ABSTRACT

JESSUP, RACHEL AMBER. Habitat Description and Experimental Augmentation of Two Rare Piedmont Prairie Species: Oak Barrens Barbara's-buttons, *Marshallia legrandii* Weakley, and smooth purple coneflower, *Echinacea laevigata* (C.L. Boynt. & Beadle) Blake. (Under the direction of Dr. Jodi Forrester)

Pervasive land use change and fire suppression driven by European colonization have made Piedmont Prairie ecosystems and their associated species subjects of conservation concern in the southeastern United States. Piedmont Prairies are often characterized by species-rich understories dominated by grasses and forbs with sparse, fire adapted trees in the canopy. This study focuses on two rare prairie-associate species: the critically imperiled Oak Barrens Barbara's-buttons (*Marshallia legrandii*) and the federally listed, smooth purple coneflower (*Echinacea laevigata*).

Our primary study site was Picture Creek Diabase Barrens, a State Natural Area and Preserve in Granville Co., NC, which houses the largest known populations of both *M. legrandii* and *E. laevigata*. Our secondary study site was Difficult Creek Natural Area Preserve, Halifax Co., VA, another site supporting *E. laevigata* and one of the two other known extant populations of *M. legrandii*.

We performed flowering head count surveys of *M. legrandii* at both sites to investigate the current population size, distribution, and status of *M. legrandii*. At Picture Creek Diabase Barrens, environmental measurements (canopy openness, soil moisture, soil properties, and surrounding vegetation) were taken to describe the habitat niches that *M. legrandii* and *E. laevigata* occupy on the site. We also measured neighboring vegetation composition and cover

around flowering *E. laevigata* at Picture Creek Diabase Barrens to describe the composition, cover, and structure of plants that grow where extant *E. laevigata* is successful.

The second focus of our study was to establish experimental augmentations of *E. laevigata* and *M. legrandii* in a restored Oak Barrens Woodland at Picture Creek Diabase Barrens outside of the powerline where their conservation could be prioritized. The *E. laevigata* augmentation allowed us to investigate how neighboring life forms (graminoids, forbs, and vines) affected the growth and success of *E. laevigata.* We planted seedlings in plots dominated by one potentially competing life form and measured their survival and growth in the following years. The *M. legrandii* augmentation focused on the light preference of the species. We planted seedlings in three different light environments (along the north side of a forest edge, in full sun, and among scattered trees).

We found that since the last flower count survey in 2012, the population of *M. legrandii* at Picture Creek Diabase Barrens grew from 459 flowering stems to 7,872 in 2021 and 5,493 in 2022. The Picture Creek Diabase Barrens *M. legrandii* population had more open canopy (57.5% avg. openness) and flowering stems than the population at Difficult Creek (36.3% avg. openness, 1,284 flowering stems).

We found that flowering *E. laevigata* plants at Picture Creek Diabase Barrens grew in a diverse mixture of graminoids and forbs that differed in cover and composition between two environments (powerline and forest glade). *Echinacea laevigata* displayed signs of plasticity in growth in the two different environments; plants in the powerline had more vegetative cover in the first quarter meter above the ground than forest glade plants, while plants in the forest glade had more cover above half a meter than in the powerline.

Length and width of the largest leaves in the *E. laevigata* augmentation plots were significantly different between each of the life form treatments. We discovered that plots with vegetation clipped prior to planting had higher survival and earlier flowering than plots that were not clipped. While initial planting survival of *E. laevigata* was high, suspected vole predation in the summer of 2021 had a devastating impact in half of our planted experimental augmentation plots. Planted *M. legrandii* seedlings had high survival in 2021 and 2022.

Future research will expand the plot design and continue to measure the effects of different light regimes on *M. legrandii* and vegetation competition on *E. laevigata*. Managers of natural populations at Picture Creek Diabase Barrens and Difficult Creek Natural Area Preserve should consider thinning the canopy and midstory along with introducing or maintaining fire to promote these two rare species.

Habitat Description and Experimental Augmentation of Two Rare Piedmont Prairie Species: Oak Barrens Barbara's-buttons, *Marshallia legrandii* Weakley, and Smooth Purple Coneflower, *Echinacea laevigata* (C.L. Boynt. & Beadle) Blake

by
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DEDICATION

This thesis work is dedicated to:

My parents, Sarah and John, who have supported my endless pursuit of plant knowledge in every way imaginable. It is not every child who asks for "garden dirt" for their birthday and certainly not every parent who would gift it.

My siblings, Jackson, Jillian, and Tucker who regularly lift my spirits with their humor and inspire me to live life to the fullest with their own adventures.

My partner, Matt, who has supported me daily through this academic journey with a healthy dose of love, encouragement, and stoicism.

All the family, friends, peers, teachers, students, and memorable acquaintances who have starred in the chapters of my life leading to this.

BIOGRAPHY

Rachel Jessup is a second year M.S. Forestry student at North Carolina State University, College of Natural Resources in Raleigh, North Carolina. She received a B.S. Forest Management and B.S. Plant Biology from the same university. She is interested in the natural history, ecology, taxonomy, care, and management of plants and their communities, especially those of conservation concern.

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Chapter 1: Introduction

Background: Piedmont Prairies

"Piedmont Prairie" is a broad term used to describe a number of unique ecosystems of conservation concern in the southeastern United States including savannahs, woodlands, and barrens (Barden 1997). They are often characterized by species rich understories dominated by grasses and forbs with sparse, fire tolerant trees in the canopy (Davis et al. 2002, Szakacs 2020). They naturally occur alongside other plant communities, governed by variations in fire frequencies, soil moisture, sun aspect, and soil properties in the rolling hills characteristic of the Piedmont region (Szakacs 2020). Piedmont Prairies are thought to have been a prominent component of the landscape, but pervasive land use change and fire suppression, driven by European colonization, have degraded and extirpated these ecosystems and their associated and endemic species throughout their range. Today, remnants of these once common ecosystems are relegated to utility rights of way and roadsides, where canopy cover remains open as a side effect of infrastructure maintenance that reduces woody vegetation such as mowing, herbicide, and clearing (Davis et al. 2002).

Primary Study Site: Picture Creek Diabase Barrens

Picture Creek Diabase Barrens, "Picture Creek," of Granville Co., NC, is a known Piedmont Prairie remnant site. Historically, the site had far more open canopy structure and was dominated by fire resistant oaks and pines as evidenced by dendrochronology and aerial photo studies (Sigmon-Chatham 2015). The nature preserve portion of the site is centered over a high-voltage transmission powerline corridor. The powerline is positioned over Iredell (Alfisol) and Picture (Mollisol) soil series. These soil series are mafic-calcareous and formed over residuum weathered from diorite and/or gabbro and/or diabase and/or gneiss parent materials that have higher pH, Mg, and Ca than soils formed over residuum from felsic parent materials (Soil Survey Staff, NRCS, USDA 2022). The soils also have vertic properties that cause them to fluctuate swiftly from hard, cracked soil to fully saturated and very malleable soil following precipitation. (Soil Survey Staff, NRCS, USDA 2022)

Picture Creek retains the remnants of a Piedmont Prairie. Fire suppression and land use change in the last half century caused portions of the site to shift from Piedmont Prairie to Xeric Hardpan Forest (XHF) (Schafale 2012; Stanley et al. 2019). This increase in canopy cover changed the composition of the understory as available light was significantly reduced. However, a number of Piedmont Prairie-associated species were able to persist underground until a powerline cut through the forest in the 1980s opened the canopy of a portion of the site shortly after the forest had reached canopy closure. Subsequent surveys by North Carolina Natural Heritage botanists, preceding consideration of the area for a placement of a supercollider, revealed that a number of now rare, threatened, and endangered Piedmont Prairie associates had survived under a section of the powerline (Legrand 1986; Smith 1993).

The site is now owned and protected by the NC Department of Agriculture and Consumer Services Research Stations Division as part of a research farm. The NC Plant Conservation Program and NC Museum of Natural Sciences are secondary custodians of the preserve. Additionally, North Carolina State University, the North Carolina Botanical Garden, and the NC Natural Heritage Program are also stakeholders that collaborate to promote research and conservation projects on the site. Current management includes returning and maintaining frequent fire to the landscape and thinning overstory and midstory vegetation to rehabilitate the plant communities there.

A number of factors make the plant community at Picture Creek especially unique and worthy of this level of conservation effort. First among these is the presence of over 30 rare and endangered species on the site, including one of the largest known populations of the federally listed smooth purple coneflower, *Echinacea laevigata* (Stanley et al. 2019). Picture Creek also hosts a number of otherwise disjunct species that are more typically associated with grasslands and woodlands of the midwestern United States, such as the charismatic prairie dock, *Silphium terebinthinaceum* (Stanley et al. 2019; Szakacs 2020). The presence of these associated species may indicate that the site is a remnant Piedmont Prairie. Specifically, the area of this study is currently a Xeric Hardpan Forest (Northern Prairie Barren subtype) (Schafale 2012). This plant community is characterized by an open canopy dominated by fire adapted trees (*Pinus echinata*, *Pinus virginiana*, *Quercus marilandica*, and *Quercus stellata*), a sparse midstory (*Cercis canadensis*, *Chionanthus virginicus*, *Cornus florida*, *Juniperus virginiana*, and *Ulmus alata*), and an understory that is more species rich than neighboring forest communities, including species such as *Marshallia legrandii*, *Solidago ptarmicoides*, and *Solidago rigida* var. *glabrata* (Stanley et al. 2019).

Secondary Study Site: Difficult Creek

Difficult Creek Natural Area Preserve, "Difficult Creek" of Halifax Co., VA, is another suspected Piedmont Prairie remnant site. As is the case at Picture Creek Diabase Barrens, land use change and fire suppression have degraded the former Piedmont Prairie ecosystem there. The majority of the property was converted from Southern Piedmont Hardpan Forest (Schafale 2012) to planted pine (primarily *Pinus taeda*) by previous owners, prior to its acquisition by the Virginia Department of Conservation and Recreation. In the vegetation analyses of Szakacs (2020), plots at Difficult Creek emerged within the Piedmont Red Cedar-Post Oak Woodlands cluster (whereas plots at Picture Creek emerged within the Piedmont Prairie Barren cluster). Importantly, Difficult Creek is home to 14 species considered rare by the state of Virginia, including the second known extant population of *Marshallia legrandii* (Townsend and Ludwig 2020). The areas with the richest diversity of Piedmont Prairie associates sit upon mafic, Virgilina formation parent material. Soils are Virgilina Series Alfisols that have high shrink-swell potential, a dense hardpan layer, relatively high base status, and a significant gravelly or stony component (Townsend and Ludwig 2020). Current management focuses on using prescribed fire and *Pinus taeda* removal to restore historic Piedmont Prairie ecosystems to the site (Virginia Department of Conservation and Recreation Staff 2022).

Project Overview

The focus of our project is the experimental augmentation and habitat description of two rare species at Picture Creek Diabase Barrens “Picture Creek”: the critically imperiled Oak Barrens Barbara’s-buttons (*Marshallia legrandii*) and the federally listed smooth purple coneflower (*Echinacea laevigata*). *Marshallia legrandii* has a global conservation rank of G1S1, indicating it is critically imperiled worldwide due to few populations or occurrences, while *E. laevigata* is G2G3, indicating it is either imperiled globally and/or vulnerable (NatureServe 2022). In North Carolina, both species are protected under the NC Plant Protection and Conservation Act.

An augmentation is the planting of a species on a site where it already occurs in order to bolster the population of the site. An experimental augmentation of a rare plant presents a unique opportunity to learn more about the biology, ecology, or growth habits of that species that might not be possible to investigate otherwise due to the size or fragility of the species population (Maschinski 2012).

We were made aware of the opportunity to help design the experimental augmentation of *E. laevigata* at Picture Creek by the North Carolina Plant Conservation Program. They, along with the North Carolina Botanical Garden, had overseen the seed collection, seedling growth, and site preparation for establishing an augmentation of *E. laevigata* outside of the powerline, where the majority of the extant population occurs. This augmentation project started as a plan to meet the requirements of the U.S. Fish and Wildlife Service 1995 recovery plan for *E. laevigata*, which specified that in order for the species to be down-listed from endangered to threatened, there must be nine stable populations in protected natural areas where the conservation of the species is the primary objective and not dually managed for human use such as utility lines or roadsides (U.S Fish and Wildlife Service 1995). Seedlings of *M. legrandii* had also been grown for augmentation because that species grew alongside *E. laevigata* in many areas on the site and could also benefit from augmentation to help secure the longevity of such a rare species.

We designed the experimental augmentation of *M. legrandii* and *E. laevigata* to learn more about the ecology of both species to inform future conservation management. The *M. legrandii* experimental augmentation allowed us to investigate the optimal light environments for the growth of the species, while the *E. laevigata* augmentation focused on how competing vegetative growth affected the growth and survival of the species.

As described in the chapters that follow, we also measured environmental variables such as light availability, canopy openness, competing vegetation, and soil properties in the augmentations and natural populations of *E. laevigata* and *M. legrandii*. This allowed us to describe the ecological niches the species currently occupy and compare these to the environment into which we planted them. Additionally, proximity of the second extant population of *M. legrandii* at Difficult Creek made surveys of the two known known populations possible. We compared the current status, population size, and habitat of *M. legrandii* in both states where it is found.

CHAPTER 2

Population Status and Habitat Description of Oak Barrens Barbara's-buttons (*Marshallia legrandii*)

Abstract

Oak Barrens Barbara's-buttons (*Marshallia legrandii*) is a critically imperiled perennial herb in the Asteraceae with just two known extant population locations: Difficult Creek Natural Area Preserve, Halifax County, VA, and Picture Creek Diabase Barrens, Granville County, NC (Weakley & Poindexter 2012). Surveys to assess the population vigor and to describe the habitat characteristics were carried out for the two locations. At the Picture Creek Diabase Barrens population, we mapped the current size and extent of the subpopulations of *M. legrandii* in two consecutive years and compared these findings to those for known occurrences surveyed nine years earlier. We described the physical environment where the species is found flowering and related these values to corresponding flowering density. More detailed environmental measurements were performed in the largest subpopulation as a case study to compare the environment of flowering *M. legrandii* to the proximate environment where its flowering and vegetative presence abruptly ends. We also implemented an augmentation of *M. legrandii* on the site to study planting survival and effect of different light environments on the growth of the plant. The positive correlation of flowering stems with vegetative stems in the largest population at Picture Creek Diabase Barrens suggests flowering stems are an appropriate index of the population size. The change in spread and flowering of *M. legrandii* in areas managed for smooth coneflower (*Echinacea laevigata*) indicate that the population at Picture Creek Diabase Barrens may be benefiting from conservation interventions such as thinning and burning that reduce canopy and midstory competition. Survival of augmented *M. legrandii* was nearly 90%

two years from planting, and 28–42% of plants bloomed in the second year, indicating that augmentation efforts are a viable tool for conservation of this rare plant.

