C/26°C long day conditions experienced excessive growth (Figure 2-5). Plants vernalized under short day conditions at 10°C/5°C developed an extensive root system. Each root mass consisted of a subterranean crown attached to an extensive system of multiple secondary roots (Figure 2-6). These vernalized plants later developed an increased number of stems per individual (between 3 to14 compared to a maximum of 3 stems on plants not vernalized).

All mature plants having an erect structure flowered either with 22°C/18°C or 26°C/22°C condition. Plants having the short and branched structure rarely produced inflorescences. More than 75% of flowers withered without forming pods, less than 10% of

flowers produced pods, and these pods did not contain any seeds (Figure 2-7). At anthesis, the anthers surround and hug the stigma such that it is totally covered with pollen when the flower opens (Figure 2-8). A few flowers were observed with stigmas extending beyond the anthers. Plants that were unsuccessful at seed production in the greenhouse produced seeds successfully in the study plot in the field the following spring.

Plant condition after winter in field-planted populations

The above-ground stems of all plants had senesced after the first killing frost. The forty-one percent of the plants that did not re-sprout in the spring were considered dead (Table 2-1). Thirty-five % of the older and fifty-five % of the younger cohort plants did not survive over-winter. There was no difference in survival between Sites 1 and 2. Logistic regression showed that plant height ($p<.0001$) and number of stems ($p=.0004$) were significantly related to over-winter survival (Table 2-2). Plant survival was not affected by age ($p=.0542$) or interspecific competition from oak saplings ($p=.1159$), wiregrass ($p=.0912$), pine trees ($p=.1787$), or pine seedlings ($p=.3581$). All surviving mature plants having an erect structure flowered in the spring and some had set seed by the middle of June.

CONCLUSIONS

Survival of *A. michauxii* plants over winter was significantly affected by plant height and number of stems. Fifty-nine percent of plants planted in the summer of 2002 survived to the following spring. Growth of sub-populations with large numbers of small individuals having fewer stem numbers will be most limited by mortality through the dormancy period.

As winter approaches, the decreasing temperatures of fall facilitate survival by increasing root growth and the development of a crown root. The more extensive root system allows faster growth and multiple stems the following spring. Age and interspecific competition were not significant to over-winter survival. It might be possible to increase plant over-winter survival rates by planting earlier in the growing season (March or April rather than July). Additionally, increased watering after out planting may increase survival.

Successful seed germination and seedling establishment under controlled conditions make artificial propagation an ideal technique for *A. michauxii*. Greenhouse propagation and out-planting of plants can be used with success to increase stem numbers in conservation programs for *A. michauxii* as well as other rare species.

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Table 2-1 Descriptive statistics for the survival of *A. michauxii* plants on sites 1 and 2 over winter. A survival value of one means the plants survived and that of 0 means they did not. CI means competition index in cm^{-1}.

Variable	#		Mean		Minimum		Maximum		Std. Deviation		P>t
.	1	0	1	0	1	0	1	0	1	0	
Survival	78	55									
Height (cm)			29.0	9.8	2.4	1.8	104.0	40.3	25.7	10.5	**<0.01**
# of Stems			4	2	1	1	14	6	3	1	**<0.01**
Age (days)			171	145	52	52	256	218	69	74	**0.05**
CI - Wiregrass			6.4	5.3	1.0	0.0	21.9	13.4	3.9	3.1	0.06
CI - Pine trees			2.0	2.6	0.3	0.0	10.5	16.4	1.8	3.1	0.32
CI - Oak saplings			1.3	0.2	0.0	0.0	26.6	3.0	34.0	0.6	0.35
CI - Pine seedlings			0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.67

Table 2-2 Logistic regression of survival over winter on height, # of stems, age, and competition indices (CI) of *A. michauxii* plants (X^2 = Wald Chi-Square).

Independent Variable	Odds Ratio	X^2	Pr> X^2
Height (cm)	1.06	18.2	**<.0001**
Number of Stems	1.55	12.7	**.0004**
Age (days)	1.01	3.7	.0542
CI - Wiregrass	1.09	2.9	.0912
CI - Oak saplings	1.28	2.5	.1159
CI - Pine trees	0.90	1.8	.1787
CI - Pine seedlings	0.02	0.8	.3581

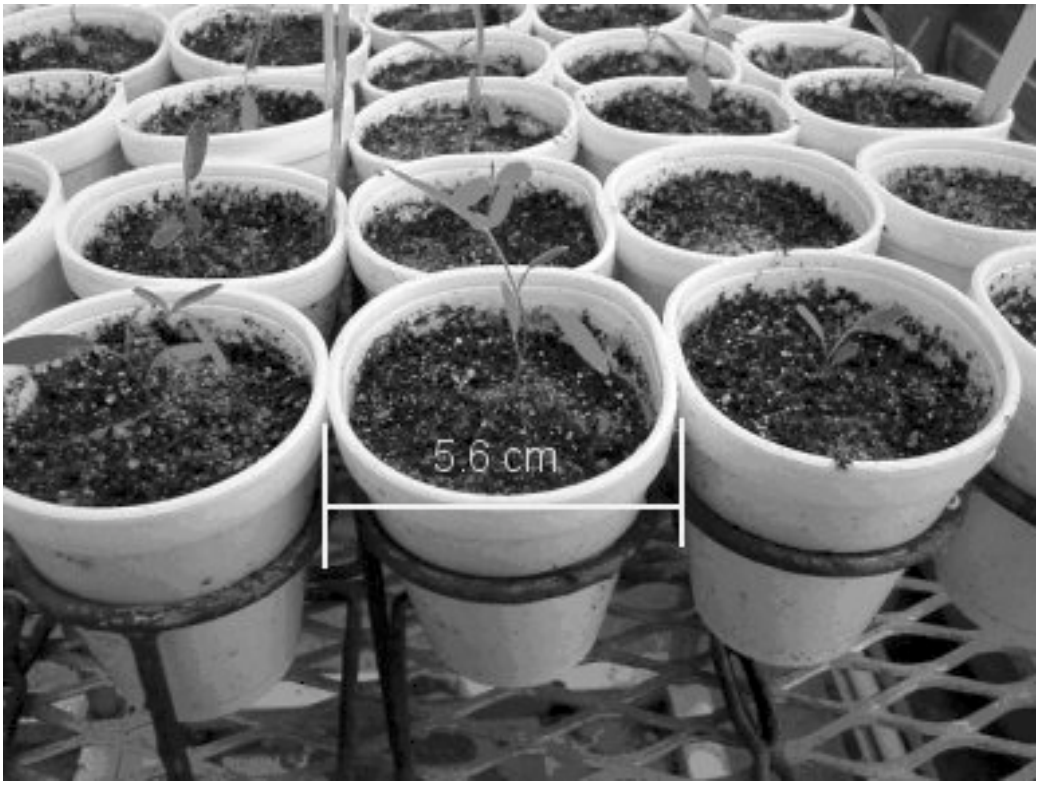

Figure 2-1 Half-sib *A. michauxii* seedlings at 52 and 60 days, showing variation in size.

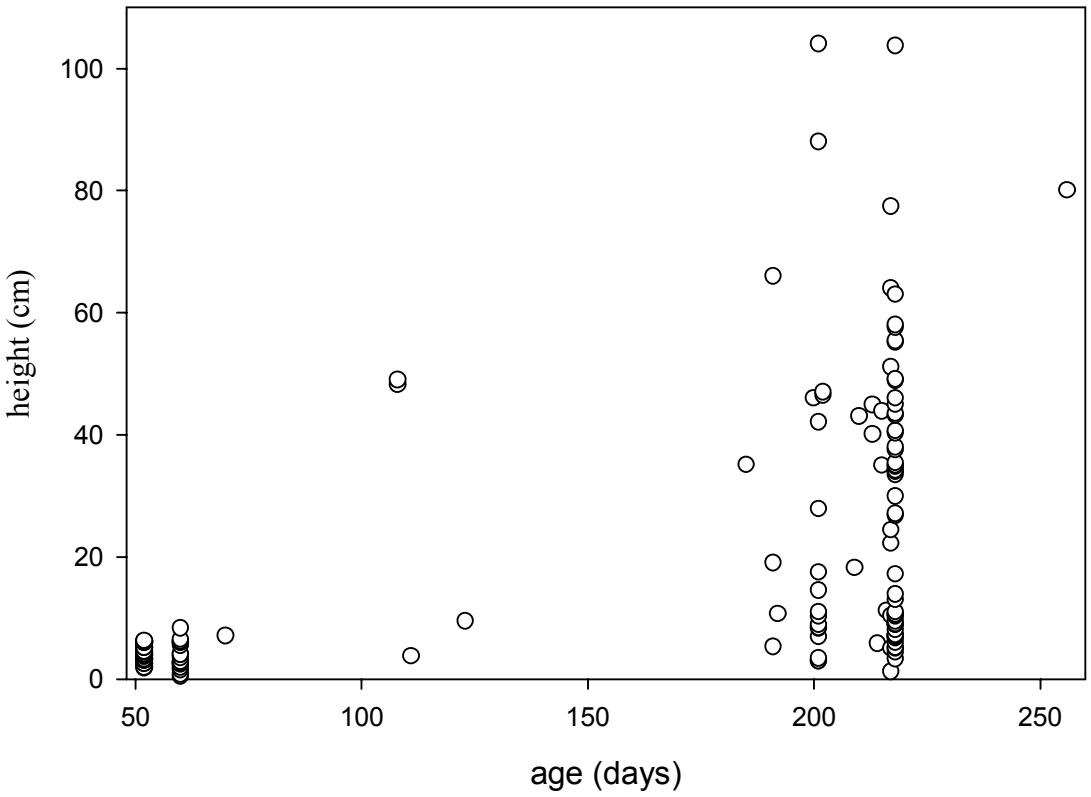

Figure 2.2 Heights of plants in younger (52-70 days) and older (108-256 days) cohorts of *A. michauxii* plants at time of planting in the field.



Figure 2-3 Mature (218 days old) *A. michauxii* plants from one maternal line. Note the variation in size and structure. These plants developed from similarly sized seeds.