Introduction

Oak Barrens Barbara's-buttons (*Marshallia legrandii* Weakley; Asteraceae) is a perennial herb endemic to the Piedmont of the southeastern United States. It exhibits a basal rosette of linear-oblanceolate leaves that can be easily mistaken for graminoids to the untrained eye. When in flower, its leaves occasionally become cauline along flowering stems and are a little shorter than the basal leaves. It flowers in late May – mid-June with 0.5 – 1 m stems terminating in a single pink-purple discoid flowering head, differing in flower size, color, and phenology from other species of *Marshallia* (Weakley and Poindexter 2012). The sites where it occurs share many characteristics, including mafic soils and remnant Oak Barrens plant communities which may have supported a number of Piedmont Prairie associates in the past. This affinity gives it its common names, "Oak Barrens marshallia" and "Oak Barrens Barbara's-buttons" (NatureServe 2022).

Little literature exists about the ecology and current status of *Marshallia legrandii* beyond the observational documentation at the time of its naming (Weakley and Poindexter 2012). The federally listed *Echinacea laevigata* co-occurs with *M. legrandii* at Picture Creek Diabase Barrens, "Picture Creek," and Difficult Creek Natural Area Preserve, "Difficult Creek." *Echinacea laevigata* and a number of other species on the sites are known Piedmont Prairie associates. Within the past century, portions of Picture Creek were an open oak barrens woodland as evidenced by historical aerial photos and dendrochronological work at the site (Sigmon - Chatham 2015). Over time, the forest has developed and portions of the site have since experienced mesophytification (a concept established by Nowacki & Abrams (2008); orthographic correction by Krings (2015)) leading to dense closed canopy forest (Stanley et al. 2019). Piedmont Prairies, like the remnant ones at Picture Creek and Difficult Creek, are unique habitats typically of conservation concern (Davis et al. 2002). They are grasslands, grassy savannas, and woodlands with widely spaced trees, and are thought to have occurred historically across the southeastern Piedmont region (Barden 1997; Davis et al. 2002; Szakacs 2020). These open canopy systems are thought to have been maintained by frequent fires and supported a high diversity of plants, pollinators, and wildlife (Frost 1998). It is common now, with pervasive land use change and fire suppression throughout the Southeast, to find that these once-open systems have developed into thick forested areas.

The most recent survey of the *M. legrandii* population at Picture Creek was performed by the North Carolina Plant Conservation Program in 2012 (Lesley Starke, personal communication, June 7th 2021). The 2012 survey found *M. legrandii* flowering only along an old roadway enclosed by a forest glade and along forest edges along a powerline that runs through the property. The population at Difficult Creek has a similar distribution, with the majority found along a roadside in the now mature pine plantations on the site. The combination of Piedmont Prairie associates and historical openness suggest that *M. legrandii* is also a remnant Piedmont Prairie species that requires some minimum threshold of sunlight to flower. Land use change and fire suppression have relegated *M. legrandii* to the patches of light that remain along roadsides and powerline utilities at the two sites where it still occurs.

There is an effort to augment some of the populations of rare plant species at Picture Creek including *M. legrandii*. In this effort, seeds were collected on site, grown at the NC Botanical Garden, and plans were made to plant them in the fall of 2019 (though this was delayed to 2020 due to the COVID-19 pandemic). This augmentation provided a unique opportunity to learn more about the plant by implementing an experimental design to test if light drives plant vigor.

To broaden our ecological understanding and inform future management, the primary goals of this study were to (1) investigate the status of *M. legrandii* at its two extant populations and (2) describe the basic properties of the environment where it occurs, and (3) augment and monitor the population of *M. legrandii* at Picture Creek Diabase Barrens. We seek to answer: (1) What is the current size and spread of the two extant populations? (2) How has the population at Picture Creek Diabase Barrens changed over time? (3) How do soil moisture, soil properties, canopy openness, and competing vegetation structure correlate with the subpopulation size and floral density where *M. legrandii* occurs? and (4) What is the survivorship and initial growth characteristics in the first two years post-planting in the augmentation?

Methods

Site Descriptions

Picture Creek Diabase Barrens is a State Dedicated Nature Preserve in Granville County, North Carolina (Stanley et al. 2019). The 165-ha acre site is managed by the Research Division of the North Carolina Department of Agriculture & Consumer Services (NCDA) in partnership with North Carolina Plant Conservation Program (NCPCP). It is located in the northeastern Piedmont of North Carolina, part of the upper Neuse River watershed, north of Stem, North Carolina.

A powerline owned by Duke Energy cuts through the site and provides canopy openness to remnants of Xeric Hardpan Forest (sensu Schafale 2012) that occur on Iredell and Picture series soils formed over diabase parent material. Xeric Hardpan Forest is characterized as having open canopy dominated by fire adapted trees (*Pinus echinata*, *Pinus virginiana*, *Quercus marilandica*, and *Quercus stellata*), a thin midstory (*Cercis canadensis*, *Chionanthus virginicus*, *Cornus florida*, *Juniperus virginiana*, and *Ulmus alata*), and an understory that is more species rich than neighboring forest communities, including Piedmont Prairie species such as *Marshallia legrandii*, *Solidago ptarmicoides, and Solidago rigida* var. *glabrata* (Schafale 2012).

The change in composition and structure of the Xeric Hardpan Forest has been documented through aerial photography over the past ca. 100 years (Sigmon-Chatham 2015), with the canopy either infiltrated by mesophytic tree species like *Acer rubrum*, *Liquidambar styraciflua*, and *Pinus taeda* or entirely removed as in the powerline. Portions of the site were previously grazed and/or farmed until the 1940s. Old-field succession likely occurred on portions of the site after agricultural abandonment, until the mid-1980s when NCDA began active management of the property. Current management focuses on thinning canopy, removing/controlling the mesophytic species, and restoring fire to remediate the plant community there.

Difficult Creek Natural Area Preserve is located in Halifax Co., VA and is another Piedmont Prairie remnant site. As is the case at Picture Creek, land use change and fire suppression have degraded the former Piedmont Prairie ecosystem here. The majority of the property was converted from Southern Piedmont Hardpan Forest (Schafale 2012) to planted pine (primarily *P. taeda*) by previous owners, prior to its acquisition by the Virginia Department of Conservation and Recreation. Current management focuses on using prescribed fire and *P. taeda* removal to restore historic Piedmont Prairie ecosystems to the site (Virginia Department of Conservation and Recreation Staff 2022).

Importantly, Difficult Creek is home to 14 species considered rare by the state of Virginia including the second known extant population of *Marshallia legrandii* (Townsend and Ludwig 2020). The areas with the richest Piedmont Prairie associate diversity occur upon the mafic Virgilina formation parent material. Soils are Virgilina Alfisols that have high shrink-swell potential, a dense hardpan layer, relatively high base saturation, and a significant gravelly or stony component (Townsend and Ludwig 2020).

Survey

Thorough surveys of *M. legrandii* at Picture Creek and Difficult Creek were conducted during their flowering period in early June of 2021 and again at Picture Creek in 2022. During each survey, the flowering subpopulation clusters were mapped at each of the sites to describe the shape and spread of each. Counts of flowering heads were taken as a proxy measure of success that could be compared to previous year flowering head surveys. We observed that the majority of the populations formed clusters that could be described by an ellipse, so we estimated the area of each subpopulation cluster by measuring the length and width of the cluster and applying the formula for calculating the area of an ellipse. Flowering clusters had to be spaced more than two meters apart by vegetation other than *M. legrandii* to be considered separate subpopulations.

At Picture Creek, the population was surveyed using known occurrence records that included points recorded in a 2012 flowering head count (NCPCP) as starting points (Lesley Starke, personal communication, June 7th 2021). These points represented clusters of flowering *M. legrandii* that were present in 2012. The area around each point was carefully searched for up to 300 meters to make sure no flowering clusters were missed and to identify new clusters. The subpopulations identified at Picture Creek in 2021 were revisited and recounted in 2022 to compare flowering head counts between years.

At Difficult Creek, help was provided by Virginia Natural Heritage botanist, John Townsend, to locate the flowering populations at the site referencing GPS points. We took new points at each found cluster using GPS and proceeded with measurements of flowering head count and cluster size in the same way as at Picture Creek.

Due to time constraints, environmental measurements were made at a subset of subpopulation clusters of *M. legrandii* at both sites. We sampled clusters that had 15 or more flowering heads during the 2021 flowering survey. This accounted for 20 of the 35 extant subpopulation clusters at Picture Creek and 9 of the 14 subpopulation clusters at Difficult Creek. Additionally, environmental measurements were taken at points along transects in the largest subpopulation at Picture Creek (Fig. 2.1).

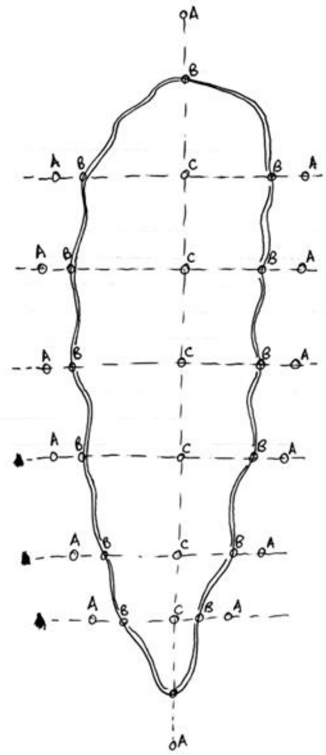

Figure 2.1. Sample population of *Marshallia legrandii* at Picture Creek Diabase Barrens and transect design. On the left, M.S. student, Rachel Jessup, is pictured standing in the narrow pathway in the largest population of *M. legrandii* at Picture Creek Diabase Barrens during peak bloom. On the right, a sketch of the transects made to establish points for measuring environmental variables in the population. Points are labeled to represent the three locations along the transect (A = "away" points one meter from the edge of the flowering population, B = "border" points between the last flowering and non-flowering M. legrandii, C = "center" points every 3.05 m in the middle of each transect) (start and end points of the main center transect were taken using GPS).

Soil Properties

Soil moisture and soil temperature were recorded five different times over the course of the growing season in 2021 (June 25th, July 14th, July 23rd, September 4th, and October 4th.) One measurement was made at each separate cluster of *M. legrandii* that had over 15 flowering heads during the 2021 flowering survey. Soil moisture and temperature were measured using the Aquaterr M-350 meter, which uses a scale of 0–100 to measure percent soil moisture with 0 being the driest and 100 being completely saturated. Measurements were taken in the same day across all points at Picture Creek to minimize changes in moisture due to rainfall or evaporation over time.

Additionally, six aggregate soil samples were each compiled from 3–5 clusters of *M. legrandii* points that were geographically near each other. Each of the aggregate samples represented multiple measurement points in a similar area that shared environmental characteristics. We recognized three distinct environments (forest glade, powerline, and edge) where *M. legrandii* was found, and two aggregate soil samples were located in each of these distinct environments (Fig. 2.2).

Samples consisted of 5+ plugs of soil taken to a depth of 15 cm with a 2.5 cm wide corer (one sample from each *Marshallia* measurement point in each geographical cluster). These samples were dried, sifted, and combined in the lab before being sent to Brookside Laboratories, Inc. (New Bremen, OH) for analysis of pH, % organic matter, carbon-nitrogen ratio (CN), sulfur (S), phosphorus (P), magnesium (Mg), calcium (Ca), potassium (K), sodium (Na), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and aluminum (Al).

<u>Light and Canopy Openness</u>

Hemispherical photos were also taken at each cluster with 15 or more flowering heads at Picture Creek (N=20) and Difficult Creek (N=10). Each photo was taken 1 meter above the ground, leveled to make sure it was parallel to the sky, and with the top of the camera facing North.

All of the transect points in the largest *M. legrandii* population (Fig 2.1) were analyzed in Gap Light Analyzer (Cary Institute of Ecosystem Studies 2020). This gave values for light properties including diffuse and direct light. Due to unintentional photo framing in camera equipment used later on, the rest of the photos were analyzed for canopy openness using ImageJ (Rasband 2022).

Competing Vegetation Structure

Vertical complexity and density of competing vegetation life forms were measured at sampling points using a modified version of the technique described by Wiens and Rotenberry (1981). A graduated pole was placed vertically at each measurement point and the vegetation life forms (graminoids, forbs, and woody plants) that touched the pole along each graduated section (0.25 m, 0.5 m, 0.75 m, 1 m, 1.5 m, and 2 m) were recorded. For example, if a grass touched the pole along the 0.25–0.5 m section, it was recorded as a hit for graminoids in that height range.

Subpopulation Case Study at Picture Creek Diabase Barrens

The largest known subpopulation of *M. legrandii* in both area and flowering head count exists along an old roadbed in the forest glade, referred to as “the glade,” northeast of the powerline at Picture Creek. The flowering individuals in this population are separated from other occurrences in the same forest glade area by several meters. Because of its unique size, we used this location as a case study to investigate the ecology of *M. legrandii* more intensively and to determine which factors were preventing the population from connecting with the other clusters of the species in the forest glade.

Transects were established at regular intervals across the subpopulation (Fig. 2.1) to compare the soil moisture, light, flowering head density, vegetation density, and competing vegetation structure both within, along the border, and away from the flowering portion of the population. The environmental measurements were recorded at the same time as the environmental measurement points for the rest of the subpopulations across the site. This allowed for comparison of the coarser site-wide plot measurements with the more intensive ones done in the largest subpopulation case study.

Experimental Augmentation

The experimental design was a factorial experiment to test three light levels (partial light along a northern forest edge, full sun in the middle of a field, and partial light under scattered woodland trees). Four, one-meter by one-meter plots, each containing eight plants, were replicated in each of the three light levels. However, a shortage of plants meant that the design could not be completely filled out. Therefore, four forest edge plots, four full sun plots, and one scattered woodland tree plot were planted on December 4th, 2020. Seedlings were sourced from a collaboration between the NCPCP and the North Carolina Botanical Garden. In late 2018, seeds were collected from the population at Picture Creek by NCPCP volunteers and grown outdoors at the North Carolina Botanical Garden overseen by Mike Kunz. Originally, these plants were intended to be planted in fall of 2019, but this schedule was delayed a year due to prescribed burn plans that fall and the COVID-19 pandemic the following spring.

Soil moisture and canopy openness were measured in each of the plots over the season so that the augmented plots could be compared to the natural population on the site. Neighboring understory vegetation was not measured because the plots were clipped prior to planting and reclipped midseason to minimize the effect of competing vegetation on plant growth and to make sure that light was the major variable being measured in each light environment block.

Each plant was tagged with an identification number. Survival of each plant was measured in July of 2021 and May and July of 2022. Besides survival, plant measurements also included presence of flowering heads, height of present flowering heads, and height of leaves on the plant in July of 2021 and 2022.

Analyses

We calculated the mean soil temperature and moisture by measurement period and also summarized the seasonal trends using z-scores. Differences in both temperature and moisture were tested using analysis of variance (PROC GLM, SAS v.9.4). Soil moisture differences between months were further explored using ANOVA (F-values and p values), followed by Tukey's multiple comparison test.

Principal Components Analysis (PCA) of soil properties derived from the soil samples taken in the three environmental areas were performed in PC-ORD (v 7.07, McCune and Medford 2018) to visualize how soil properties related to the environmental locations. A secondary matrix of environmental variables, including flowering density and canopy openness was used with the ordination to explore relationships between each variable and soil properties. Regression analysis was used to further explore the relationship between flowering and canopy openness evident in the PCA, for both the Difficult Creek and Picture Creek occurrences.

Analysis of variance (ANOVA) was used to test for differences in mean moisture, canopy openness, flowering density, and among grouped point locations in the large subpopulation case study. A summary of average soil moistures in each location were compiled in tables using Microsoft Excel. This allowed us to investigate environmental differences between the away, center, and border point locations.

Results

Survey

During the 2021 Survey, we identified flowering clusters of *M. legrandii* in three distinct habitat types at Picture Creek: the treeless powerline, the forest glade surrounding an old road, and the forest edge between the powerline and the forest (Fig. 2.2). At Difficult Creek, flowering clusters of *M. legrandii* were found in two distinct habitat types: mature pine plantation stands and along a roadside (Fig. 2.3). In 2021, there were 7,872 flowering heads at Picture Creek and 1,284 flowering heads at Difficult Creek (Table 2.1). Picture Creek had the largest flowering footprint (area covered by plants with flowering stems) at 709 m^2, while Difficult Creek's flowering footprint covered 135 m^2.

At Picture Creek, relative to a known historical flowering head count of 459 from 2012, flowering head count increased 1,615% in 2021. The powerline flowering clusters we identified in 2021 and in 2022 were not present during last survey done in 2012. During our second flowering head count survey in 2022, we found that there were 5,493 flowering heads, a decrease of 30% from the previous year but still a 1,097% increase from the flowering head count in the same areas a decade earlier (Fig. 2.4). Change in flowering head count from 2021 to 2022 was not uniform across the three different environments on the site (Fig. 2.5). There was a larger decrease in flowering head count in the forest glade (35%) and powerline (32%) compared to the edge (2%).

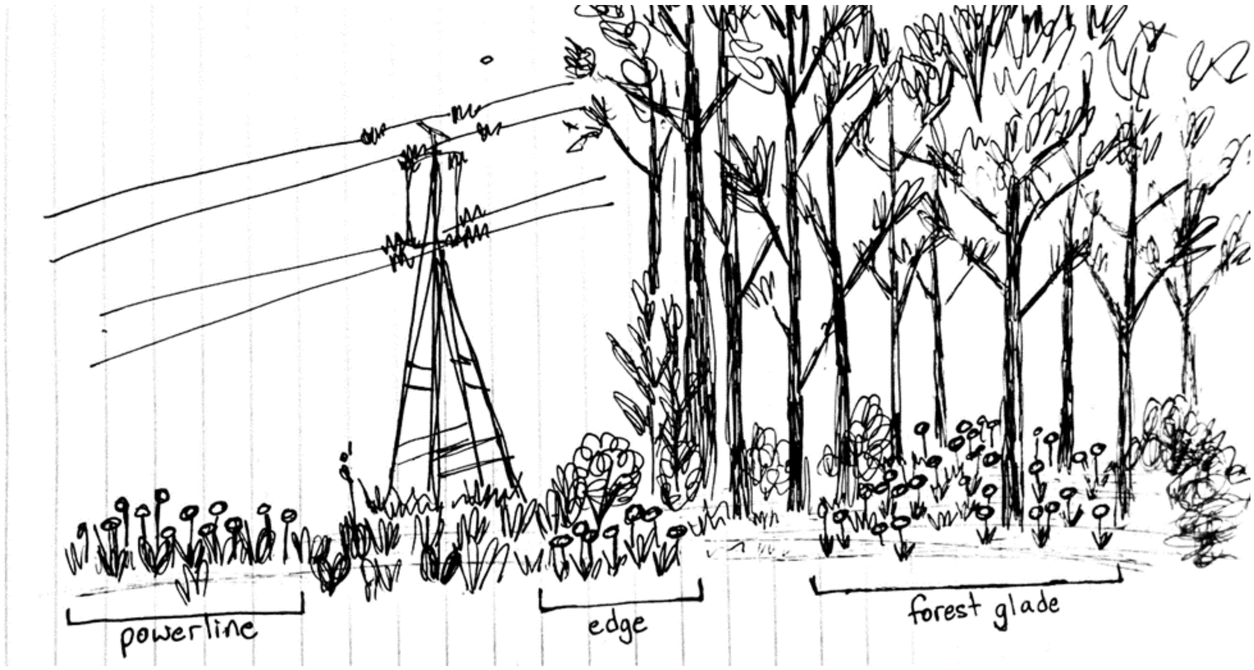

Figure 2.2. Sketch of habitat locations where *Marshallia legrandii* is found flowering at Picture Creek Diabase Barrens, Granville Co. NC. Picture locations not to scale.

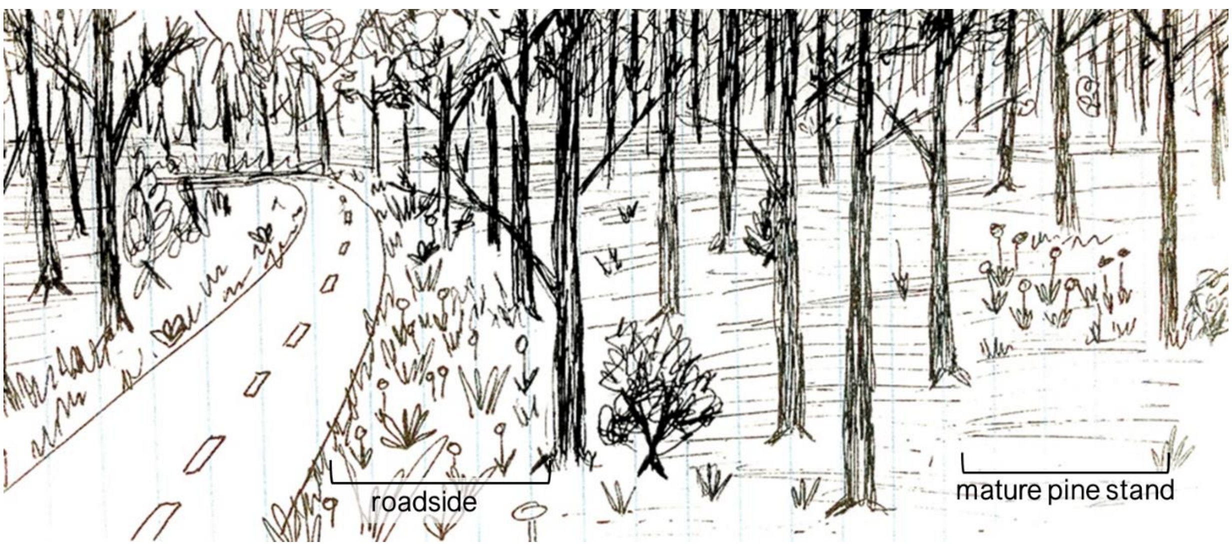

Figure 2.3. Sketch of habitat types where flowering clusters of *M. legrandii* were found at Difficult Creek Natural Area Preserve, Halifax Co. VA. Most flowering *Marshallia* is found along a roadside, others in mature loblolly pine stands. Picture locations not to scale.

Table 2.1. A summary of the flowering head counts and area of flowering population of *Marshallia legrandii* for both Picture Creek Diabase Barrens and Difficult Creek Natural Area Preserve in 2021.

	Difficult Creek	**Picture Creek**	**World Population**
Flowering Head Count	1,284	7,872	9,156
Area m^2	135	709	844

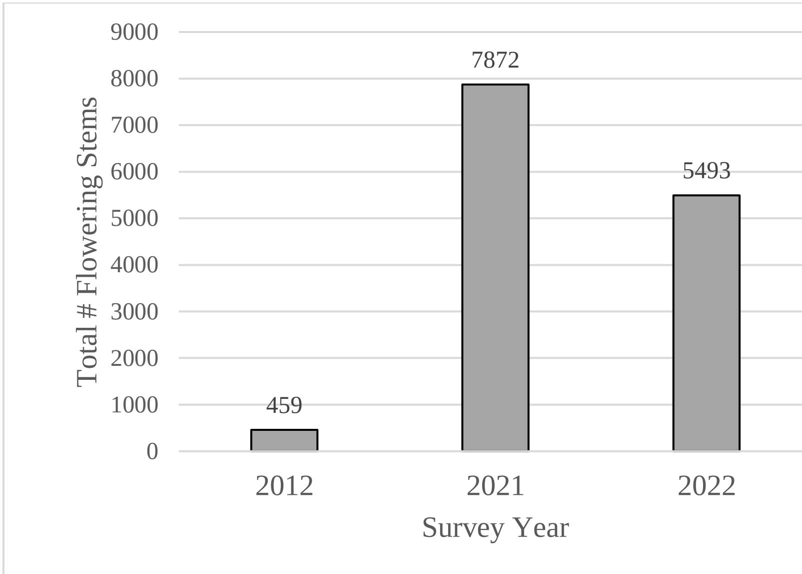

Figure 2.4. Total flowering head count of *Marshallia legrandii* at Picture Creek Diabase Barrens, Granville Co., NC, by survey year. 2012 data from Lesley Starke with NCPCP.

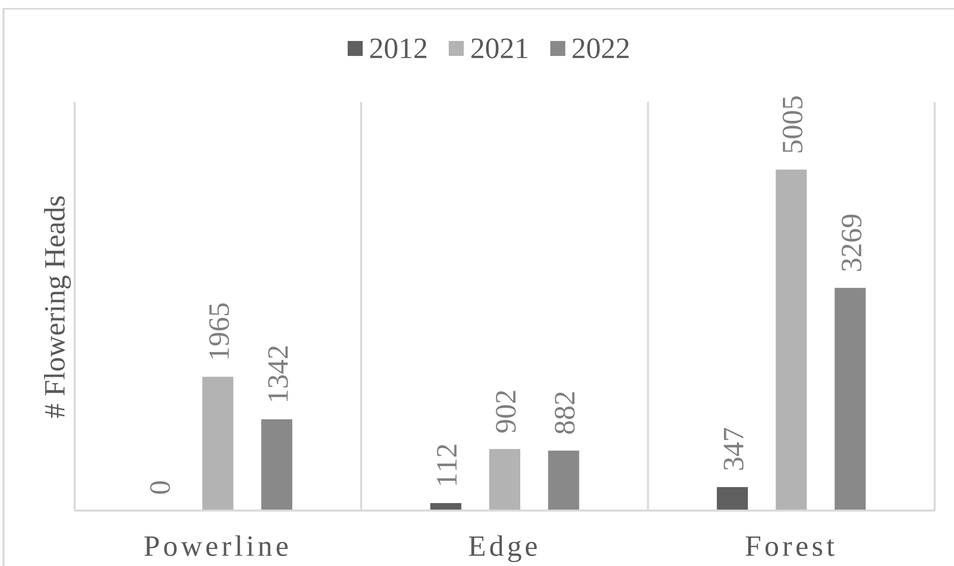

Figure 2.5. Flowering head count of *Marshallia legrandii* in each environmental location (powerline, edge, and forest glade) at Picture Creek Diabase Barrens, Granville Co. NC by year. 2012 data form Lesley Starke with NCPCP.

Environmental Factors

The driest soil moisture measurements at Picture Creek in 2021 were captured during early June (87%) and September (74%). Late July had the highest soil moisture (97%). Comparison between the three different environmental locations (forest glade, edge, and powerline) shows that there is a significant difference between them on some months. In June, and July, the powerline had higher soil moisture on average than the forest glade (by 10% and 16% respectively) (Table 2.2). In June and late July there was a significant difference ($p<0.05$) in the soil moisture for the edge *M. legrandii* compared to the forest glade. For June, the edge populations had 16% higher soil moisture value than the forest glade. In late July, the forest glade populations had 4% higher soil moisture on average than the edge population. Early July had the only significant difference between the powerline and the edge population soil moistures with a 9% higher soil moisture in the powerline than the edge (Table 2.2).

Soil chemistry varied across the location gradient, with soils in the powerline containing higher organic matter, phosphorous, and potassium. Soils in the forest glade had higher aluminum, iron, magnesium, and carbon to nitrogen ratio. Differences in pH occurred across the site, ranging from 5.8-6.3, but did not correspond to the habitat locations. Soil properties can explain 73% of the variance between the three environmental locations on just two axes of the ordination (Fig. 2.6) The arrangement of the soil samples in the PCA ordination shows a clear relationship between the soil samples taken from the same environments with the forest glade soil samples in the lower left and the powerline samples in the upper right respective to each other. The edge soil samples are between the other two, showing that the soil properties of the edge share similarities with both the powerline and the forest glade.