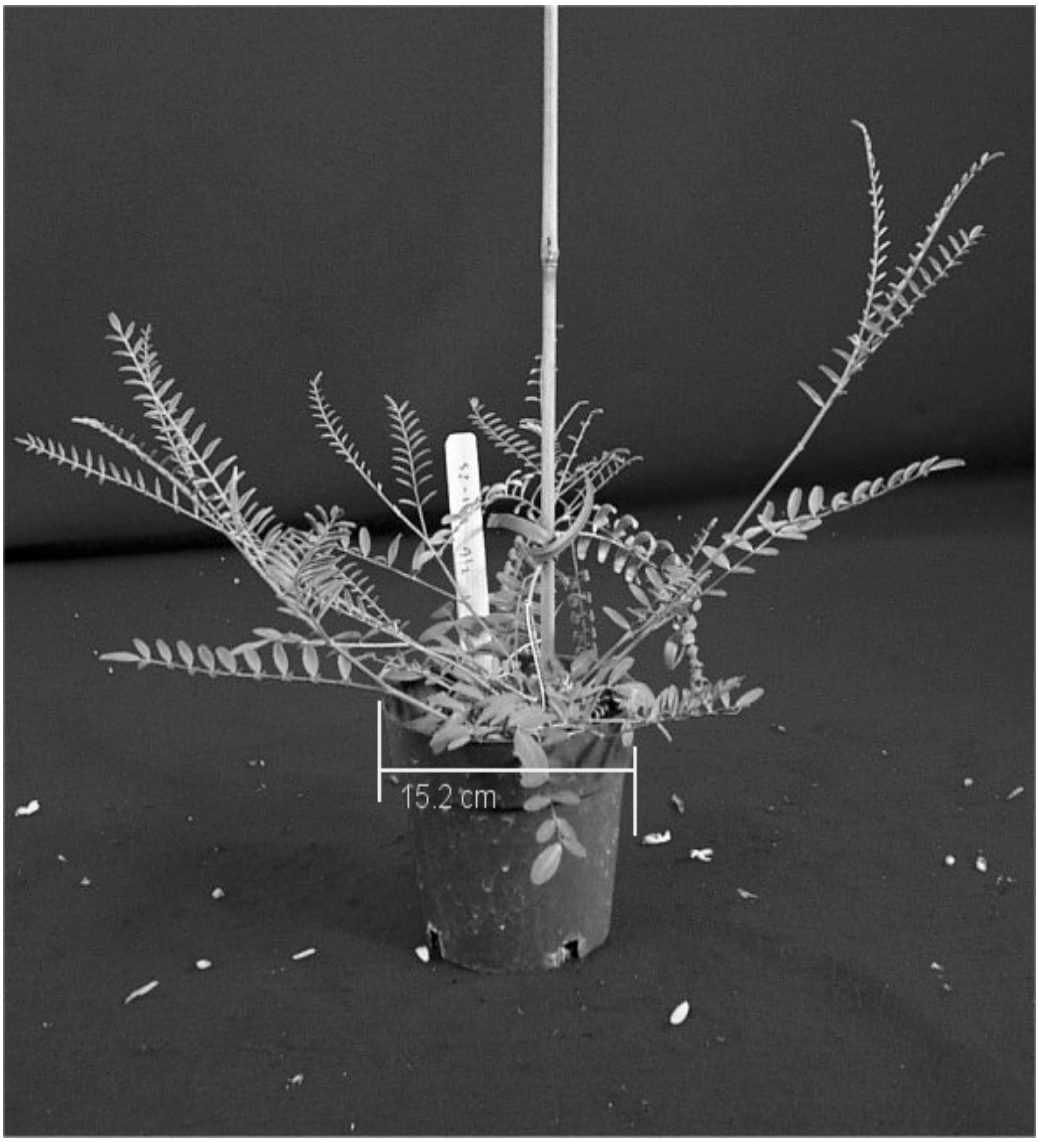

Figure 2-4 *A. michauxii* plant showing a short and bushy structure. Plants with this growth form did not produce any inflorescences and did not occur in the field.



Figure 2-5 Mature (217 days old) *A. michauxii* plants from the 30°/26°C treatment have a more robust size than plants in other treatments.



Figure 2-6 *A. michauxii* plants under short day conditions at 10ºC/5ºC showing root structure. An extensive root structure consists of a crown with multiple secondary roots below ground. Stems arise above ground out of crown root.



Figure 2-7 *A. michauxii* inflorescence showing formed pods.



Figure 2-8 *A. michauxii* flowers after anthesis showing anthers with pollen grains surrounding the stigma.

CHAPTER 3

Effects of fire and competition on vegetative growth, reproductive effort, and population persistence in *Astragalus michauxii.*

ABSTRACT

I investigated limitations to population growth of *Astragalus michauxii* (Kuntze) Hermann by fire history and competition for resources in the long-leaf pine/wiregrass habitat at Fort Bragg Military Reservation, North Carolina. Ground cover, canopy cover, vegetative growth and reproductive effort data were collected from 48 sub-populations. Fire history and population persistence data were obtained from the Endangered Species Branch of Fort Bragg. Trends in population persistence were analyzed in response to time-since-burn, fire frequency, and competition. Sub-populations with lower percentages of understory species covers other than pine, oak, and wiregrass were more likely to persist. Next, I determined the effects of fire history and competition on plant growth and reproduction. Plants in more frequently burned habitats were more likely to grow taller. Third, factors limiting the number of individuals in a cluster or sub-population were investigated. The number of *A. michauxii* plants in a 4 m^2 plot increased with % oak cover. Low regression coefficients for effects of competition and fire history indicate that, not one, but rather a number of factors, may work together to impact growth and reproduction. Further studies need to be carried out to determine limitations to seed set. In addition, management should investigate experimental fire regimes to test for fire impacts on habitat quality, plant growth and reproduction, and population growth and persistence.

INTRODUCTION

Astragalus michauxii (Kuntze) Hermann (Sandhills milk-vetch) is a rare North Carolina Threatened Species (TNC 1993) ranked as category S3 as well as a Federal Species of Concern (USFWS 1990). The species is a regional endemic, with populations recorded in 11 Sandhills and Coastal Plain counties in North Carolina and in South Carolina, Georgia, Tennessee, and Florida. An erect perennial in the Fabaceae family, *A. michauxii* is found in xeric to dry-mesic, nutrient poor, fire-dependent long-leaf pine (*Pinus palustris* Miller) /wiregrass (*Aristida stricta* Michaux) ecosystems of the Atlantic coastal plains (Figure 3.1) (Schafale and Weakley 1990). These communities were historically burned by regular low- to moderate-intensity fires which maintained the open understory necessary for *A. michauxii* and other species. The open understory, along with the intact ground cover, provides optimum habitat conditions for rare flora (Hawkes and Menges 1996).

The largest *A. michauxii* population is located on the federally owned Fort Bragg Military Reservation (Figure 3.2) in the North Carolina Sandhills. This population is popularly thought to be threatened primarily by habitat loss as a result of fire suppression and habitat fragmentation (TNC 1993, TNC 1991-1993) due to housing and military-related land development. Although there is no direct evidence that *A. michauxii* is limited by fire suppression, the absence of fire allows overgrowth of the understory, which can lead to the loss of numerous pyrophytic rare species in the herbaceous layer when they are shaded out (Frost 1998). In addition, Fort Bragg as a military base exerts additional threats to this and other rare plant species. For example, excessive trampling during military activities and pine straw raking either physically destroys the plants or disrupts fire movement and nutrient cycling (TNC 1993).

Because of the fragmented condition of the landscape and consequent reduction in fire movement, long-leaf pine ecosystems require carefully planned management to simulate natural fire regimes (Leach and Givnish 1996). Fort Bragg currently uses growing season prescribed fire programs as a management tool for the conservation of *A. michauxii* and other rare plants. Prior to 1960, there was no prescribed fire program at Fort Bragg, although wildfires were frequent (Sewell *et al*. 1993). Between the mid-1960s and 1989, managed areas were burned once every five years, using winter dormant season fuel reduction fires. Beginning in 1990, areas managed for rare plants on Fort Bragg were put on a prescribed fire regime to be burned once every three years during summer months (TNC 1991-1993; Sewell *et al*. 1993). Although the natural fire frequency is unknown, *A. michauxii* is thought to respond well to this fire regime (TNC 1993; TNC 1991-1993).

Although Fort Bragg uses growing season prescribed fire programs as a management tool, species-specific features of natural fires need to be incorporated into managed fire regimes in order to maintain viable populations (Hiers *et al*. 2000). Population persistence has been reported to increase with fire frequency in a suite of rare species (Gray 2001). Growing season fire may increase flowering and seed set in herbaceous species of long-leaf pine savannahs (Davies and Platt 1984; Platt *et al*. 1988). On the other hand, a fire not properly timed to reproductive cycles can reduce population resilience of fire-adapted species, resulting in deleterious effects on growth, reproduction, and persistence of small populations (Kirkman *et al*. 1998). The proper timing of fire to support *A. michauxii* populations is not know, but ideally, a fire should not disrupt or hinder flowering, seed set, or seed germination. For example, a summer fire occurring after flower production but before seed dispersal destroys the current year's seed production. The ideal timing of fires to

reproduction is however not known. Fire maintains a balance between *A. michauxii* and other species in the understory. A too long or short fire return interval can decrease the resilience of small rare populations. A fire return interval that is too long may allow litter to accumulate, increasing understory competition. A two-year minimum fire return interval has been proposed for maintaining a balance between colonization and extinction of rare vascular flora in the long-leaf pine ecosystem at Fort Bragg (Gray 2001). However, a species-specific fire return frequency has not yet been determined for *A. michauxii*.

A. michauxii individuals occur in sub-populations of one or more small clusters of one to ten plants spread across a site. In the event of stochastic environmental or demographic disturbance, these small populations may not be able to recover (Walker and Peet 1983). High stem numbers are important for population viability and persistence because they increase the probability of successful seedling establishment (Hiers *et al*. 2000) and decrease inbreeding. Although population persistence has been linked to stem number in a suite of rare species (Gray 2001), however, it is not known if this is true for *A. michauxii*. If stem number is important to population persistence in these sub-populations, they may be at risk of becoming extirpated, especially in the absence of adequate and successful colonization. Extinction and colonization across metapopulations are important determinants of rare plant population persistence (Walker 1993). In 1993, there were 88 *A. michauxii* sub-populations at Fort Bragg, with a mean stem density of 6.6 per sub-population (Appendix A-2). The most recent count before this study in 1999 was 87 sub-populations with a mean stem density of 10.7. Of the 87 sub-populations found in 1999, 69 had persisted and 18 were colonized since 1993. Another 19 sub-populations that existed in 1993 had been extirpated

by 1999. Understanding population dynamics and turnover rates of individual species is important, especially when life history information is lacking (Walker 1993).

Although generalized information documenting the phenology, habitat, and range of *A. michauxii* has been recorded, there is a broad lack of knowledge pertaining to its reproductive ecology. The role of fire in population persistence in this fire-dependent ecosystem is unknown. With population persistence likely to be directly linked to both the number of individuals in a population and fire frequency (Gray 2001), the factors limiting plant growth and reproduction need to be identified. The motivation for my research was to provide information useful to *A. michauxii* management and conservation. I attempted to answer the following questions:

1) What are the factors limiting population persistence?
2) What are the factors limiting plant growth and reproduction?
3) What are the environmental factors limiting the number of individuals in a cluster of plants or a sub-population?