There is a strong directional relationship between canopy openness and location. The direction of the canopy openness vector in an overlay on the soil ordination (Fig. 2.7) shows the forest glade has the least canopy openness (33% on average) and powerline has the most at (79% on average). Flowering density, graminoid vertical density, and *M. legrandii* vegetative vertical density also increase from the forest glade towards the edge and powerline.

There were slight positive trends between canopy openness and flowering density at both Difficult Creek and Picture Creek (Fig. 2.8). Canopy openness ranged from 21% to 50% at Difficult Creek and 29% to 81% at Picture Creek where clusters with 15 or more flowering heads of *M. legrandii* were found blooming.

Table 2.2. Differences in mean soil moisture content (%) among locations of *Marshallia legrandii* measured from June through October, 2021. Mean moisture is presented by time and location. Differences were tested with ANOVA, followed by Tukey's multiple comparison test. Values sharing the same letter or not lettered are not statistically different at alpha <0.05 level.

Time	Forest glade	Edge	Powerline	F value	*p* value
June	77.1 a	91.4 b	90.7 b	24.42	<0.001
Early July	83.5 a	84.4a	92.0 b	5.31	0.016
Late July	98.3 a	95.0 b	97.2 ab	3.43	0.056
September	79.0	68.2	73.3	1.73	0.210
October	85.3	86.0	88.0	0.30	0.746

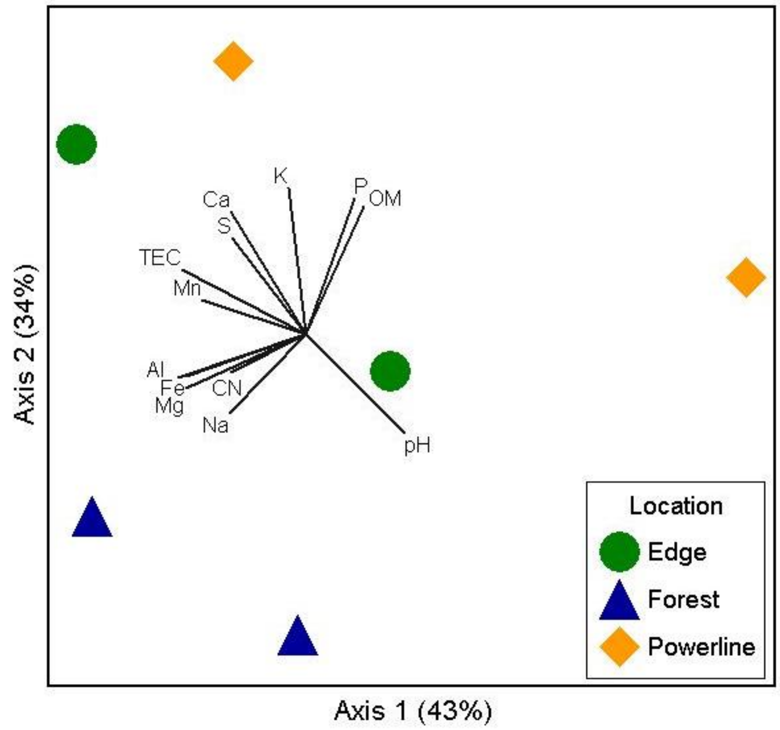

Figure 2.6. PCA displays arrangement of augmentation plots from three locations at Picture Creek Diabase Barrens based on soil properties. The PCA described cumulative variance of soil properties (Ca, K, P, Organic Matter(OM), pH, Na, CN, Mg, Fe, Al, Mn, Total Exchangeable Cations (TEC), and S) to explain the differences between samples taken from the Forest glade, Edge, and Powerline *Marshallia legrandii*.

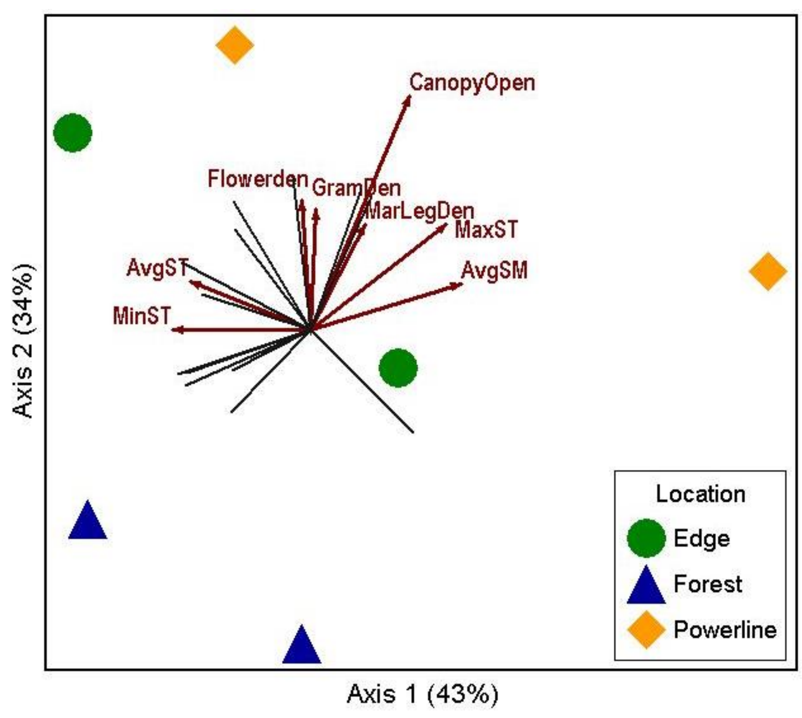

Figure 2.7. Overlay of environmental variables (red arrows) show relationships between those variables and the arrangement of soil samples across the site. Black lines depict the same soil property vectors from the Figure 2.6). (AvgST =average soil temperature, MinST =minimum soil temperature, MaxST = Maximum soil temperature, AvgSM = Average Soil Moisture, Flowerden = flowering density of *M. legrandii*, GramDen = Graminoid vertical density as measured in veg pole, MarLegDen = *M. legrandii* vegetative density, CanopyOpen = canopy openness.)

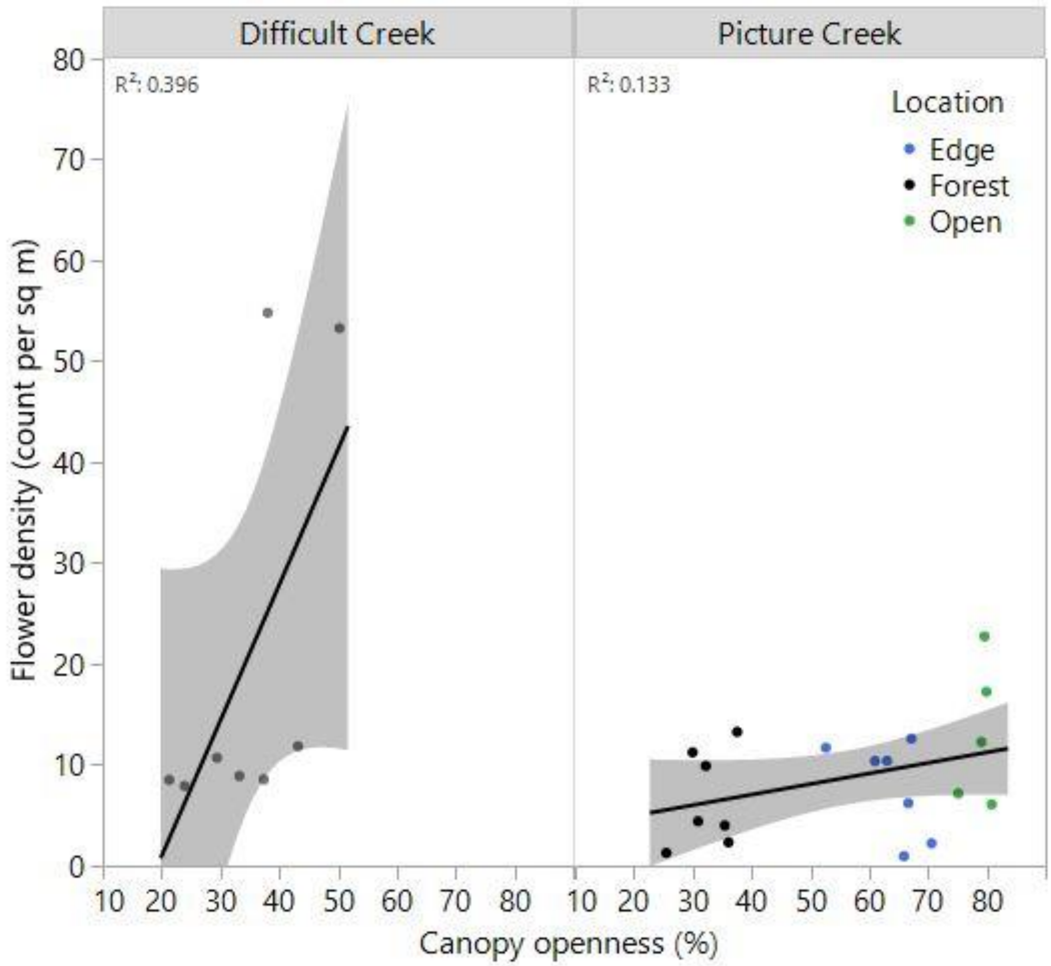

Figure 2.8. Relationships of plot flowering stem density and percent canopy openness for the subset of *Marshallia legrandii* clusters represented by 15 or more flowering heads at Difficult Creek Natural Area Preserve (N=10 of 14 total flowering clusters) and Picture Creek Diabase Barrens (N=20 of 35 total flowering clusters). Each point represents the plot of flower density and percent canopy openness for an occurrence of flowering *Marshallia legrandii* at Difficult Creek Natural Area Preserve and Picture Creek Diabase Barrens in 2021. Grey zone surrounding fitted line shows the confidence interval.

Subpopulation Case Study at Picture Creek Diabase Barrens

The largest flowering subpopulation, located at Picture Creek, had a footprint area of 1,005 m^2 and 4,072 flowers in 2021. For comparison, the next largest population only had an area of 220 m^2 and 665 flowering stems in 2021. Transect points were grouped into three types: those in the center of the flowering population, those on the border, and those one meter away from the last flowering stem.

The center points had the highest flowering and vegetative stem density of the three groups, followed by the border points. The away points systematically had the fewest flowers because they were selected away from the flowering population, but they also had the lowest number of *Marshallia* vegetative sprouts, revealing a relationship between flowering stems and vegetative sprouts (Fig. 2.9). Soil moisture was lower in the one meter away points than in the border and center of the flowering population in early and late July at the 0.06 level (Table 2.3). Plants in the center and border of the flowering population received more diffuse and direct light than the away points ($p = 0.001$) (Fig. 2.10). There was 93% more average vertical density of woody plants among the away points than in the border of the flowering population where there was little to no woody vegetation. There was no vertical density of woody plants in the center points where flowering was the most prolific (Fig. 2.11).

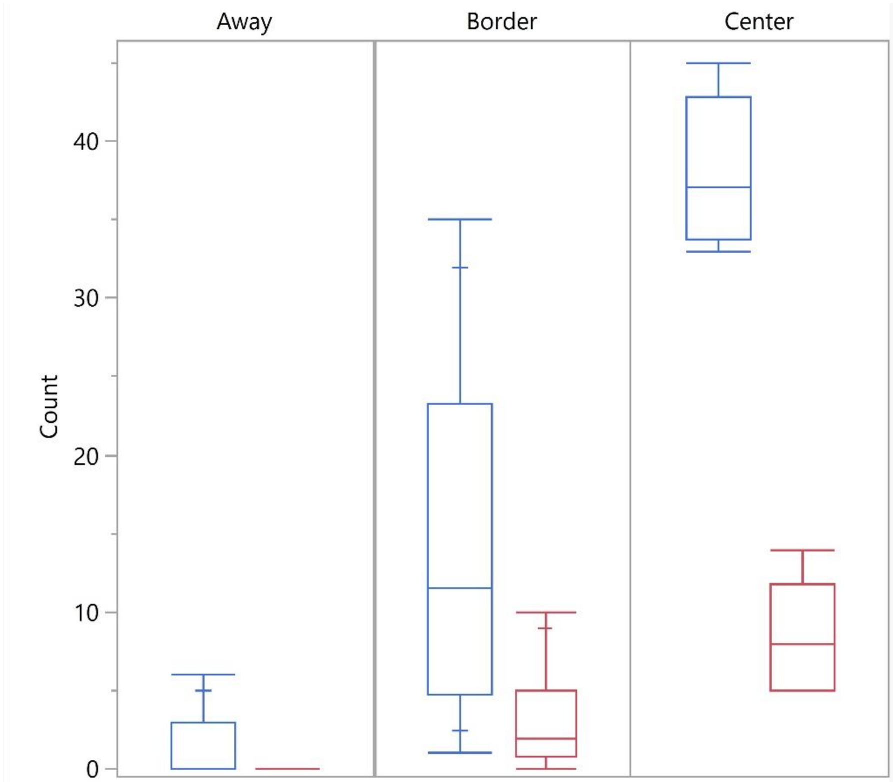

Figure 2.9. Comparison of the counts of flowering stems (red) and individual vegetative sprouts/plants (blue) of *Marshallia legrandii* from the 2021 survey in the largest flowering subpopulation at Picture Creek Diabase Barrens. Counts are broken up by Away, Border, and Center points in the transects for further comparison.

Table 2.3. Differences in soil moisture content (%) among locations of *Marshallia legrandii* in the largest flowering subpopulation at Picture Creek Diabase Barrens measured from June through September, 2021. Values are mean moisture by month and location. Differences were tested using ANOVA (F-values and p values), followed by Tukey's multiple comparison test. Values sharing the same letter or not lettered are not statistically different at alpha <0.05 level.

Time	Away	Border	Center	F value	*p* value
June	70.9	75.8	75.5	1.6	0.221
Early July	81.3	86.1	85.8	0.9	0.408
Late July	86.2 a	95.9 b	94.5 b	6.21	0.005
September	67.5	67.9	72.8	0.7	0.515

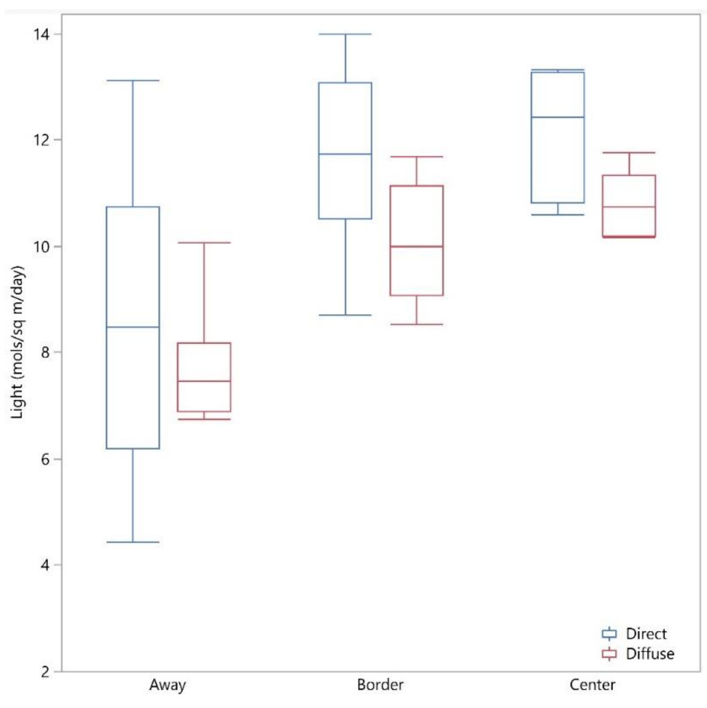

Figure 2.10. Comparison of diffuse and direct light among the three transect groups of the largest flowering subpopulation at Picture Creek Diabase Barrens: Away (measurements one meter from the last flowering stem in the transect) Border (measurements at the last flowering stem in the transect) and Center (measurements in the center of the subpopulation.)

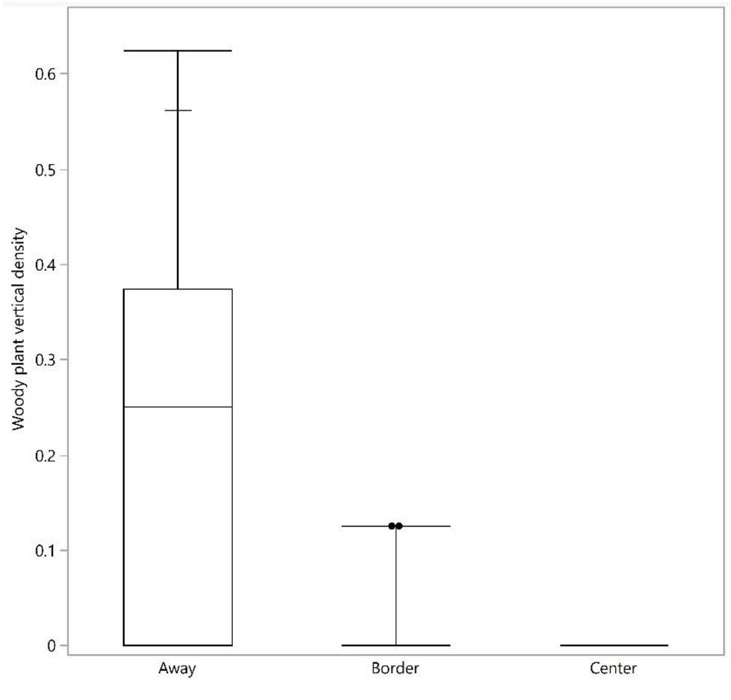

Figure 2.11. Comparison of woody plant vertical density in the three transect groups of the largest flowering subpopulation of *Marshallia legrandii* at Picture Creek Diabase Barrens.

Experimental Augmentation

The survival of plants in the augmentation was very high, with 97% of 72 two-year-old plants surviving the first year after outplanting and 89% surviving in the second year from outplanting. Four plants flowered for the first time in 2021 with two flowering in separate edge plots, and two flowering in the scattered trees plot. More flowering occurred in 2022 with 21 plants of the 64 surviving plants (33%) flowering for the first time. None of the plants that flowered for the first time in 2021 flowered again in 2022. Flowering varied between the three different plot treatments with 28% of living plants flowering in the forest edge plots, 32% flowering in the full sun plots, and 43% flowering in the scattered tree plot. Average flowering height in 2021 was 37 cm and ranged from 22.4 cm to 44.5 cm. Average flowering height in 2022 was 64 cm and ranged from 37cm to 82 cm. There was a 64% difference between the maximum height of leaves on flowering plants and non-flowering plants.

Discussion

Survey and Environmental Description

The large increase in flowering number and spread at Picture Creek from the last survey in 2012 to the current survey in 2021 appears encouraging for performance in the short term for the population there. The appearance of *Marshallia legrandii* in the powerline since 2012 shows that there has been spread and also challenges the prior conventional wisdom that this plant has some niche constraints relegating it to the edges of the powerline and dappled shade of the northern forest glade.

We found that *M. legrandii* is able to successfully flower in a range of light conditions from partial shade to full sun (Fig. 2.8). The lowest canopy openness value where it was found flowering was 25% at Picture Creek and 21% at Difficult Creek. *Marshallia legrandii* is found in residual light gaps along roadsides and powerlines at both sites, which indicates that there is some environmental pressure keeping the plants from thriving in the forest. Another rare plant, *Echinacea laevigata*, occurs at both Difficult Creek and Picture Creek and interestingly, the increase in *M. legrandii* flowering stems in the forest glade at Picture Creek from 2012 to 2021 mirrors a similar increase in flowering for *E. laevigata* following a thinning of the midstory in 2012 by the NC Plant Conservation Program (Szakacs 2020). This suggests that management for *E. laevigata* benefited the population of *M. legrandii*. Thus, while the plant is able to flower at lower light conditions, its ability to spread is likely inhibited in the forested environment.

Fortunately, managing to reduce cover of mature *P. taeda* and reintroduce frequent fire supporting Piedmont Prairie ecosystems is already an objective for Difficult Creek management (Townsend 2020). If completed around areas where *M. legrandii* is found, we expect that *M.*

legrandii will expand into the interior of the site away from the roadside in a way similar to how the population at Picture Creek has increased and spread following burning in the forested subpopulations. One of the observations that was not captured in the data collected was that two of the larger populations at Difficult Creek had little flowering relative to their vegetative presence. These were populations deeper in the stands of mature pine where the overhead canopy closure was high.

During some sampling periods, there was a significant difference ($p<0.05$) between the soil moisture of the powerline, the forest glade, and the edge. The forest glade was drier than the powerline during these times. If these differences occur often enough during some rain events, then the difference in soil moisture could affect the growth of *M. legrandii* over time in these different environments. That said, the presence of successful flowering subpopulations in all three of these environments may mean that the range of soil moisture is sufficient in all three environments even if they do occasionally fluctuate.

Subpopulation Case Study at Picture Creek Diabase Barrens

The case study of the largest population at Picture Creek reveals that soil moisture, soil temperature, vertical woody density, and light all correlate with changes in flowering, but in different degrees. We found that presence of woody plants in the understory and midstory seemed to have the largest impact on flowering. The presence of woody plants negatively correlated with the presence of flowers. It appears that since the population is hemmed in by *Liquidambar styraciflua* saplings and *Rubus* spp., it cannot connect to the populations farther into the forest glade due to a combination of lack of light and possibly the lower soil moisture from competition with tree roots. Conservation management for *M. legrandii* may benefit from removing that competitive midstory woody cover. The case study also gives evidence that the use of flower counts for population surveillance are relatively effective for determining the success of the populations since flowering is strongly associated with the density of vegetative presence of the plant.

Experimental Augmentation

This is the one of the first experimental augmentations of *M. legrandii*. According to best management guidelines observed by the North Carolina Plant Conservation Program, in order to be an effective augmentation, the augmentation needs to establish and maintain a viable population that requires a minimal amount of management intervention, use an experimental design approach, and establish a monitoring protocol and schedule for both short- and long-term objectives (NC Plant Conservation Program 2005). We met some of these criteria by establishing a population of *M. legrandii* in a restored area at Picture Creek that still had high survival two years post planting, using an experimental design to investigate which light conditions favor the success of the plants, while tagging the plants and setting up a schedule for monitoring in the long term.

The high survival in the first two years is promising for future conservation of this species especially with the flowering of many plants by year 2. Maximum leaf height was likely highest on flowering plants, because flowering plants have primarily cauline leaves on the flowering stem while non-flowering plants have only basally disposed leaves. Future research will expand the design to determine the robustness of the individuals in the three light environments.

Conclusion

The global population of *M. legrandii* remains limited to just 2–3 populations. The population at Picture Creek appears to be stable. The population trends at Difficult Creek cannot be determined without access to prior data, but the species may be especially threatened by the dense canopy in some areas when compared to the population at Picture Creek, since it had significantly less canopy openness and less flowering. A third extant occurrence is said to be in Virginia not far from the Difficult Creek population, but little is reported about it (NatureServe 2022).

The trends in light effects on flowering density in the subpopulation case study mirror the trends that canopy openness had on flowering density in the rest of the population. This shows that *M. legrandii* flowers best in partial to full sun. It needs some minimum threshold of sunlight in order to flourish. As is the case with many of its Piedmont Prairie associates, past land use changes that have led to denser overstories and midstories may have relegated this plant to more open roadsides and utility lines (Davis et al. 2002; Szakacs 2020). Opening up the canopy to create 25% or more openness and removing midstory woody cover (e.g., thinning, burning, and hand pruning, etc.) is likely an effective way to help this species thrive *in situ*. Due to the small range of this species, local augmentation may be an important conservation tool, but longer-term data that include genetic analyses are needed.

CHAPTER 3

Experimental augmentation and habitat description of *Echinacea laevigata* at Picture Creek Diabase Barrens

Abstract

Smooth purple coneflower (*Echinacea laevigata*) is a federally-listed perennial herb endemic to Piedmont Prairies of the southeastern United States. It flowers in late May to August with large, single-stem, pink or white, composite blooms that stand up to a meter above the leaves of its basal rosette. Fire suppression and land use change have reduced it to a handful of scattered populations in Georgia, South Carolina, North Carolina, and Virginia.

Our study site is Picture Creek Diabase Barrens ("Picture Creek"), Granville Co., NC, home to the largest known population of *E. laevigata*. We designed and established an experimental augmentation at Picture Creek using *E. laevigata* seedlings grown from seeds collected at the site. The augmentation involved planting four seedlings each into meter-square plots, each dominated by a different life form (graminoids, forbs, and vines), to investigate how different life forms affect survival and success of *E. laevigata*. A fourth treatment involved nine seedlings planted into meter-square plots where any competing vegetation was clipped before planting to see how intraspecific competition affects success and survival of *E. laevigata*. Additional measurements in the extant population of *E. laevigata* at Picture Creek were completed to describe and compare neighboring plant competition and environmental variables in the naturally occurring population to the augmentation.

We found that flowering plants of *E. laevigata* at Picture Creek grew in a diverse mixture of graminoids and forbs that differed in cover and composition between two environments (powerline and forest glade). *Echinacea. laevigata* displayed signs of plasticity in growth in the

two different environments; plants in the powerline had more vegetative cover in the first quarter meter above the ground than forest glade plants, while plants in the forest glade had more cover above half a meter than in the powerline.

Length and width of the largest leaves in the *E. laevigata* augmentation plots were significantly different among the life form treatments. We discovered that plots that were clipped prior to planting had higher survival and flowered earlier than plots that were not clipped. While initial planting survival of *E. laevigata* was high, suspected vole predation in the summer of 2021 had a devastating impact in half our planted experimental augmentation plots. Fortunately, the remaining *E. laevigata* seedlings had high survival in 2022. Augmentation continues to be an effective tool for conservation of *E. laevigata*. Future augmentations should consider clipping vegetation prior to planting and avoiding planting the species near woody plant species, brambles, and vines. Future research will expand the plot design and continue to measure the effects of interspecific and intraspecific vegetation competition on *E. laevigata*.

Introduction

Smooth purple coneflower (*Echinacea laevigata*; Asteraceae) is a federally-listed perennial herb with large, showy, pink-white, radiate heads that bloom in late spring- to summer. Its leaves are typically abaxially glabrous and usually basally disposed, elliptic to lanceolate-ovate, with serrate-dentate margins (Urbatsch et al. 2019). It is currently found in a patchy distribution on mafic-calcareous soils of the Piedmont and mountains of Georgia, North Carolina, South Carolina, and Virginia and believed to be extirpated from Pennsylvania (U.S. Fish and Wildlife Service 2011; Flora of North America 1993). Prior to European colonization, the species is thought to have existed in sunny habitats such as Piedmont Prairies and post oak-blackjack oak savannahs that were maintained by fire (U.S. Fish and Wildlife Service 1995). Land use change and fire suppression have likely dramatically reduced the available habitat for this species, resulting in its rarity in the landscape. Augmentation and reintroduction of *Echinacea laevigata* into favorable sites are some of the many tools that are being used to help conserve this species (Alley 1997).

Many of the current remnant populations of *Echinacea laevigata* exist in powerline rights-of-way and roadsides, where encroaching woody vegetation is controlled as a side effect of utility maintenance (Davis et al. 2002). The population at Picture Creek Diabase Barrens ("Picture Creek") is a dramatic example, where nearly the entire population, with thousands of individuals, is contained within a powerline (Lunsford 2001) first cut through the property in 1984 (Sigmon-Chatham 2015). One of the stipulations of the recovery plan for *E. laevigata*, is that at least nine out of the twelve required self-sustaining protected populations be in natural habitats before it can be reclassified from endangered to threatened (U.S. Fish and Wildlife Service 1995). Therefore, since a number of current protected populations exist in unnaturally managed landscapes, including the population at Picture Creek, augmentation of naturally occurring populations is being used to expand the range of the species into protected areas outside of utility zones like powerlines and roadsides.