I examined some of the factors likely to affect population persistence, and plant growth and reproduction of *A. michauxii* sub-populations at Fort Bragg. First, I examined trends in population persistence in response to time-since-burn, fire-frequency, canopy cover, and ground competition from neighboring species in the habitat, including various oak species (*Quercus* spp.), most of which were turkey oak (*Quercus laevis* Walter), long-leaf pine, wiregrass, and other species cover. Other species include pignut hickory (*Carya glabra* (Miller) Sweet), love grass (*Eragrostis* spp.), dwarf huckleberry (*Gaylussacia dumosa* (Andrz) T. & G.), winged sumac (*Rhus coppalina* L.), false poison sumac (*Rhus michauxii* Sargent), *Solidago verna* M.A.Curtis, poison ivy (*Rhus radicans* L.), and

muscadine (*Vitus rotundafolia* Michaux). Second, I determined limitations to population growth, including the causes of reduced plant growth and reproductive effort. Measures of growth were plant height and the number of stems per individual. 'Stem' refers to each stem arising from a rootstock that makes up one individual plant. Reproductive effort was determined by the number of inflorescences, flowers, and pods per plant. Plant growth and reproduction were examined in response to: 1) time-since-burn, 2) fire frequency, 3) ground cover, and 4) canopy cover. Third, I explored how these factors might restrict or enhance the number of *A. michauxii* individuals in a cluster or a subpopulation.

MATERIAL & METHODS

Description of Study Species

A. michauxii is a perennial legume which blooms from the beginning of May to late June. The flowers are bisexual and the highly specialized tubular corolla is differentiated into a banner, wings, and a keel (Figure 3-3). *A.michauxii* sets fruit in early June and is presumed to require an insect pollinator since insects have been seen visiting the flowers. Seed production for the species is thought to be at its peak one or two years after a summer burn. At this time, flower production is high, flowering is synchronized and pollinators are abundant, probably due to increased light, water and nutrients. Seeds are dispersed from July to October by apical dehiscing of the pods, which sometimes persist until the next growing season. (The information in this paragraph is from TNC 1991-93.)

Selection of study sites

The most recent survey taken in 1998-1999 reported 1090 stems among 87 sub-populations (Appendix A-2). Stem count numbers can be ambiguous because a 'stem' can be either one individual having many stems or a single-stemmed plant, depending on the surveyor's classification (Gray 2000, personal communication). Forty-eight *A. michauxii* sites on Fort Bragg were selected for data collection. Sites are habitats that contained *A. michauxii* sub-populations at the 1993 survey of Fort Bragg. In 1993, total stem count of the Fort Bragg population was estimated to be 701 stems distributed among 88 sub-populations (Russo 1993). Study sites were chosen if they were not scheduled for a prescribed burn during the summer of 2003, and were not in temporarily off-limit areas, such as bombing or impact areas. Sub-populations scheduled to be burned in the summer of 2003 were not surveyed because fire destroyed the above-ground vegetation. Individuals in habitats that are

burned in late summer cannot achieve full size and reproductive maturity before the dormant season arrives.

Data collection

Data were collected between June and July of 2003. Each of the 48 sites was surveyed for the presence of *A. michauxii* sub-populations. A sub-population was considered present if at least one individual was present. If a sub-population was present, a single square 4 m^2 plot including as many of the individuals as possible was established. If the sub-population was absent, a square 4 m^2 plot was established surrounding the original location, located by the coordinates recorded during the 1993 census. I searched within a 10-meter radium of the recorded coordinates to compensate for discrepancies of the GPS. One of the study sites contained areas of two distinctly different levels of ground competition. One area had two times as much ground cover as the other and was more representative of all other sites. One plot was established in each of the two areas.

Canopy cover was determined within each plot using a spherical densiometer by the method of Lemmon (1956). % Canopy cover was determined by taking densiometer readings facing each of the four cardinal directions at the center of each plot, and then averaging the four readings. Percent ground cover was estimated for all herbaceous/shrub vegetation rooted within the square 4 m^2 plot for all plants up to one meter high. Ground cover estimates included % oak sapling, % pine seedling, % wiregrass, and % other species. Vascular plant nomenclature follows Radford (1964). The numbers of *A. michauxii* individuals located within the study plots were counted. Stems rising out of a common crown root were considered to be one plant.

The location of each *A. michauxii* plant was marked. The total number of stems arising from the underground crown was determined and recorded as the number of stems. An individual includes all stems of that plant. The height of the tallest stem of each individual plant was measured from ground level to the apical meristem.

The numbers of inflorescences, flowers, and pods per plant were counted. To collect seeds, selected inflorescences were bagged before the pods dehisced. Each bag was sewn from nylon netting and secured around an entire inflorescence using fabric-coated wire ties. Not more than 25% of inflorescences with pods on each plant were bagged to ensure that an adequate supply of seeds remained on site for reproduction. After collection, the seeds were stored in brown paper envelopes at room temperature until use. The actual number of seeds per plant could not be determined because the holes in the netting of the pod collection bags, while allowing good ventilation, allowed seed loss.

The fire histories of the 48 sites were examined to explain the effect of time-since-burn and fire-frequency on vegetative and reproductive efforts, number of *A. michauxii* individuals, and population persistence from 1999 until 2003.

Statistical Analysis

A t-test was used to establish differences between habitat characteristics of sub-populations that persisted from 1999 until 2003 and those that did not. Logistic regression was used to investigate effects of competition (canopy cover, oak sapling, pine tree, wiregrass cluster, and pine seedling) on population persistence. Logistic regression is the statistical analysis of choice for modeling data when the dependent variable is dichotomous. I determined correlations between all independent and non-dichotomous dependent variables. Linear analysis of variance models were used to determine significant

relationships between growth, reproductive effort, and number of individuals in sub-populations, fire frequency, time-since-burn, % canopy cover, and % ground cover. Significance was determined at $\alpha = 0.05$. All statistical analyses were performed with the Statistical Analysis System (SAS 1999-2001) software.

RESULTS AND DISCUSSION

Population Persistence

Thirty-nine of the 48 sub-populations I surveyed that were recorded in 1993 were also present in 1999 (Appendix A-2). By 2003, only 21 were present (Table 3-1), signifying an alarming rate of sub-population loss between 1999 and 2003. However, the survival of a metapopulation depends on extinction and colonization rates across the sub-populations, and I did not search for newly colonized subpopulations. It is not known if these plants sprout from the underground crown each and every year after dormancy. Sub-populations absent in one year may be present in a later year. Also, germination of seeds in the seed bank may bring in new recruits to re-colonize a site.

Sub-populations of *A. michauxii* were more likely to persist with lower rather than higher % other species cover (Table 3-1, 2). Sites where sub-populations failed to persist had eight times as much other species cover when compared to sub-populations that persisted. There were no differences between other habitat characteristics, including fire frequency and years-since-burn. Among the 48 sub-populations surveyed, there were no significant effects of total ground cover, canopy cover, or fire history on population persistence.

Variations in Growth and Reproductive Effort

Plant heights, stems per individual, numbers of inflorescences, flowers, pods, and *A. michauxii* individuals within sub-populations varied widely (Table 3-2). Plant height among individuals varied by more than 20 times. Numbers of inflorescences per plant varied as much as 50 times between plants. Average flower numbers per plant ranged from zero on plants with one to few stems, to more than 800 on plants having multiple stems.

Ninety-two percent of flowers withered without developing pods. High flower abortion rates are common in legumes. For example, only 14% of self pollinated peanut flowers produced mature pods (Smith 1954). Soybeans have floral abortion rates ranging from 20 to 80% depending upon the variety and environmental conditions (Hardman 1970; Reese 1993; Tenuta 2002). There are three possible explanations for the high flower loss in *A. michauxii* sub-populations. One, there was a pollinator limitation, two, *A. michauxii* has a physiological limitation, or three, the environment during flowering periods was too hot and dry (Tenuta 2002). The number of seeds collected from 88 plants in the summer of 2003 totaled 2100. Since only 25% of pods were collected, each plant produced at least 95 seeds. However, I have no way of estimating the number of seeds lost. In addition, all plants were not mature enough to reproduce.

Factors Affecting Vegetative and Reproductive Effort

Although Fort Bragg management aims to burn each rare plant site once every three years, actual fire frequencies between 1991 and 2002 ranged from 0.2 to 0.4 fire / year on *A. michauxii* sites with sub-populations that were present and from 0.2 to 0.3 fire / year on sites from which previous subpopulations were absent in 2003 (Appendix A-2, Table 3-1).

Plant height was negatively correlated with % other species cover but positively correlated with fire frequency (Table 3-3). Plant height and numbers of inflorescences and flowers were negatively correlated with % other species cover (Table 3-3). Fire is important to prevent reduction in plant growth rates as a result of understory overgrowth and shading out of smaller *A. michauxii* individuals. However, neither % other species cover nor fire frequency accounted for much of the variation in height, numbers of inflorescence, or numbers of flowers (Table 3-3, Figures 3-4, 3-5). A number of factors including, but not

limited to fire, competition for space and nutrients interact to influence plant growth. The removal of competing species and the thick litter layer from the understory by fire allows more of the very slow growing individuals to survive.

Time since burn did not affect vegetative or reproductive effort. Fire is not related to flowering in a broad suite of legumes species (Leach and Givnish 1996; Hiers *et al*. 2000). The ability to flower and fruit during years without fire may be necessary for population persistence. During years with no fire, plants need to reproduce despite the increased competition.

Factors Affecting the Number of Individuals in Clusters/Sub-Populations

Percent oak cover accounted for only a small amount of the variation in the number of *A. michauxii* plants and is not a determining factor (Table 3-4, Figure 3-6, note that a single outlier drives the relationship). *A. michauxii* and oak saplings may both increase in abundance after a fire when resources are plentiful and competition is limited. I found no dependence of the number of stems per individual on time-since-burn, fire frequency, ground cover or canopy cover (Table 3-4).

CONCLUSIONS

Sub-populations with lower percentage covers of understory species other than pine, oak, or wiregrass were more likely to persist. However, there were no significant effects of ground cover, canopy cover, or fire history on population persistence. None of the environmental factors tested was independently important to vegetative or reproductive effort, or the numbers of individuals clustered together or in a sub-population. However, these factors may work together and with other environmental factors to be important determinants of population growth of this species. It is important to control competition for space and soil nutrients, while increasing the probability of germination and seedling survival. The timing of fire and its physical effects may work together with the resulting changes in soil chemistry to facilitate increases in plant growth and reproduction.

Maintenance of the habitat by fire may be the key to population growth and persistence of *A. michauxii* and other rare plants. To optimize the effectiveness of fire, varying fire regimes should be tested for impacts on vegetative and reproductive efforts and population persistence. Determining impacts on pollination, particularly pollinator and other limitations to seed set, will help to properly time fires to maximize seed production while limiting competition. Though I did not find that persistence of *A. michauxii* sub-populations increases with fire frequency, alternating fire intervals may be more effective than a consistent interval at maintaining habitats for *A. michauxii* and other rare species.

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Table 3-1 Ground and canopy cover for 48 sub-populations of *A. michauxii* on Fort Bragg. P means a sub-population was present. A means the sub-population was absent. Significance was determined at $\alpha = 0.05$.

Variable	N		Mean		Minimum		Maximum		Std. Dev.		P>t
	P	A	P	A	P	A	P	A	P	A	
Persistence	21	27									
% Pine cover			1	1	0	0	7	20	2	4	0.06
% Oak Cover			20	13	0	0	60	50	19	17	0.32
% Wiregrass cover			12	16	0	0	50	80	13	19	0.35
% Other species cover			2	16	0	0	42	70	4	19	<0.01
% Total ground cover			44	51	12	5	93	93	22	21	0.39
% Canopy cover			62	61	57	35	65	65	2	6	0.84
Years-since-burn			2	2	1	1	6	9	1	2	0.48
Fire frequency			0.3	0.3	0.2	0.2	0.4	0.3	0.1	0.1	0.37

Table 3-2 Vegetative and reproductive effort of *A. michauxii*.

Variable	N	Mean	Minimum	Maximum	Standard Deviation
Total number of plants	88				
Height (cm)		64	6	132	22
# Stems / plant		2	1	10	2
# Inflorescence / plant		9	0	55	10
# Flowers / plant		150	0	863	158
# Pods / plant		7	0	64	12
# *A. michauxii* plants		9	1	19	6

Table 3-3 Correlation matrix of 11 parameters for 88 *A. michauxii* plants. Height is in cm, years-since burn is the number of years since the last fire, fire frequency is fires/year.

	# stems	# inflorescences	# flowers	# pods	yrs-since-burn	fire frequency	% pine cover	% oak cover	%wiregrass cover	% other species cover	% total cover	% canopy cover
height	**0.26**	**0.48**	**0.54**	**0.36**	-0.08	**0.25**	0.14	0.01	-0.03	**-0.38**	-0.06	-0.08
	0.01	<.001	<0.01	0.00	0.44	0.02	0.21	0.95	0.76	0.00	0.57	0.47
# stems		**0.77**	**0.68**	**0.54**	-0.15	0.02	**0.21**	0.11	-0.06	-0.20	0.13	0.10
		<0.01	<0.01	<0.01	0.15	0.87	0.05	0.32	0.59	0.07	0.25	0.34
# inflorescences			**0.94**	**0.67**	-0.16	0.04	**0.22**	0.04	0.08	**-0.28**	0.09	0.01
			<0.01	<0.01	0.15	0.74	0.04	0.69	0.48	0.01	0.40	0.91
flowers				**0.74**	-0.17	0.07	0.15	0.03	0.08	**-0.31**	0.06	0.03
				<0.01	0.12	0.55	0.15	0.80	0.44	0.00	0.58	0.77
pods					-0.14	0.19	0.18	0.00	0.10	-0.19	0.11	-0.12
					0.19	0.07	0.10	0.99	0.37	0.08	0.31	0.29
years-since-burn						0.12	-0.08	0.06	-0.02	0.20	0.06	0.00
						0.28	0.48	0.59	0.86	0.07	0.60	1.00
fire frequency							**0.29**	**-0.32**	**-0.25**	-0.01	**-0.37**	0.03
							0.01	0.00	0.02	0.91	0.00	0.82
% pine cover								**-0.39**	-0.15	-0.12	**-0.35**	0.15
								0.00	0.18	0.27	0.00	0.18
% oak cover									**-0.21**	**-0.23**	0.88	-0.11
									0.05	0.03	<0.01	0.29
% wiregrass cover										**-0.30**	0.05	**-0.34**
										0.00	0.64	0.00
% other cover											-0.01	0.01
											0.93	-0.90
% total cover												**-0.25**
												0.02

Table 3-4. Correlation matrix (r, probability of p>t) of 10 parameters for 49 plots within 48 *Astragalus michauxii* sub-populations surveyed. Height is in cm, years-since burn is the number of years since the last fire, fire frequency in fires/year.

	Fire frequency	Mean # *A. michauxii* in sub-population	#*A. michauxii* in 4 m^2 plot	% pine cover	% oak cover	% wiregrass cover	% other species cover	% total ground cover	% canopy cover
Years-since-burn	0.07 0.65	-0.12 0.40	-0.25 0.08	0.05 0.74	-0.10 0.48	-0.10 0.48	0.19 0.18	-0.05 0.75	0.20 0.18
Fire frequency		-0.23 0.11	-0.17 0.23	0.06 0.67	0.09 0.56	0.01 0.93	-0.17 0.24	-0.05 0.75	-0.05 0.75
#*A. michauxii* in sub-pop			**0.62** <0.01	0.17 0.23	0.25 0.09	0.03 0.83	-0.22 0.13	0.15 0.30	0.12 0.39
#*A. michauxii* in 4 m^2 plot				0.15 0.30	**0.28** 0.05	0.03 0.83	-0.21 0.15	0.25 0.09	0.12 0.40
% pine cover					-0.17 0.25	-0.08 0.58	-0.15 0.30	-0.16 0.28	0.09 0.55
% oak cover						-0.17 0.24	-0.27 0.06	**0.52** 0.00	0.05 0.75
% wiregrass cover							-0.21 0.15	**0.44** 0.00	-0.14 0.35
% other species cover								**0.31** 0.03	-0.09 0.56
% total ground cover									-0.10 0.50



Figure 3-1 *A. michauxii* in its habitat with long-leaf pine, shrub oak, and wiregrass. Stems are erect, leaves pinnately compound. Inflorescences grow out of leaf axes.

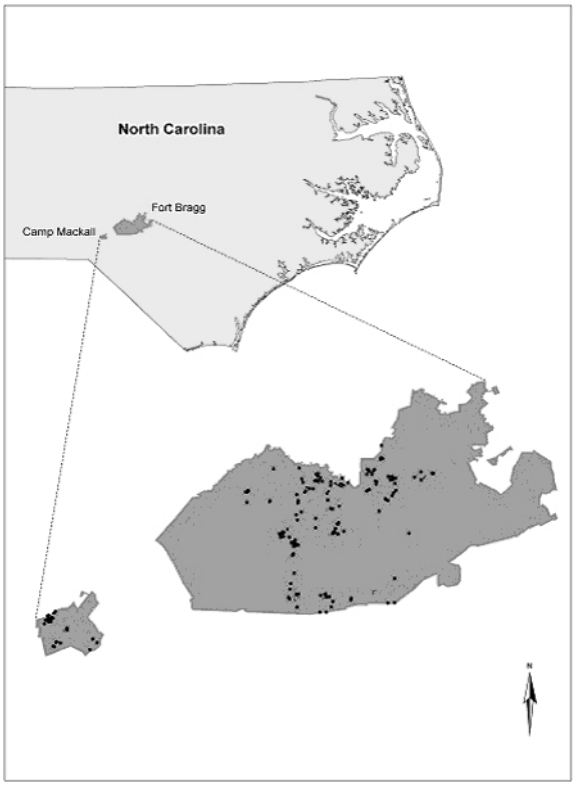

Figure 3-2 Element occurrences of *A. michauxii* on Fort Bragg and Camp MacKall Military Reservations in North Carolina.

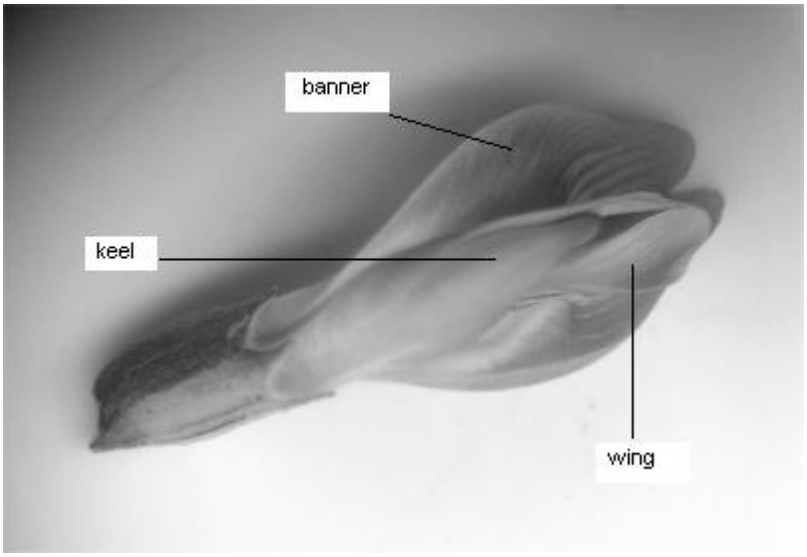

Figure 3-3 *A. michauxii* flower with banner, wings and keel.

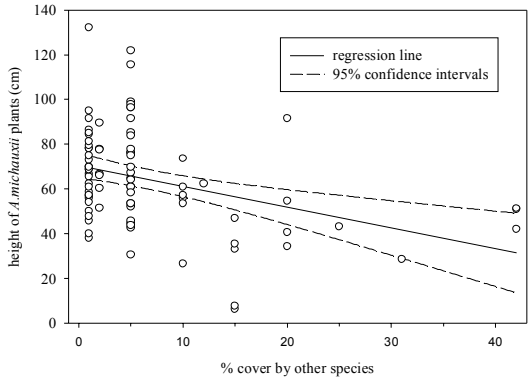

Figure 3-4 Graph of the effect of % other species cover on heights of 88 *A. michauxii* plants in 48 sub-populations at Fort Bragg. P = 0.0003, R^2 = 0.14.

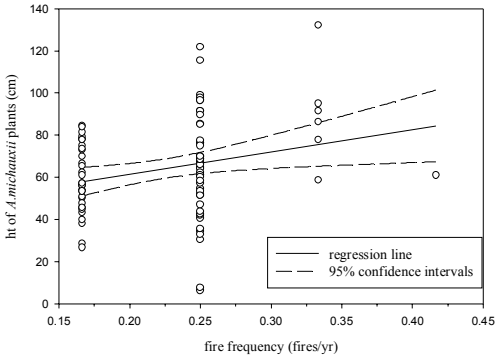

Figure 3-5 Graph of the effect of % fire frequency on heights of numbers of flowers on 88 *A. michauxii* plants in 48 sub-populations at Fort Bragg. P=0.0170, R^2 = 0.06.
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Figure 3-6 Graph of the effect of % oak cover on the number of *A. michauxii* plants in 49 4-m2 plots in 48 sub-populations at Fort Bragg. P=0.0277, R^2 square = 0.10.

CHAPTER 4

A Demographic Model for *Astragalus michauxii*

INTRODUCTION

Astragalus michauxii (Kuntz) Hermann (Sandhills milk-vetch) is a rare and threatened perennial legume (USFWS 1990, TNC 1993) occurring in open understory habitats of nutrient poor, fire-dependent, long-leaf pine (*Pinus palustris* Miller) /wiregrass (*Aristida stricta* Michaux) communities of the southeastern Atlantic coastal plains (Schafale and Weakley 1990). The largest *A. michauxii* population is located on the federally owned Fort Bragg Military Reservation in the North Carolina Sandhills. This population is mostly threatened by habitat loss as a result of fire suppression and habitat fragmentation due to land development.