Picture Creek Diabase Barrens was established in the early 1990s after a survey by the North Carolina Natural Heritage Program uncovered the presence of several threatened and endangered plant species at both the state and federal level on the site. Some species of conservation concern now recognized at the site include: *Echinacea laevigata*, *Marshallia legrandii*, *Cardamine douglassii*, *Baptisia aberrans*, *Ruellia humilis, Scutellaria, leonardii*, and *Solidago ptarmicoides* (Stanley et al. 2019). Currently there is an agreement with the powerline company not to spray certain sections with herbicides in the southern end of the powerline corridor due to the presence of *E. laevigata* (per. Comm, with David Schnake [formerly with NCDA]). In the areas surrounding the powerline, a dense closed canopy forest filled in open fields, beginning in the 1940s. In many portions of the site, mesophytic trees were able to grow due to the land use change and fire suppression in the area. Since acquisition of the site by the North Carolina Department of Agriculture and Consumer Services, a series of selective harvests have been conducted and some fire has been introduced to benefit many of the imperiled taxa on the site (Sigmon-Chatham 2015; Szakacs 2020).

The primary goal of the current project was to establish a population of *E. laevigata* outside of the powerline in some of the woodland restoration blocks on the same protected property. A successful establishment would not only help to meet the requirements of the 1995 species recovery plan, but also make the population more secure long term by expanding its range and establishing the species in an area that is managed solely for its conservation, rather than dually for the utility of the powerline. The secondary goal was to design and implement an experimental augmentation that would further the knowledge of ecology and biology of *E. laevigata*. Specifically, how does *E. laevigata* perform when grown among different competing vegetation life forms, and how do moisture and light influence *E. laevigata*'s growth in the field? A third goal was to describe patterns in plant composition and abiotic variables in the naturally occurring populations of *E. laevigata* at Picture Creek in order to compare to the experimental augmentation.

Methods

Site Description

Picture Creek Diabase Barrens "Picture Creek" is a State Dedicated Nature Preserve in Granville County, North Carolina. The 165-ha acre site is owned by the North Carolina Department of Agriculture and Consumer Services (NCDA) and managed in partnership with the North Carolina Plant Conservation Program (NCPCP). It is located in the northeastern Piedmont of NC, part of the upper Neuse River watershed, north of Stem, NC.

A powerline owned by Duke Energy cuts through the site and provides an open-canopy habitat for remnants of Xeric Hardpan Forest (Schafale 2012) that occur on Iredell and Picture series soils formed over diabase parent material. Xeric Hardpan Forest is characterized as having an open canopy dominated by fire adapted trees (*Pinus echinata*, *Pinus virginiana*, *Quercus marilandica*, and *Quercus stellata*), a sparse midstory (*Cercis canadensis, Chionanthus virginicus, Cornus florida*, *Juniperus virginiana*, and *Ulmus alata*), and an understory that is more species-rich than that of neighboring forest communities, including Piedmont Prairie species such as *Marshallia legrandii*, *Solidago ptarmicoides*, and *Solidago rigida* var. *glabrata*. The site was previously grazed and farmed in some sections until the 1940s. NCDA began active management of the property in the 1980's, at which point the majority of the property was forested outside of the powerline (Stanley et al. 2019).

Much of the historic Xeric Hardpan Forest has begun to be succeeded by mesophytic tree species like *Acer rubrum*, *Liquidambar styraciflua*, and *Pinus taeda* or entirely removed, as in the powerline. Current management focuses on thinning canopy, removing mesophytic species, and restoring frequent fire to remediate the desired plant community there.

Augmentation Establishment

A collaboration between the Plant Conservation Program (NCPCP) and the North Carolina Botanical Garden (NCBG) allowed several hundred individuals of *E. laevigata* to be grown from seeds collected from the population at Picture Creek Diabase Barrens by NCPCP volunteers on site in 2018. The seeds were planted at NCBG that December and germinated in the spring of 2019 after stratifying during winter outside in planting trays. While it is not known exactly how many matrilineal lines there are in the material collected, the seeds were collected from several dozen different adult plants on the site in both the powerline and the forested glade populations. Genetic studies within *E. laevigata* populations, including the Picture Creek population, have shown a considerable amount of genetic diversity (Peters et al. 2009) and the species is known to be mostly self-incompatible (Stucky et al. 2012), which makes a substantial genetic bottleneck in the augmented population unlikely, especially given the near distances to the source population (Collins and Foré 2009).

A section of the northeast side of Picture Creek with the same soil type (Iredell) as the majority of the natural population was designated for the augmentation. This area had been managed with thinning and burning in 2018 and 2019 to restore the area to woodland. We split the designated area into four different blocks corresponding to visual changes in landscape due to stream management zones and elevation change. Within each block, randomized points were created using ArcGIS software to minimize bias in plot placement. Each point was located and local vegetation life form was assessed to determine if it met requirements for a planting plot (as specified below). This was repeated until all the plots were placed in each block. Each block included two plots with over 60% graminoid cover, two plots with over 60% forb cover, one plot with over 60% vine cover, and one additional plot that was not dominated by a particular life form and which would be clipped to reduce competing interspecific vegetation. This created six plots in each block, or 24 plots total. Plots were oriented to face cardinal directions on each side and marked with flagging and conduits on each corner.

Planting was originally planned for the fall of 2019 but was delayed because the site needed a scheduled prescribed fire before the planting could occur. Unfortunately, the altered operation of NCBG the following spring and summer due to the 2020 COVID-19 Pandemic meant that a portion of the plants died over the summer. Just over 100 plants (now ca. 1.5 years old) remained to be planted in fall of 2020 for the augmentation. Volunteers with the Friends of Plant Conservation assisted with planting on December 4th, 2020. Each graminoid, forb, and vine plot was planted with four *E. laevigata* plants, one in each quadrat corner (Fig. 3.1). The intraspecific plots were clipped prior to planting and then planted with nine *E. laevigata* seedlings, in three-by-three rows. Consistent with recommendations by Alley (1997), plants were watered immediately after planting.

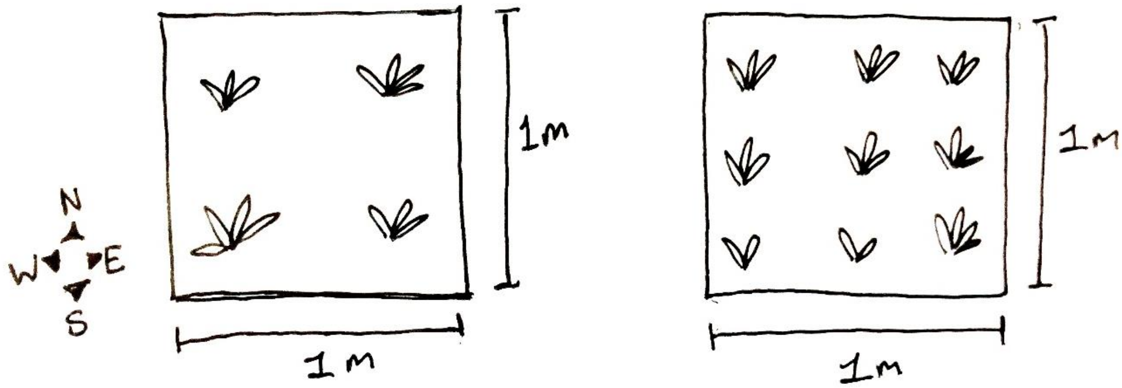

Figure 3.1. Sketch of plot planting designs for augmentation of *Echinacea laevigata* at Picture Creek Diabase Barrens. On left, plot design for life form treatments (graminoid, forb and vine plots; 4 individuals of *E. laevigata* planted per plot). On right, plot design for intraspecific plots (9 individuals of *E. laevigata* planted per plot).

<u>Plant measurements</u>

Initial, post-outplanting survival was assessed in March 2021, and each plant was marked with a numbered metal tag. Each plant's position, status, and identification number were recorded for tracking the plants and their corresponding measurements later in the year. Status included "live" if the plant was alive and present, "dead" if the plant was visibly dead and present, and "missing" if no remnants of the plant were found. In March 2021, most of the plants were still budding and had not leafed out yet, so a second survival assessment was undertaken in early July 2021 when plants were more fully leafed out. Second year survival was measured in July 2022 (of plants then ca. 3.25 years old).

Plant characteristics were measured in mid-July in 2021 and 2022, when the plants were nearing the end of the flowering. Plant measurements included basal plant height (not including flowering stems if present), total plant width (longest leaf tip to longest leaf tip of basal leaves), largest leaf length of basal leaves (including petiole), largest leaf width (at the widest part of leaf), number of leaves (including stem leaves), number of flowering heads, and flowering stem height, if present.

Natural Population Plots

The naturally occurring population of *E. laevigata* was assessed using plots of the same dimensions as the augmented plots, in order to compare vegetation of the two and to describe the environment in which *E. laevigata* naturally occurs at the site. The sample area was divided into two distinct habitat types where *E. laevigata* occurs on the site, the powerline cut and the forest glade. Points from a previous *E. laevigata* flowering survey performed by the NCPCP (Lesley Starke, personal communication, June 7th 2021) were randomly selected and located for plot placement. At each selected point, two flowering *E. laevigata* within two meters of the point were selected and each placed in a one meter by one-meter plot. Each plot was oriented to the cardinal directions just like the augmented plots and marked with flagging. In total, ten temporary plots were placed in the powerline population, and eight in the forest glade population.

Vegetation Measurements

Vegetation in the natural and augmented plots was measured in two ways. First, percent cover of the different life forms (graminoids, forbs [not including *E. laevigata*], vines, and *E. laevigata*) was estimated at four different heights in each plot (0–0.25m, 0.25–0.5m, 0.5–0.75m, and 0.75–1m). Second, competing vegetation was identified to species and total species cover and height measured in each plot. Species names follow Weakley (2022), and plants were identified in the field using a combination of The Guide to the Vascular Flora of Picture Creek

Diabase Barrens (Stanley et al. 2019) and online resources, including Lady Bird Johnson Wildflower Center Native Plants Database (https://www.wildflower.org/plnts/), North Carolina State University Extension (https://gardening.ces.ncsu.edu/gardening-plants/), North Carolina State University Herbarium website (https://herbarium.ncsu.edu/), and USDA Plants Database (https://plants.usda.gov/home). Photos were taken of most plants during identification, but vouchers were not. Cover classes were used to estimate percent cover in both vegetation measurements (0.0–4.99%, 5–24.99%, 25–49.99%, 50–74.99%, 75–94.99%, and 95–100%). Data for life forms and species were analyzed using the midpoint between each cover class.

Environmental Measurements

Light, soil moisture, and soil nutrient properties were also assessed at each of the augmented and natural plots to account for basic factors influencing seedling growth. Light was indirectly measured using hemispherical photos that were taken one meter above each plot. These photos were processed in ImageJ (Rasband 2022) to estimate canopy openness values. Soil moisture and temperature were measured at four different times throughout the growing season using the Aquaterr M-350 meter, which uses a scale of 0-100 to measure percent soil moisture, with 0 being the driest and 100 being completely saturated. The natural and augmented plot soil moisture was measured within the same day so that they could be compared to each other. Soil samples were collected from each plot and analyzed at Brookside Laboratories, Inc. (New Bremen, OH) for pH, % organic matter, S, P, Mg, Ca, K, Na, B, Fe, Mn, Cu, Zn, and Al.

Analyses

We calculated the mean soil temperature and moisture by measurement period and also summarized the seasonal trends using z-scores. Differences in both temperature and moisture were tested using ANOVA (PROC GLM, SAS v.9.4). The relative percent cover of each species was calculated within four groupings: 1) natural population plots, 2) powerline plots, 3) forest glade plots, and 4) experimental plots. Relative percent cover of each life form was also calculated for each experimental plot to compare their composition to each other and relate to the natural plots.

To visualize potential differences in species composition (based on cover) among the forest glade and powerline plots, a Non-metric Multidimensional Scaling Analysis (NMS) was performed in PC-ORD. Life form and canopy openness variables were overlayed in this ordination to see what relationship these variables had to the species composition of the plots. In a separate NMS, a second overlay of soil properties was placed on this ordination to see what relationship soil variables had to the species composition of the plots. A Bray-Curtis ordination, also performed in PC-ORD, included species composition in the experimental plots in addition to the species composition of the natural plots to visually compare how the species composition of different life form treatments related to the natural population.

Results

Species and Life Form Cover: Natural Population

In the natural extant population plots, over 44 species were found growing alongside *E. laevigata* (Table 3.1). Composition varied between the two natural plot environments with some species more prevalent in the powerline than the forest glade. The powerline plots had higher total richness (33 species) than the forest glade plots (17 species). In the powerline, the 10 species with the most overall percent cover made up 70% of the composition of competing vegetation around *E. laevigata*. In order of decreasing importance, they are *Schiyzachyrium scoparium*, *Aristida purpurea*, *Sorghastrum nutans*, *Liatris squarrosa*, *Rudbeckia triloba*, *Silphium terebinthinaceum*, *Andropogon virginicus*, *Lespedeza cuneata*, *Elymus virginicus*, and *Solidago altissima* (Table 3.1). In the forest glade plots, the top ten cover species made up 85% of the composition of competing vegetation around *E. laevigata*. In order, they were *Marshallia legrandii*, *Rubus* spp., *Carex* spp., *Liquidambar styraciflua*, *Lespedeza cuneata*, *Muscadinia rotundifolia*, *Cercis canadensis*, *Dichanthelium* spp., *Lespedeza procumbens*, and *Sorghastrum nutans* (Table 3.1). Cover of *E. laevigata* across all population natural plots ranged from 15% to 85%, with a median of 37.5% cover; it did not significantly differ between the powerline and the forest glade population plots (Student's t-test two tailed, unpaired, unequal variance: p-value=0.1305).

When species cover composition was ordinated for all the natural plots, the first axis was strongly related to a high occurrence of *Dichanthelium* sp. (r^2 = 0.369), *Lespedeza cuneata* (r^2 = 0.399), *Rubus trivialis* (r^2 = 0.5), *Silphium terebinthinaceum* (r^2 = 0.235), *Monarda* sp. r^2 = 0.264), *Potentilla canadensis* (r^2 = 0.252), *Acer rubrum* (r^2 = 0.264), and *Quercus alba* (r^2 = 0.264). The second axis was strongly related to *Andropogon virginicus* (r^2 = 0.309), *Solidago* sp.