Stem numbers in *A. michauxii* sub-populations are small with individuals occurring in isolated clusters of one to ten plants. Increasing stem number is important for population viability and persistence because it increases the probability of successful seedling establishment (Hiers *et al*. 2000). Also, in the event of stochastic environmental or demographic disturbance, small populations may not recover (Walker and Peet 1983) and so are at risk of extirpation. Although there is as yet no evidence linking population persistence of *A. michauxii* to stem numbers, these sub-populations may be at risk of becoming extirpated.

The growth and persistence of a plant population depends on germination, survival, and fecundity. Fire increases germination (Chapter 1, page 22). Heat from fire disrupts the integrity of the outer seed coat allowing imbibition. Fire related smoke chemicals interacting within the soil may facilitate germination after imbibition. The understory habitat is maintained by fire to minimize competition, enhance soil nutrient and water content, and increase the chances that a newly germinated seed will become established. The limited

number of *A. michauxii* seedlings present on *A. michauxii* sites relative to the number of seeds produced indicates either a lack of seeds in the seed bank or the lack of 'safe sites' where they can become established. 'Safe sites' are those areas in the habitat that have ideal environmental conditions optimal for germination and establishment (Harper 1977). The particular features of the environment determine the success or failure of a seed to becoming an established seedling. Both seed germination rates (Chapter 1, page 22) and number of seeds in the seed bank surrounding mature individuals (Chapter 1, page 17) were found to be less than adequate.

Plant mortality strongly influences population size and can lead to local extinction. As perennials, survival of *A. michauxii* individuals over winter and stem emergence the following growing season are important for population growth. High mortality rates over winter result in reduced stem counts available the following year for reproduction. At 41%, over-winter mortality rates in *A. michauxii* sub-populations were moderate (Chapter 2, 38). Increased vegetative growth increases plant survival over-winter to the following growing season (Chapter 2, 38). Growth is in turn indirectly linked to fire, since fire maintains the habitat and reduces competition for resources. Population persistence in *A. michauxii* is also linked to fire (Chapter 3, page 61). Populations with lower rather than higher percentages of species other than oak (*Quercus* spp), pine, or wiregrass in the understory, were more likely to persist. Regular prescribed fire is used as a management tool to reduce the percentages of other species cover in the habitat.

Identification of factors influencing population growth provides some direction for conservation efforts. However, it is more useful to determine if the population is stable or declining and gain some insight into where management should best focus conservation

efforts. The attributes of germination, survival and fecundity can be useful to determine whether a rare plant population is declining, increasing, or stable (Schemske *et al*. 1994). Modeling of demographic processes can give insights into population behavior, which could otherwise require long term field observations. Demographic modeling can be invaluable in directing conservation efforts to the types of field observation most likely to be meaningful. Manipulations to a demographic model can be made to determine how variations in parts of the life cycle of a species can change and thereby affect population growth.

I developed a demographic model for the *A. michauxii* population at Fort Bragg and used the model to calculate the population growth rate (λ). I then determine proportional and absolute importance of germination, survival, fecundity, and seed survival in the seed bank in effecting changes in population growth. I attempt to answer the following questions:

1) Is the population increasing, stable or declining?
2) How can management efforts be best directed to increase population growth of *A. michauxii*?

MATERIAL & METHODS

A Stage-structured Demographic Model

Plant populations can be thought of as existing in two parts. While one is growing in a vegetative state and producing seeds, the other is stored as seeds in a viable dormant state in the seed bank. The stage-structured demographic model developed by Schmidt and Lawlor (1983) for plant species with seed banks can be used as a springboard to model the demographics of the *A. michauxii* sub-populations at Fort Bragg (Figure 4-1).

The *A. michauxii* model defines transitions between life cycle stages for seeds and for plants:

Plants

p_r = probability of winter survival of mature plants

m = seeds produced per mature individual

Seeds

$p_{sb} = p_s p_w$ = probability of survival of seeds in the seed bank

Where:

p_s = probability of summer survival of seeds in the seed bank

p_w = probability of winter survival of seeds in the seed bank

p_{ge} = germination expressed as the probability of germination after a fire

The seed stage can be extracted from the model (Figure 4-1) and used to construct a graph with one node (Figure 4-2, Elbert 1999). The probabilities of germination (p_{ge}) and of not germinating ($1-p_{ge}$) are modeled. The top arrow represents plant survival through winter to flowering (p_r), reproduction (m), and seed survival in the seed bank (p_{sb}). P_{sb} includes seed survival in the summer, through winter, to the following summer.

The model makes two assumptions:

1) Since pods dehisce to disperse seeds (page 54), all seeds are assumed to undergo transformation from the plant to the seed bank before any mortality takes place.
2) Since the number of seeds in the seed bank is so small, assume mortality is high and the number of seeds entering the seed bank from plants is much greater than seeds already stored in the seed bank (page 85, Table 4-1).

Calculations

To analyze the life table graph, each transition is multiplied by λ^{-1} in a Z-transformation to normalize the transitions.

Loop 1: $\lambda^{-1} p_{ge}p_r m p_{sb}$ Equation 1

Loop 2: $\lambda^{-1} (1-p_{ge}) p_{sb}$ Equation 2

The two products are summed, and 1 minus this sum set to zero gives the population growth rate equation:

$$1 - \lambda^{-1} p_{ge}p_r m p_{sb} + \lambda^{-1} (1-p_{ge}) p_{sb} = 0 \quad \text{Equation 3}$$

Multiplying the entire equation by λ^1 to clear the equation of the λ^{-1} term gives:

$$\lambda - p_{ge}p_r m p_{sb} + (1-p_{ge}) p_{sb} = 0 \quad \text{Equation 4}$$

and

$$\lambda - p_{ge}p_r m p_{sb} + p_{sb} - p_{ge} p_{sb} = 0 \quad \text{Equation 5}$$

$$\lambda = p_{ge}p_r m p_{sb} - p_{sb} + p_{ge}p_{sb} \qquad \text{Equation 6}$$

Then the values for p_{ge}, p_r, m, and p_{sb} (Table 4-1) can be used to calculate λ:

$$\lambda = (0.02) \times (0.65) \times (343) \times (0.04) - (0.04) + (0.02) \times (0.04)$$

$$\lambda = 0.14\ yr^{-1}$$

Next, a sensitivity analysis is used to explore the impact of variations in parts of the life cycle on λ. The characteristic equation for the life cycle graph is:

$$f(\lambda,a_i) = \lambda - p_{ge}p_r m p_{sb} + p_{sb} - p_{ge}p_{sb} \qquad \text{Equation 7}$$

The partial derivatives which determine the sensitivity (Table 4-2) of λ to changes to the various parameters a_i are:

$$\partial f(\lambda,a_i) / \partial \lambda = 1$$

$$\partial f(\lambda,a_i) / \partial p_{ge} = - p_r m p_{sb} - p_{sb} = - 8.96$$

$$\partial f(\lambda,a_i) / \partial p_r = - p_{ge} m p_{sb} = - -0.274$$

$$\partial f(\lambda,a_i) / \partial m = - p_{ge}p_r p_{sb} = - 5.2 \times 10^{-4}$$

$$\partial f(\lambda,a_i) / \partial p_{sb} = - p_{ge}p_r m - p_{ge} = - 4.46$$

Then the sensitivities are calculated by:

$$\partial\lambda / \partial a_i = - \partial f(\lambda,a_i) / \partial \lambda \qquad \text{Equation 8}$$

The contributions of each factor in the life cycle graph are calculated as the proportionality of sensitivity or "elasticity" (Caswell *et al*. 1984). Elasticities (Table 4-2) are calculated by:

Elasticity = e_i

$$e_i = (a_i / \lambda) / (\partial\lambda / \partial a_i) \qquad \text{Equation 9}$$

Next, I calculate the maximum attainable value of λ (Table 4-1) when the parameters p_{ge}, $p_{r,}$ and p_{sb} are maximized at a value of 1 in the population growth equation (Equation 6).

RESULTS

The estimate of λ at 0.14 yr^{-1} indicates that the population declined between 2002 and 2003. Calculated elasticities for the four parameters in the life cycle of *A. michauxii* sub-populations are similar (Table 4-2). Larger elasticities correspond to a larger contribution of that attribute. However, the similar elasticities indicate that increasing the proportions of any one of the four parameters, p_{ge}, p_r, m, or p_{sb}, will not greatly influence the growth rate of the population.

When the probability of germination is maximized to 1, λ attains a value of 16. When the probability of seed bank survival is maximized to 1, λ has a value of 3.5 (Table 4-1). These results provide some insight into where to best focus management efforts. Increasing either p_{ge} or p_{sb} can potentially increase the population growth rate to greater than one which indicates a growing population. Increasing the probability of survival over winter to 1 can only raise λ to 0.24 which would not result in population growth.

Fire is a management tool used by Fort Bragg and is implicated in both germination and reproductive processes. Fire maintains the understory habitat, keeping competition at a reduced level to facilitate both growth and seed production. Fire also increased germination rates by facilitating imbibition (Chapter 1, page 22). However, while the heat from fire promotes germination, there is a risk of seed mortality due to over-heating. As an alternative, seeds of *A. michauxii* can be germinated artificially with great success (Chapter 1, page 26). Seedlings can also become well established under controlled conditions (Chapter 2, page 36). One conservation strategy with good potential for success is to collect seeds, germinate them artificially, grow the plants in the greenhouse until they are well established, and then transplant them into the established sub-populations.

The subsequent increase in stem number from this sort of undertaking, while not directly increasing fecundity, will ultimately increase the total number of seeds produced by each sub-population. A management strategy to increase fecundity directly is not straightforward because seed production is influenced by many factors which are either unknown or difficult to control because of interdependencies. Reproductive effort was not determined to be influenced by any one environmental factor (Chapter 3, page 59).

Although λ could be theoretically increased by improving the probability of seed survival (Table 4-1), controlling seed survival in the seed bank is not a practical means of trying to increase stem numbers. There are so many factors interacting in the soil that it would be difficult to control seed mortality. In addition, if the number of seeds surviving in the seed bank increases, seeds will still have to germinate to eventually lead to recruitment. Seeds remaining dormant in the seed bank will not increase stem number. Maximizing plant survival over winter is not indicated to increase λ to a level of population growth (Table 4-1). However, over-winter survival rates used in this analysis may have been deceptively low because the plants used were planted late in the growing season and were not kept well-watered. Also, the data were obtained over one winter and from only two plots, lacking both temporal and spatial variability (Chapter 2, page 31). Therefore, a high level of sensitivity of the population growth rate to changes in plant survival over winter can not be ruled out.