($r^2 = 0.202$), *Aristida purpurascens* ($r^2 = 0.221$), *Scutellaria integrifolia* ($r^2 = 0.232$), *Rosa caroliniana* ($r^2 = 0.202$), *Liquidambar styraciflua* ($r^2 = 0.313$), *Potentilla canadensis* ($r^2 = 0.220$), and *Echinacea laevigata* ($r^2 = 0.531$). A third axis was strongly related to *Schizachyrium scoparium* ($r^2 = 0.202$), *Silphium terebinthinaceum* ($r^2 = 0.202$), *Sorghastrum nutans* ($r^2 = 0.202$), *Liatris squarrosa* ($r^2 = 0.202$), and *Marshallia legrandii* ($r^2 = 0.202$). Arranged by trends of compositional variation, the powerline plots clustered together, indicating that they were more similar in species composition to other powerline plots than to the majority of the forest glade plots. An overlay of life form cover and canopy openness indicates that powerline plots tended to have more openness and graminoid cover than the forest glade plots. The forest glade plots had a much wider range of vegetation composition. Some plots containing mostly forbs and other plots containing only woody vines (Fig. 3.2).

Comparisons of total overall life form cover (graminoids, forbs, and vines) revealed that there was a significant difference between total graminoid cover between the powerline and forest glade population plots (Student's t-test two tailed, unpaired, unequal variance: p-value=0.0274). Graminoid cover in the powerline ranged from 37.5% to 85%, with a median of 50% and a mean of 56.8%. Graminoid cover in the forest glade ranged from 15% to 85%, with a median of 15% and a mean of 29.4% cover (Fig. 3.3). There was not a significant difference between total cover of forbs or vines (p-value=0.308 and p-value=0.23, respectively).

Table 3.1. A summary of % species composition (not including *Echinacea laevigata*) among the different plot locations at Picture Creek Diabase Barrens (All natural plots, powerline plots, forest glade plots, and experimental plots). The ten species with the highest cover are bolded in each category below.

	Percent Composition by Plot Location			
Species	**All Natural Plots**	**Powerline Plots**	**Forest Glade Plots**	**Experimental Plots**
Acer floridanum	0.75	0	2.03	0
Agalinis purpurea	0.75	1.18	0	0
Andropogon sp.	3.48	**5.50**	0	2.46
Aristida purpurascens	**6.72**	**10.61**	0	0
Baptisia sp.	0	0	0	1.84
Bidens aristosa	0.75	1.18	0	0
Carex spp.	3.48	0	**9.49**	0
Ceanothus americanus	0.75	1.18	0	0
Cercis canadensis	1.87	0	**5.08**	0
Coleataenia anceps	0.87	1.38	0	**3.23**
Conoclinium coelestinum	0	0	0	**5.22**
Coreopsis major	0.12	0.20	0	0
Dichanthelium spp.	**3.61**	2.75	**5.08**	**5.99**
Diospyros virginiana	0.75	0	2.03	0
Elymus virginicus	2.74	**3.14**	2.03	0.31
Eupatorium hyssopifolium	0	0	0	0.15
Galactia regularis	0	0	0	**6.14**
Helianthus angustifolius	0	0	0	0.92
Houstonia tenuifolia	0.37	0.59	0	0
Hypericum spp.	1.00	1.57	0	0
Lespedeza cuneata	**5.60**	**4.13**	**8.14**	2.00
Lespedeza procumbens	1.49	0	**4.07**	0
Lespedeza repens	0	0	0	0.92
Lespedeza virginica	0	0	0	0.15
Liatris squarrosa	**4.23**	**6.68**	0	0
Liquidambar styraciflua	3.36	0	**9.15**	
Marshallia legrandii	**8.46**	0	**23.05**	0
Monarda sp.	0.75	0	2.03	0
Muscadinia rotundifolia	2.61	0	**7.12**	**11.37**
Packera anonyma	0	0	0	0.15
Parthenocissus quinquefolia	0	0	0	0.92
Potentilla canadensis	0.87	0	2.37	0
Potentilla simplex	0.12	0.20	0	0.46
Pycnanthemum tenuifolium	0.87	1.38	0	**5.22**

Table 3.1 (Continued)

Quercus alba	0.75	0	2.03	0
Rhus copallinum	0.12	0.20	0	0
Rosa caroliniana	0.37	0.59	0	0
Rubus spp.	**5.35**	2.55	**10.17**	**3.53**
Rubus trivialis	**4.60**	**6.09**	2.03	0
Ruellia caroliniensis	0	0	0	0.92
Schizachyrium scoparium	**11.32**	**17.88**	0	**26.57**
Scleria oligantha	1.87	2.95	0	**10.75**
Scutellaria integrifolia	0.37	0.59	0	0
Scutellaria sp.	0.12	0.20	0	0
Silphium terebinthinaceum	**3.73**	**5.89**	0	0
Smilax bona-nox	0	0	0	0.92
Smilax rotundifolia	0	0	0	0.92
Solidago altissima	1.99	**3.14**	0	0
Solidago caroliniana	0.25	0.39	0	0.92
Solidago ptarmicoides	1.62	2.55	0	0.92
Solidago rigida	1.49	2.36	0	0
Solidago rugosa	1.74	2.75	0	**5.22**
Sorghastrum nutans	**5.97**	**7.07**	**4.07**	0
Symphyotrichum sp.	1.62	2.55	0	0
Fabaceae sp.	0.12	0.20	0	0
Tridens flavus	0.25	0.39	0	1.84
SUM	100.00	100.00	100.00	100.00

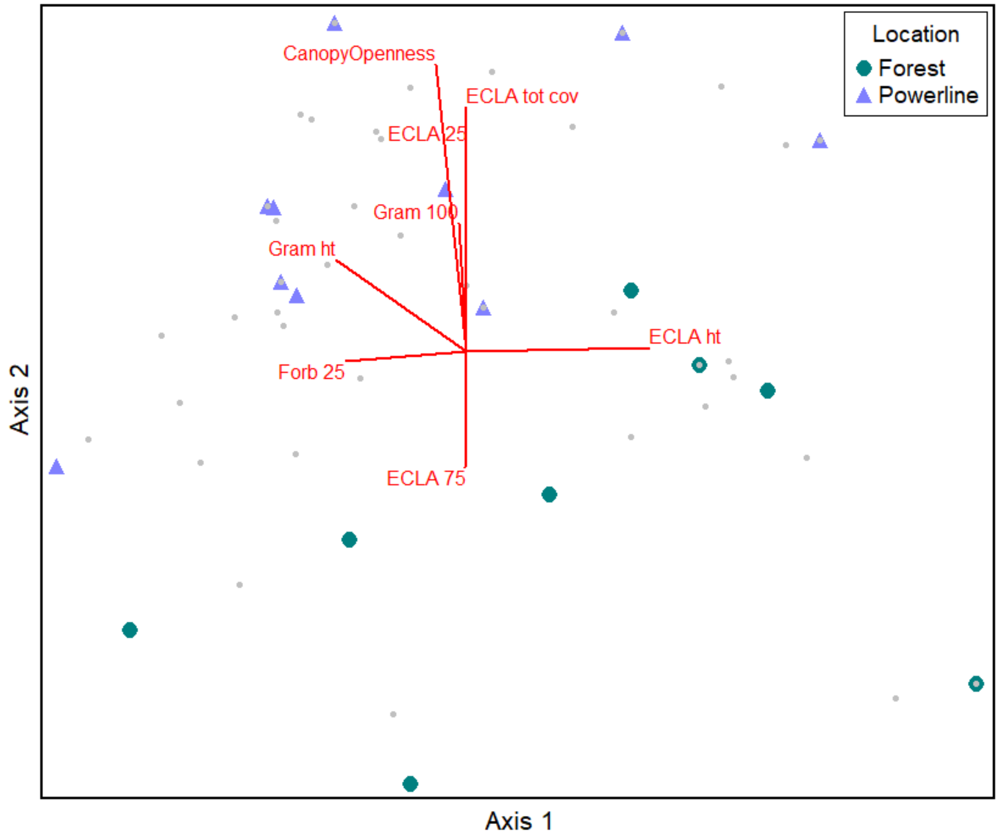

Figure 3.2. The composition of plant species separated between forest glade and powerline locations (NMS ordination using percent cover). Vectors display relationships of life form cover [Gram 100= Graminoid cover at 1 m, Forb 25= Forb cover at .25 meter], *E. laevigata* cover [ECLA 25= *Echinacea laevigata* cover at 0.25 m, ECLA 75 = *Echinacea laevigata* cover at 0.75 m, ECLA totcov = total cover of *Echinacea laevigata*] *Echinacea laevigata* height [ECLA ht], Graminoid height [Gram ht], and percent canopy openness [Canopy Openness] with the species composition. The final stress of the ordination is 10.04 for a 3 axis solution; only axis 1 and axis 2 are shown here. Small grey circles in background represent the plant species in the ordination.

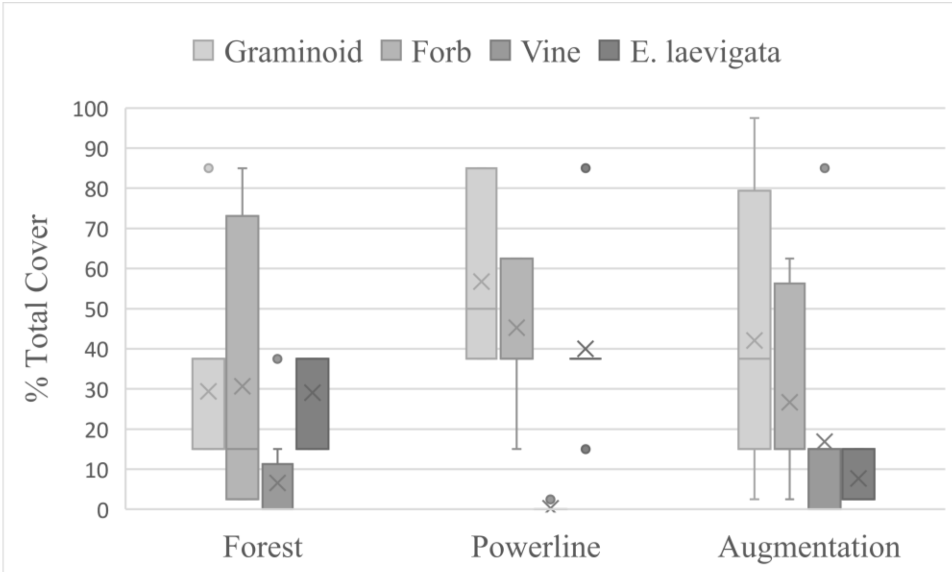

Figure 3.3. Comparison of percent total life form cover (graminoids, forbs, vines) and *Echinacea laevigata* in the forest glade plots (left), powerline plots (center), and augmentation plots (right).

<u>Species and Life Form Cover: Augmentation</u>

The majority of the experimental life form treatment plots (Graminoid, Forb, and Vine) still met the composition requirements from plot establishment in 2020 when measured in 2021 (Graminoid, Forb, and Vine plots having over 60% cover in their respective life forms). One Forb plot no longer met the 60% composition threshold, with just 46% of the percent composition containing forbs; however, forbs were still highest proportion of the composition in that plot. Intraspecific plots had no initial composition requirements because they were clipped before planting and were not dominated by any one life form when measured in 2021 (Table 3.2)

Experimental plots grouped together by life form when they were ordinated by species composition along with the natural plots. For example, Graminoid plots were more similar to other Graminoid plots than to Forb or Vine plots. Twenty-nine percent of the variation in the plots could be explained on axis one and axis two. Overlays of life form cover showed that the experimental plots tended to have more of their dominant life form than seen in the natural species composition. For example, experimental Vine plots were positioned along the extreme of the vine vectors in the ordination and had more vine cover than any natural plots. Forb cover remained very similar between powerline, forest glade, and Forb augmentation plots (Fig. 3.4).

Table 3.2. Summary of percent composition of life forms in experimental plots. Forbs do not include *Echinacea laevigata*, the values of which are shown separately.

Plots by Life Form	Graminoids	Forbs	Vines	*E. laevigata*
Forb	18.2	45.5	18.2	18.2
Forb	16.2	67.6	0.0	16.2
Forb	36.6	61.0	0.0	2.4
Forb	36.6	61.0	0.0	2.4
Graminoid	78.1	18.8	0.0	3.1
Graminoid	65.4	11.5	11.5	11.5
Graminoid	95.1	2.4	0.0	2.4
Graminoid	82.9	14.6	0.0	2.4
Vine	2.7	2.7	91.9	2.7
Vine	12.8	12.8	72.3	2.1
Intraspecific	31.6	31.6	5.3	31.6
Intraspecific	55.6	22.2	0.0	22.2

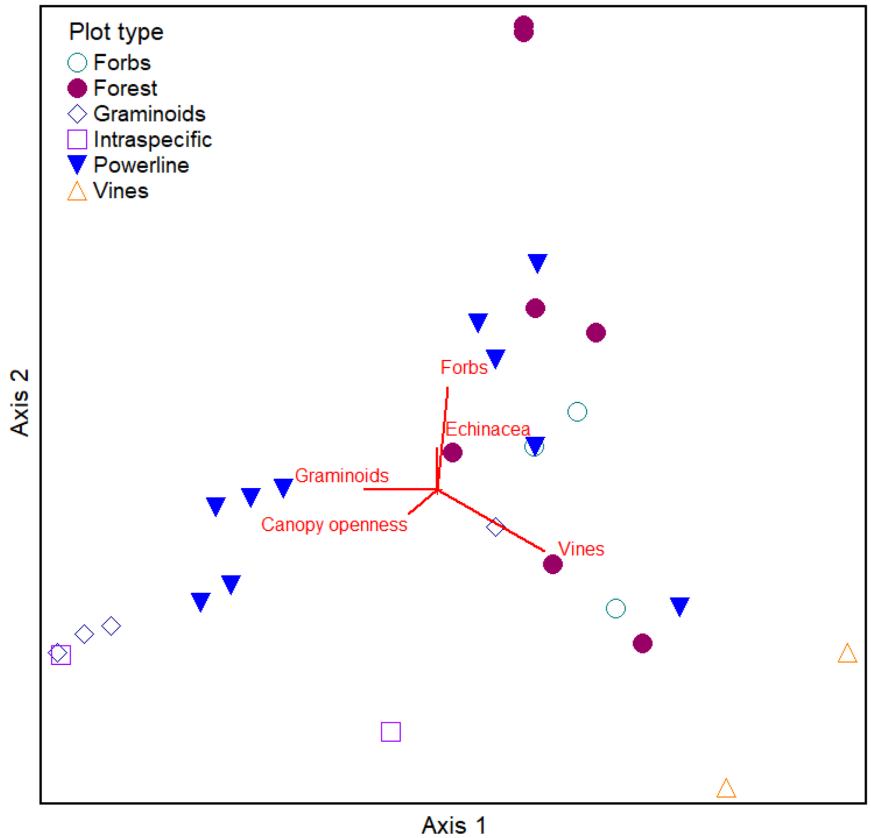

Figure 3.4. Bray-Curtis ordination of species composition in each plot to explain the variation between the plots in the experimental blocks (Forb, Graminoid, Intraspecific, and Vine) and the original plots (Forest Glade and Powerline). Overlay shows how the total cover of different life forms (Forbs, Graminoids, Vines, *Echinacea*) and Canopy openness relate to the plots.