DISCUSSIONS & CONCLUSIONS

The population growth rate, λ, was estimated at 0.14 yr^{-1}. Based on λ, the population declined during the period from summer of 2002 to summer of 2003. With respect to proportionality change in λ, elasticity, $m = p_{ge} = p_r = p_{sb}$. The population growth rate is equally sensitive to changes in all four parameters of the life cycle of *A. michauxii*. Population growth can increase by enhancing germination rates or seed survival in the seed bank. Management efforts for the conservation of *A. michauxii* can best be directed by:

1) *The collection and artificial germination of seeds*. When well established, seedlings can be transplanted into existing sub-populations. Increasing stem count will increase the population growth rate by increasing the probability of seedling survival and overall reproductive effort of the sub-population. Sub-populations with higher, rather than lower stem counts can better withstand year to year environmental stochasticity. The introduction of taller and established seedlings will increase the probability of survival to maturity. Seedlings that were not well established were less likely than established plants to survive over winter (Chapter 2, page 38).

2) *Experimental testing of the impacts of varying fire regimes on vegetative and reproductive effort*. The wearing away of the outer coat, penetration of the inner coat and leaching of germination inhibiting substances from the embryo happen slowly over time in the soil. Although seed dormancy reduces the rate of increase of the population though reduction in germination rates, the entire population is not put at risk of extirpation as a result of synchronous germination. Since the

environment is always changing, the future of a population may be better secured by waiting for a favorable period to germinate. Nonetheless, seed production, germination and seedling establishment need to be adequate to ensure population growth. Fire may be an important tool for management of new recruits into the population. It is also necessary to maintain habitat quality so that competition levels are reduced and resource needs are met for maximum growth and reproduction and subsequent population growth. The proper timing of fire to keep competition at a reduced level can potentially manage for a suite of rare plant species.

The population growth model employed here could be improved by 1) the acquisition of seed germination and over-winter survival data from natural sub-populations, 2) obtaining more accurate seed production and seed bank seed survival, and 3) introducing temporal and more spatial variation in the data. For example, over-winter survival data will be more accurate when collected over a number of winters and for a number of natural sub-populations. These implementations will allow a more accurate determination of population growth rate and subsequent identification of those factors that can best be manipulated in conservation efforts for population growth.

A population viability analysis (PVA) using count data is a potentially useful tool for predicting population growth rate as well as the likely future status of a population. Since the Fort Bragg population is a metapopulation consisting of patches of sub-populations, the PVA will need to take into account single sub-population viability, movement rates between populations, and correlation in factors that affect the different sub-populations (Groom 1999). Data will have to have temporal as well as spatial variation, and should include stem

counts for at least 10 years. Data needed to perform a PVA on the *A. michauxii* population are currently lacking.

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Table 4.1 Probabilities of life cycle parameters of *A. michauxii* in 2003 and the value of **λ** when parameters are maximized.

Parameter	**Descriptions and Sources**	**Actual Values in 2003**	**λ_{max} when Parameter = 1**
P_{ge}	Probability of germination, Table 1-1, page 22	0.02	16
P_r	Probability of mature plant over-winter survival, page 38	0.65	.24
m	Seeds produced per flowering individual, page 6	343	-
p_{sb}	Probability of summer and winter seed survival, page 15	0.04	3.58

Table 4-2 Sensitivities and elasticities for the life cycle of *A. michauxii* sub-populations.

Parameter	$\partial\lambda/\partial a_i$	**Elasticity**
P_{ge}	8.96	1.28
P_r	.27	1.28
m	5.2×10^{-4}	1.27
p_{sb}	4.46	1.27

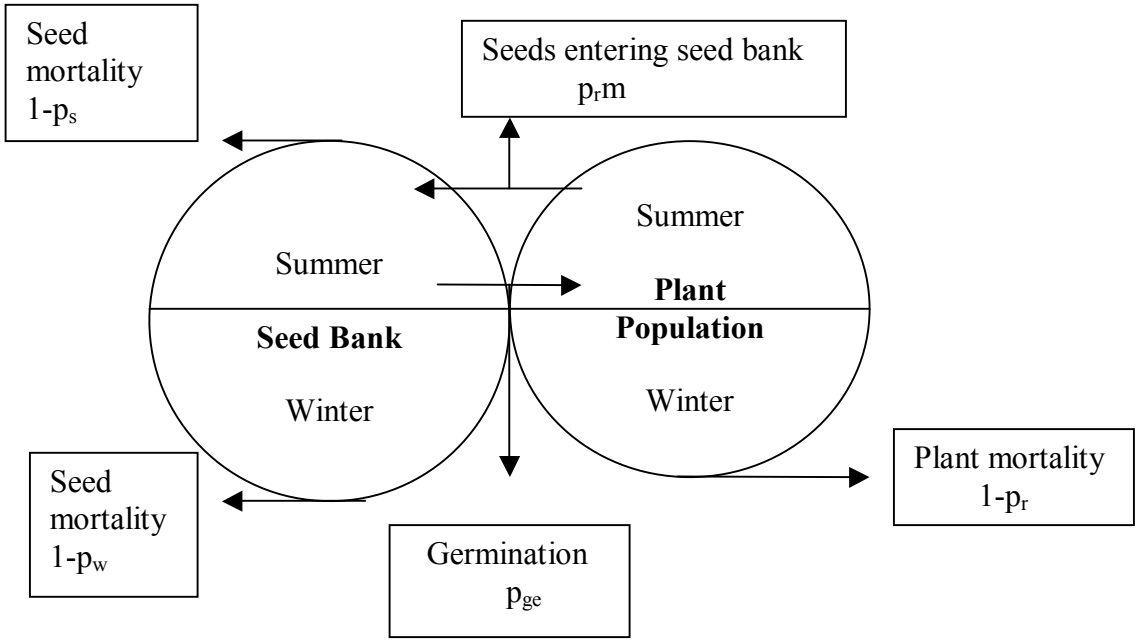

Figure 4-1 Life cycle of *A. michauxii* based on the model of Schmidt and Lawler (1983). The circle on the left represents the seed stage and the one on the right the vegetative stage of plant population. Each stage is sub-divided into summer and winter seasons. Seeds produced in by the plant population are transferred to the seed bank. Germinated seeds in turn move from the seed stage into the plant stage as seedlings. Boxes represent probabilities in the life cycle. The parameters are probabilities of germination (p_{ge}), seed survival in the seed bank in summer (p_s), seed survival in the seed bank in winter (p_w), plant survival over winter (p_r), and fecundity (m).

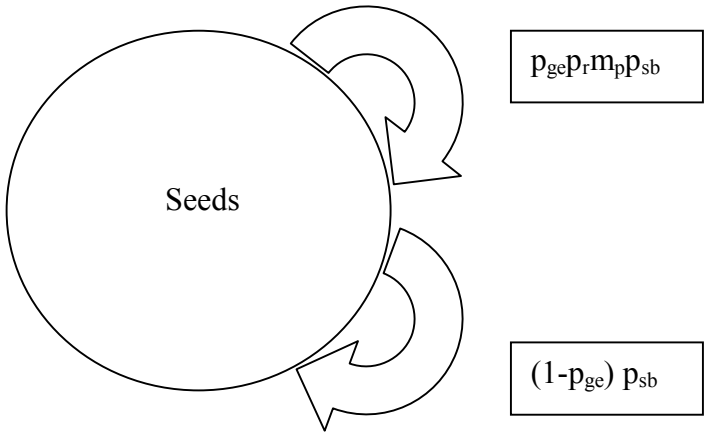

Figure 4-2 Demographic model recast as a graph with one node for *A. michauxii*. P_{ge} is the probability of germination and $(1-p_{ge})$ is the probability of not germinating. The top arrow represents survival through winter to flowering (p_r), reproduction (m), and seed survival in the seed bank (p_{sb}).

APPENDICES

Appendix A: Supplemental Tables

Table A-1 Average monthly recorded readings in ° C from 1971-2000 from Fayetteville Pope Air Force Base in Cumberland County, NC.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Ave. High	11.5	13.8	18.2	22.9	27.1	30.8	32.6	31.3	28.4	23.3	18.3	13.3
Ave. Low	0.6	1.8	5.7	9.9	15.0	19.6	22.2	21.4	17.8	10.7	5.7	2.1

Table A-2 Survey data taken in 1991-93 and 1998-99 for all *A. michauxii* sites with sub-populations recorded by Element Occurrence Number (EONUMBER), on Fort Bragg, including area, density, and burn history of each sub-population. PA91 and PA99 –presence/absence in the 1991-93 and 1998-99 surveys. AREA91 and AREA99 represent areas of sub-populations recorded in the 1991-93 and 1998-99 surveys in acres. Sub-populations listed as present but showing a zero area indicates very low densities. Sub-populations were entered into the GIS program as point features instead of polygons, therefore areas were not calculated. DEN91 and DEN99 indicate recorded stem count per area surveyed in 1991-93 and 1998-99. B91 through B02 indicate burn treatment of each site, if any, for the years 1991 and 2002. N, G, and W indicate no-burn, growing season burn, and wildfire, respectively. * indicates sites which were surveyed. All EO Numbers have ASMI before the number.

EONUMBER	PA91	PA99	AREA 91	AREA 99	DEN91	DEN99	B91	B92	B93	B94	B95	B96	B97	B98	B99	B00	B01	B02
*ASMI020A	1	1	0	0.74	5	46	N	N	G	N	G	N	N	N	N	G	N	N
ASMI020B	1	1	0	0.05	1	1	G	N	N	N	D	N	G	N	N	D	N	W
*ASMI020C	1	1	0	0.06	9	2	N	N	G	N	G	N	N	N	N	N	G	N
ASMI020D	1	1	0	0.52	7	20	N	N	G	N	G	N	N	N	N	N	G	N
*ASMI022A	1	0	0	0	10	0	N	N	N	N	G	N	N	G	N	N	G	N
*ASMI022B	1	1	0	0.25	1	7	N	N	N	N	G	N	N	G	N	N	G	N
ASMI022C	1	1	0	0.25	3	7	N	N	N	N	G	N	N	G	N	N	G	N
ASMI022D	1	1	0	0.25	10	7	N	N	N	N	G	N	N	G	N	N	G	N
ASMI022E	1	1	0	0.03	2	3	N	N	N	N	G	N	N	G	N	N	G	N
ASMI023A	1	1	0	2.7	6	75	N	N	N	N	G	N	D	N	N	N	G	N
ASMI023B	1	1	0.01	2.7	6	75	N	N	N	N	G	N	D	N	N	N	G	N
*ASMI023C	1	1	0	0.06	2	1	N	N	N	N	G	N	D	N	N	N	G	N
*ASMI023D	1	1	0	0.41	3	13	N	N	N	N	G	N	D	N	N	N	G	N
*ASMI030A	1	1	0.26	0.15	0	0	N	G	N	N	N	D	N	N	D	N	G	N
ASMI031A	1	0	0	0	1	0	G	N	N	N	D	N	N	N	N	N	N	D
ASMI031B	1	1	0	0.01	2	1	N	N	N	D	N	G	N	N	N	N	N	G
ASMI031C	1	1	0	0.02	4	5	G	N	N	N	D	N	N	N	N	N	W	N
*ASMI032A	1	1	26.51	4.41	139	104	N	N	N	N	G	N	N	N	N	N	G	N
ASMI032C	1	1	15.37	2.98	48	51	G	N	N	G	N	N	N	N	N	G	G	N
ASMI032D	1	1	0.25	0.45	16	9	D	G	N	G	G	N	G	N	N	N	G	N

EONUMBER	PA91	PA99	AREA 91	AREA 99	DEN91	DEN99	B91	B92	B93	B94	B95	B96	B97	B98	B99	B00	B01	B02
*ASMI032E	1	1	0	0.03	4	5	N	G	N	G	G	N	N	N	N	N	G	N
*ASMI032F	1	1	0	0.31	2	5	N	N	N	N	N	G	N	N	G	G	N	G
*ASMI033A	1	1	0	0.04	1	4	N	D	N	N	N	N	N	D	N	N	G	N
*ASMI033B	1	1	0	0.03	1	2	N	D	N	N	N	N	N	D	N	N	G	N
*ASMI034A	1	1	1	0.1	10	2	N	N	N	N	N	G	N	N	N	N	N	G
*ASMI034B	1	1	8.29	7.52	14	31	N	N	N	D	N	G	N	N	D	N	G	W
*ASMI035A	1	1	0	0.02	2	2	N	D	N	N	N	N	G	D	N	N	G	N
*ASMI035B	1	1	0	0.02	0	0	N	D	N	N	N	N	G	D	N	N	G	N
*ASMI035C	1	1	0	0.04	4	5	N	D	N	N	N	N	N	N	N	N	G	N
*ASMI035D	1	0	0	0	2	0	N	D	N	N	N	N	N	N	N	N	G	N
*ASMI035E	1	1	0	0.04	1	2	N	D	N	N	N	N	N	N	N	N	G	N
*ASMI035F	1	0	0	0	3	0	N	D	N	N	N	N	N	N	N	N	G	N
*ASMI035G	1	1	0	0.02	4	4	N	D	N	N	N	N	N	D	N	N	G	N
*ASMI036A	1	1	0	0.01	1	1	N	N	G	N	N	G	N	N	N	N	N	G
*ASMI036B	1	0	0	0	1	0	N	N	G	N	N	G	N	N	N	N	N	G
ASMI036C	1	0	0	0	1	0	N	N	G	N	N	G	N	N	N	N	N	G
*ASMI036D	1	0	0.03	0	4	0	N	N	G	N	N	G	N	N	N	N	N	G
ASMI037A	1	1	0	0.01	1	1	D	N	N	N	N	N	G	N	N	G	N	N
ASMI038A	1	0	0	0	1	0	D	N	D	N	N	N	N	N	G	N	N	G
ASMI038B	1	1	0	0.01	4	1	N	N	G	N	N	G	N	N	N	N	N	G
*ASMI039A	1	0	0	0	2	0	N	N	N	N	G	N	N	G	N	N	G	N
ASMI040A	1	1	0	0.01	2	2	N	N	N	N	N	G	N	N	G	N	N	N
ASMI041A	1	1	0	0.02	3	1	G	N	N	N	D	N	G	N	N	N	N	N
ASMI041B	1	1	0	0.02	1	2	G	N	N	N	N	N	G	N	N	N	N	N
ASMI045A	1	1	0	0.02	2	6	D	N	N	N	N	N	N	N	N	N	N	N
ASMI045B	1	0	0	0	1	0	D	N	N	N	N	N	N	N	N	N	N	N
ASMI045C	1	1	0	0.08	1	1	D	N	N	N	N	N	N	N	N	N	N	N
ASMI045D	1	1	0	0.02	7	2	N	N	N	N	N	N	N	N	N	N	N	N
ASMI045E	1	1	0	0.04	2	8	G	N	N	N	G	N	N	N	N	N	N	G
ASMI046A	1	1	0.22	0.04	1	4	N	N	N	N	N	N	N	N	N	N	G	N

EONUMBER	PA91	PA99	AREA 91	AREA 99	DEN91	DEN99	B91	B92	B93	B94	B95	B96	B97	B98	B99	B00	B01	B02
ASMI047A	1	1	0	0.1	7	5	G	N	N	G	N	N	G	N	N	N	N	G
*ASMI048A	1	0	0	0	1	0	N	N	D	N	G	N	N	G	N	N	G	N
*ASMI049A	1	1	3	0.37	30	11	N	N	N	G	N	D	N	N	N	N	N	G
*ASMI050A	1	1	5.9	1.53	30	45	N	N	G	N	N	G	N	N	G	G	N	G
ASMI050B	1	1	0	0.01	2	2	N	G	N	N	G	N	N	N	N	D	G	N
*ASMI050C	1	1	12.14	0.81	5	16	N	G	N	N	N	N	N	N	N	N	G	G
ASMI050D	1	1	0	0.07	1	7	G	N	N	N	N	N	G	N	N	G	N	N
*ASMI051A	1	1	2.49	2.83	1	130	N	G	N	N	N	N	N	G	N	G	N	N
*ASMI051B	1	1	0.25	0.15	3	3	N	N	G	N	N	G	N	N	N	N	N	G
*ASMI052A	1	1	0	0.11	2	11	N	G	N	N	G	N	N	N	N	N	N	G
ASMI053A	1	1	1.27	0.16	50	28	N	G	N	N	N	N	G	D	D	N	G	N
ASMI054A	1	1	0	0.05	3	9	G	N	N	N	N	N	G	N	N	N	N	N
ASMI054B	1	1	7.5	0.01	4	3	N	N	N	D	D	D	D	D	D	N	G	N
ASMI055A	1	0	0	0	2	0	N	N	N	N	N	N	N	N	N	N	D	D
ASMI055B	1	1	1.71	0.79	0	0	N	N	N	N	N	N	N	N	N	N	D	D
*ASMI056A	1	0	0	0	1	0	G	N	N	N	N	N	G	N	N	N	D	N
*ASMI056B	1	1	0.45	0.07	4	7	G	N	N	N	G	N	N	N	N	N	G	N
*ASMI056C	1	1	18.85	0.99	7	40	G	N	N	N	N	N	N	N	N	N	G	N
*ASMI056D	1	1	16.8	0.34	15	90	N	N	D	N	G	N	N	G	N	N	G	N
*ASMI056E	1	0	10	0	2	0	N	N	D	N	G	N	N	N	N	N	N	G
*ASMI056F	1	1	0	0.14	1	4	N	N	G	N	N	N	N	N	G	N	N	G
ASMI056G	1	1	15.35	9.39	23	34	G	N	N	G	N	N	G	N	N	G	N	N
*ASMI056H	1	1	10	0.02	3	2	N	N	N	N	G	N	N	N	D	N	G	N
ASMI057A	1	1	8	0.01	14	4	N	D	N	N	N	N	N	N	N	N	G	N
ASMI057B	1	1	8	0.01	14	2	N	D	N	N	N	N	N	N	N	N	G	N
ASMI057C	1	1	8	0.03	14	6	N	D	N	N	N	N	N	N	N	N	G	N
*ASMI057D	1	1	8	0.26	14	10	N	N	G	N	N	G	N	N	N	N	N	G
ASMI057E	1	1	8	0.02	25	3	N	D	N	N	N	N	N	N	N	N	N	N
ASMI057F	1	0	8	0	25	0	N	D	N	N	N	N	N	N	N	N	N	N
*ASMI057G	1	1	8	0.06	0	0	N	D	N	N	N	N	N	N	N	N	G	N

EONUMBER	PA91	PA99	AREA 91	AREA 99	DEN91	DEN99	B91	B92	B93	B94	B95	B96	B97	B98	B99	B00	B01	B02
*ASMI057H	1	1	8	0.08	14	10	N	N	G	N	N	G	N	N	N	N	N	G
ASMI059A	1	0	0	0	2	0	N	N	N	N	N	G	N	N	G	N	N	G
ASMI059B	1	0	0	0	1	0	G	N	N	G	N	N	G	N	N	N	N	N
ASMI060A	1	1	0	0.03	5	5	G	N	N	D	N	N	D	N	N	N	N	N
ASMI061A	1	0	0	0	8	0	G	N	N	G	N	N	G	G	N	N	N	N
ASMI061B	1	1	0	0.1	2	3	G	N	N	G	N	N	G	G	N	N	N	N
ASMI061C	1	1	0	0.02	5	4	G	N	N	G	N	N	G	G	N	N	N	N
ASMI061D	1	0	0	0	3	0	G	N	N	G	N	N	G	G	N	N	N	N
ASMI085A	0	1	0	0	0	1	N	N	N	N	G	N	N	G	N	N	G	N
ASMI086A	0	1	0	0	0	3	D	N	N	N	N	N	N	N	N	N	N	N
ASMI087A	0	1	0	0	0	2	N	G	N	N	N	N	G	D	D	N	G	N
ASMI088A	0	1	0	0	0	5	N	N	N	N	N	N	N	N	N	N	N	N
ASMI089A	0	1	0	0.04	0	4	G	N	N	G	N	N	G	N	N	N	N	N
*ASMI090A	0	1	0	0	0	1	N	N	G	N	G	N	N	N	N	N	G	N
*ASMI091A	0	1	0	0.64	0	25	N	N	N	N	N	G	N	N	N	N	N	G
*ASMI092A	0	1	0	0.01	0	2	N	N	N	N	N	G	N	N	N	N	N	G
ASMI093A	0	1	0	0.13	0	7	G	N	N	G	N	N	G	G	N	N	N	N
ASMI094A	0	1	0	0.01	0	1	G	N	G	N	N	N	G	N	G	N	W	N
ASMI095A	0	1	0	0.01	0	1	D	N	N	N	N	N	G	N	N	G	N	N
*ASMI096A	0	1	0	0	0	5	N	G	N	G	G	N	N	N	N	N	G	N
ASMI097A	0	1	0	0.02	0	4	D	N	N	N	N	N	G	N	N	N	N	N
*ASMI098A	0	1	0	0	0	1	D	N	N	N	N	N	N	G	N	N	G	G
*ASMI099A	0	1	0	0.01	0	2	N	N	G	N	N	N	N	N	N	G	N	G
ASMI100A	0	1	0	0.04	0	5	N	G	N	N	N	N	G	D	D	N	G	G
ASMI101A	0	1	0	0.01	0	2	N	G	N	N	N	N	G	D	D	N	G	G
ASMI102A	0	1	0	0.19	0	7	G	N	N	N	G	N	N	N	N	N	N	G

Appendix B: Supplemental Figures

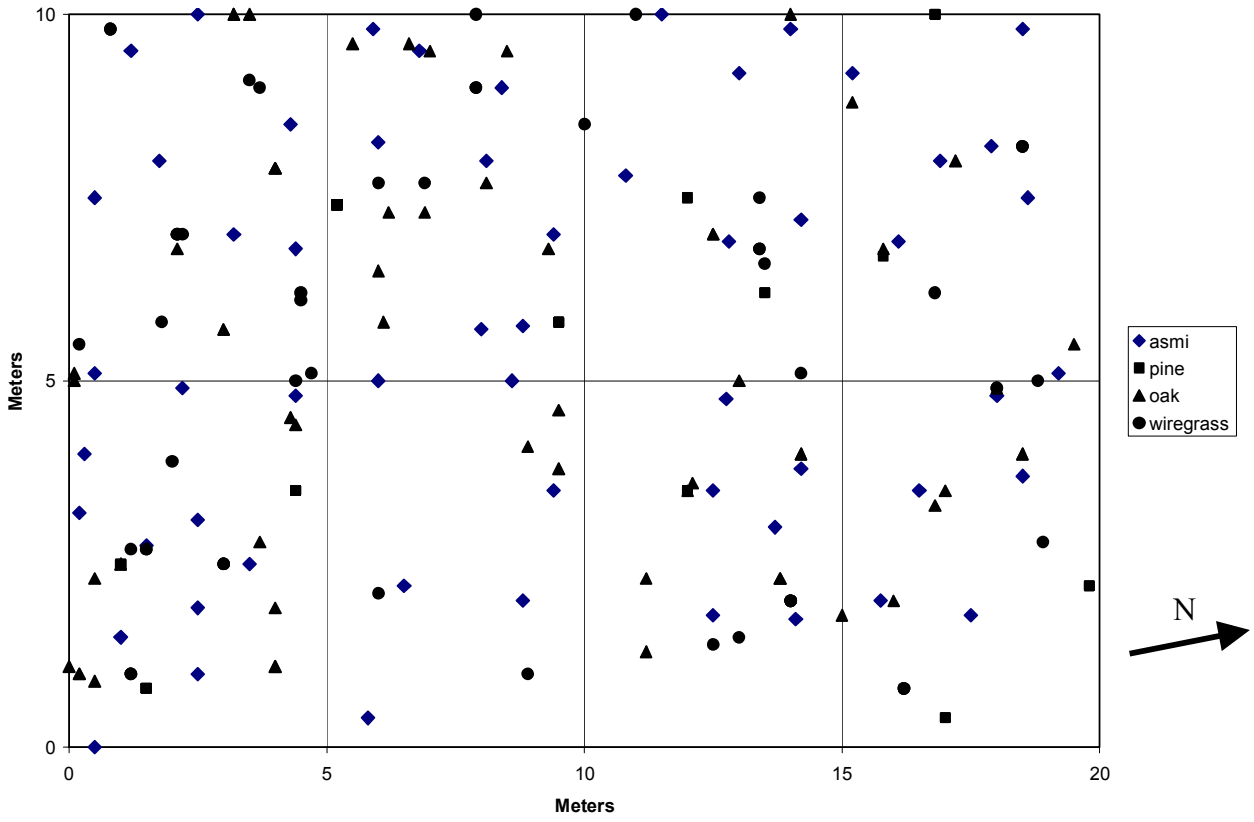

Figure B-1 Plot layout of Site 1 showing spatial relationships between *A. michauxii* and competing species. Asmi stands for *A. michauxii*

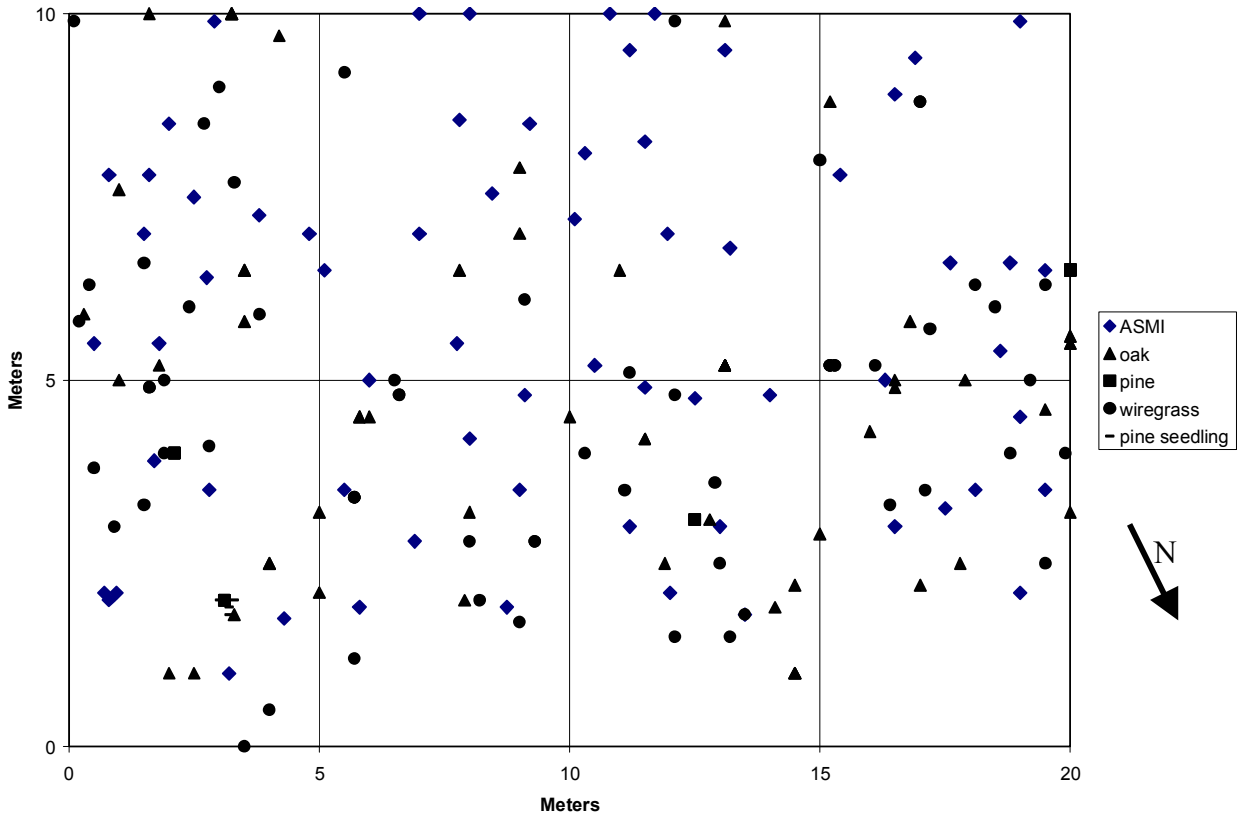

Figure B-2 Plot layout of Site 2 showing spatial relationships between *A. michauxii* and competing species. Asmi stands for *A. michauxii*.

Appendix C: Production of reproduction of plants used in the survival experiment.

Production of younger and older cohorts of plants

One hundred and twenty germinated seeds were planted in 225 ml Styrofoam cups filled with 1:1:2 peat moss/vermiculite/pea gravel substrate at a depth of approximately 1 mm. Following planting, the seedlings were watered and placed in an unshaded 26°C/22°C greenhouse. This temperature was chosen based on the average monthly high growing season temperatures in the North Carolina Sandhills (Appendix A-1). At the time of initial transfer, all seedlings were watered daily with nutrient solution[2] and flushed once a week with deionized water. As a result of continual seedling mortality, the plants were watered without nutrients and only as needed. When well established, and between 4 and 5 cm tall, the plants were transferred to 600 ml plastic pots filled with the same 1:1:2 peat moss/vermiculite/pea gravel substrate used in initial seedling planting, and allowed to grow in the 26°C/22°C greenhouse. Plants were transferred to 1650 ml and then 4000 ml pots whenever their roots began to protrude out of the holes at the bottom of the pots. Growth characteristics and vegetative traits were observed. This set of plants was the source of the older cohort.

One hundred additional germinated seeds were planted in 225 ml Styrofoam cups in the same 1:1:2 peat moss/vermiculite/pea gravel substrate as above. The seedlings were watered as needed and placed in the same unshaded 26°C/22°C greenhouse. Growth characteristics were observed. The surviving plants from this group made up the younger cohort.

[2] NCSU Phytotron Procedural Manual

Reproduction of older cohort of plants

To observe reproductive traits, I determined optimal temperature and photoperiodic conditions suitable for flowering and fruiting as follows. After the youngest plants reached maturity, 100 plants were evenly divided into 4 groups, taking care to include representative plants of the different sizes and structures in each group. I considered a plant to have reached maturity at the termination of vegetation height growth and in some cases, at the onset of reproduction. One set of plants was placed into growth chambers under each of the following four conditions:

1. 9/15 hr light/dark at 30°C/26°C, with a three-hour interruption of the dark period, to create long day conditions.
2. 9/15 hr light/dark) at 26°C/22°C, with a three-hour interruption of the dark period, to create long day conditions.
3. 9/15 hr light/dark at 22°/18°C, with a three-hour interruption of the dark period, to create long day conditions.
4. 9/15 hr light/dark, at 10°C/5°C, as short day conditions.

The short day treatment at 10°C/5°C represents cooling fall temperatures as winter approaches. The light period of 9 hours was provided by cool-white fluorescent and incandescent lamps. Light from incandescent lamps was used for the dark period interruption. The plants remained in the growth chambers for thirty days and were then removed. The plants under long day 30°C/26°C and short day 10°C/5°C conditions were transferred to the 26°C/22°C greenhouse, where they remained until they were taken to the field to be planted in the study plots. The plants were not allowed to remain longer under

conditions of long day 30°C/26°C because of accelerated growth and weakened vine-like stems. After treatment, these plants had to be supported with wooden stakes.

Plants were removed from the short day 10°C/5°C conditions at four weeks in order to induce flowering. These vernalized plants were transplanted to larger pots because of extensive root growth observed after treatment. Roots structure and volumes were observed during transplanting. The plants under long day 22°C /18°C and 26°C/22°C conditions were allowed to remain under those conditions. The plants were observed upon removal from the treatment chambers and then every other day for growth and reproductive traits.

All pods produced in the controlled-environment rooms or in the temperature-controlled greenhouses contained ovules that had enlarged to about 0.5 mm diameter but no seeds. A pollen grain viability test was carried out to ensure that the pollen grains were alive and could germinate. Following the methods of Brewbaker and Kwack [3], three pieces of filter paper were moistened with deionized water and placed in three Petri dishes. A drop of Brewbaker-Kwack nutrient medium was placed on each of three microscope slides and the slides placed on top of the filter paper. A mixture of pollen grains of three flowers from three plants was sprinkled on the nutrient drops. The Petri dishes were placed in a 30°/26° C germinator and observed once a day for pollen tube growth. At the end of three days, the average % germination was estimated and the observations terminated due to microbial infection.

[3] Brewbaker, J. L., and B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50(9):747-858.