Canopy Openness

The canopy was more open in the powerline plots than in the forest glade plots with an average of 76% openness compared to the forest's 24% openness (p-value <0.0001). Canopy openness in the powerline ranged from 56% to 88% while canopy openness in the forest glade ranged from 15% to 33% (Fig. 3.5). Canopy openness in the experimental plots ranged from 34% to 85%, with a mean of 60%. The canopy openness of the experimental plots was both higher than the openness in the forest glade and lower than the openness in the powerline ($p<0.0001$ and $p<0.05$ respectively). The experimental plots' median percent canopy openness

(64%) was lower than the powerline plots' median (79%) and higher than the forest glade plots' median (26%) (Fig 3.5).

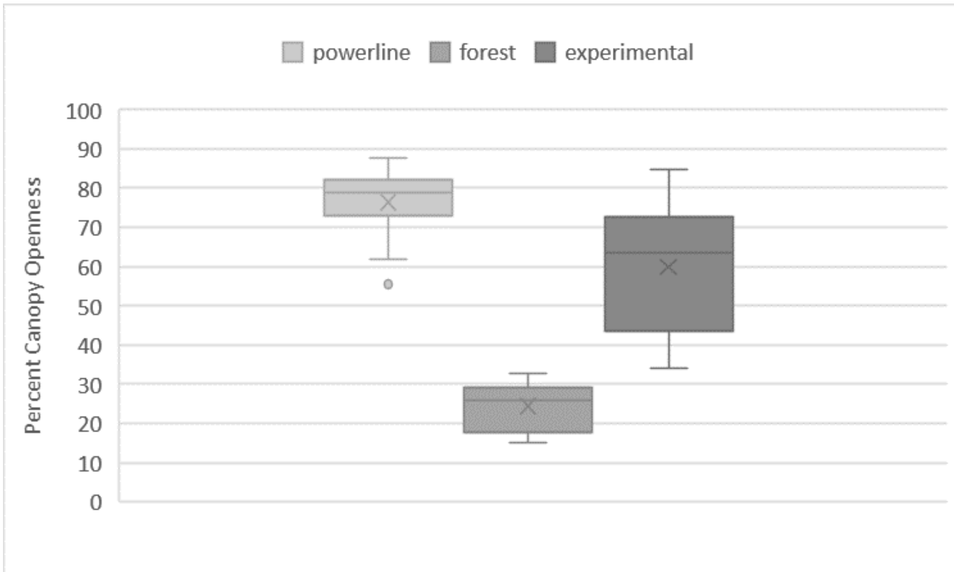

Figure 3.5. The range, max, min, median, mean and interquartile values for the canopy openness over flowering *Echinacea laevigata* occurrence within the forest glade, powerline, and augmentation plots.

Soil Properties

Soil moisture between the powerline and the forest glade where *E. laevigata* was growing was not significantly different (Pr>t = 0.67). Soil moisture was similar across the site from sampling period to sampling period. When separated by monthly measurements, soil moisture between the powerline and the forest glade differed significantly only in June (Pr >t = 0.01) (Fig. 3.6) when average soil moisture in the powerline was 94.2% of soil water capacity, and in the forest glade was 85.5% of soil water capacity.

Soil moisture among the augmented plots and the forest glade and powerline natural plots when averaged across the season measurements were similar (Pr> F= 0.74). However, when split among individual measurement days, there was a significant difference among the three plot locations in June (Pr>F=0.0154) and July 13th (Pr>F= 0.012). In June, the powerline had the highest soil moisture capacity at 94.2%, while the forest glade was the lowest at 85.5%, and the experimental plots fell between the two at 88.59%. During July 13th, the experimental plots had the highest average soil moisture capacity at 95%, while the powerline was the lowest at 78.5%, and the forest glade fell between the two at 87.875%. There was no significant difference between the three locations on July 22nd, or September 2nd (Fig. 3.6).

When ordinated, plot locations in the powerline and forest glade are clearly separated based on the plant community composition. An overlay of soil properties correlated revealed that there were some relationships between soil properties and the plot locations arranged by plant community composition. Forest glade plots had more iron, sodium, and phosphorus than the powerline plots, while powerline plots had more calcium and potassium (Fig. 3.7).

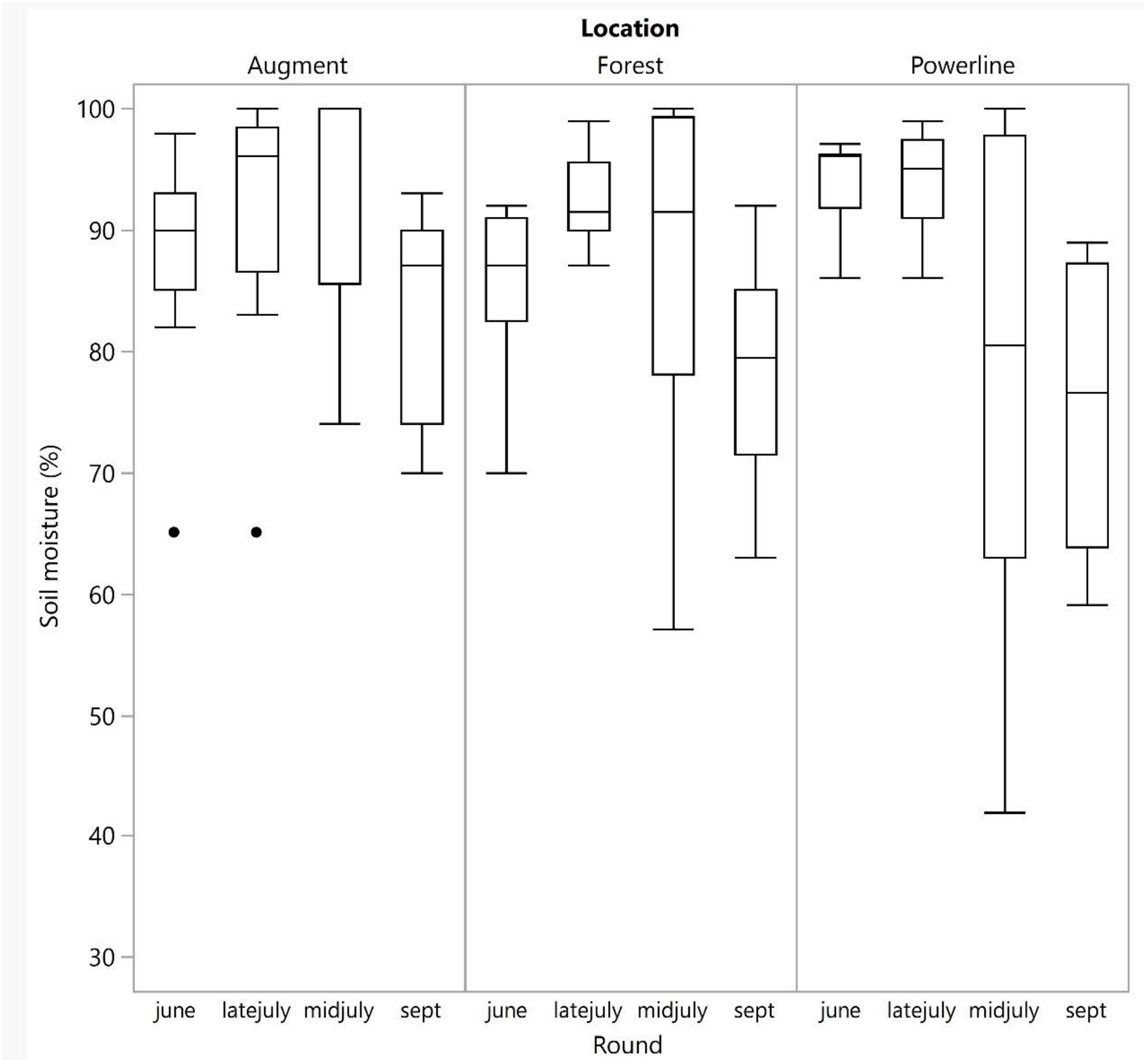

Figure 3.6. The distribution and quartile ranges of average soil moisture values in the augmented plots, powerline plots and the forest glade across measurement rounds at Picture Creek Diabase Barrens in 2021.

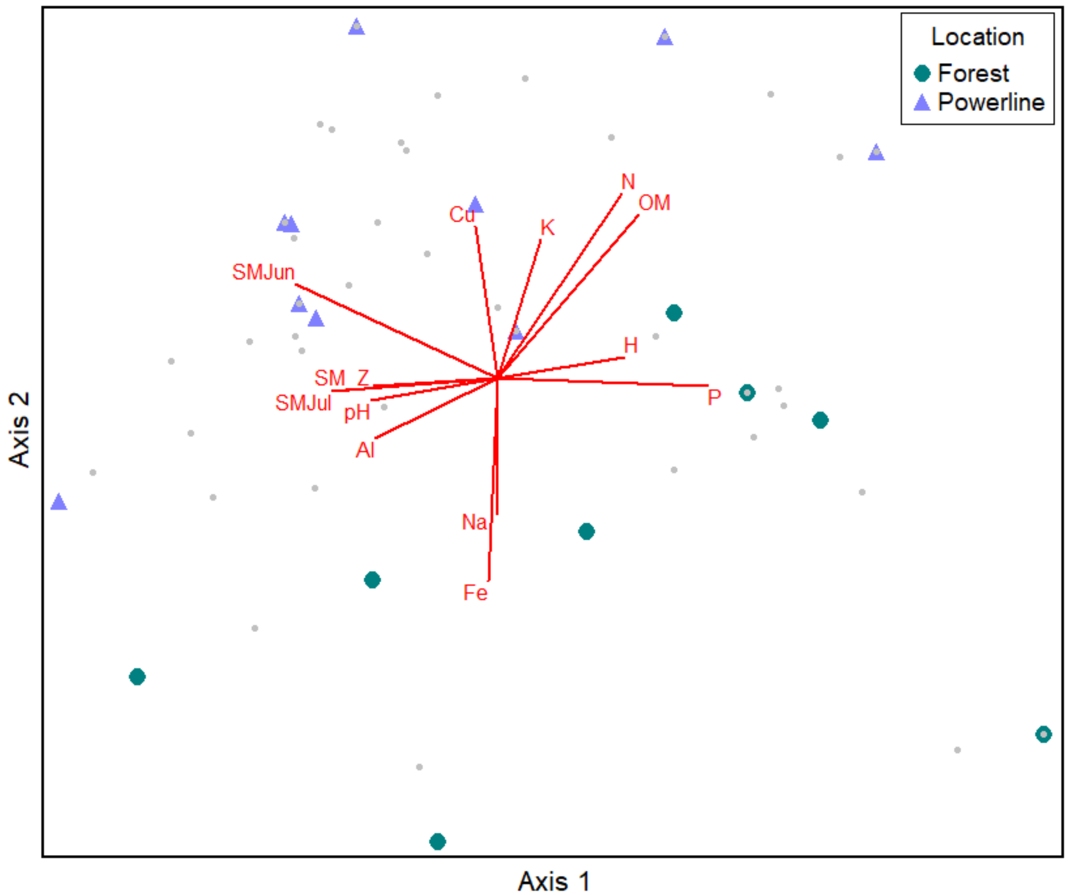

Figure 3.7. NMS ordination of forest glade and powerline plots arranged by plant abundance. Overlay shows how soil properties (soil moisture in June (SMJun), soil moisture z-scores (SM_Z), soil moisture in July (SM Jul), copper (Cu), potassium (K), nitrogen (N), organic matter (OM), pH, H, aluminum (Al), sodium (Na), phosphorus (P), iron (Fe)) relate to the species composition differences between the two environmental locations. Forest glade and Powerline plots separate from each other indicating differences in plant community composition. (Same ordination as Figure 3.2, just with different vector overlay)

Augmented Plant Measurements

Over 75% of the augmented *E. laevigata* seedlings planted in December 2020 were alive three months later when initial survival was taken. This was early in the season when most plants were just budding (Fig 3.8), so a second survival was taken in May of 2021 after plants leafed out. During this survival count, 5 plants that were not found or believed dead in the initial count were found alive, while 2 plants that were revisited in May were missing or dead. This made the overwinter survival since planting closer to 78% (Table 3.3). Block 3 contained 80% of the missing and dead plants in the May 2021 count. There was evidence of vole damage in this block with plants replaced by an underground hole and eaten from the tap root upwards.

When plants were revisited for measurements in July of 2021, two months later, survival had decreased by 42% since the last measurement (Table 3.3). No surviving plants were found in blocks 3 and 4. There was evidence of vole damage throughout blocks 3 and 4 (holes under where plants were, leaves left behind, eaten from tap root upwards) (Fig. 3.8). Plant survivorship remeasured in blocks 1 and 2 the following year (May 2022), showed little change from the previous measurement. Two plants that were missing in July 2021 were relocated and four plants were newly dead or missing, leaving a total of 51 plants from the 116 originally planted in December 2020. Again, during July 2022 measurements, survival was still high with 50 plants remaining, one plant missing from the spring.

During the first individual *E. laevigata* plant measurements in July 2021, plant height in Vine and Graminoid treatments was higher than in Forb and Intraspecific plot treatments (p=0.05). The same pattern was found with leaf length, where Vine and Graminoid plots had longer biggest leaves than Forb and Intraspecific plots (p=0.03). Just one plot, the Block 1 with Intraspecific treatment, contained flowering stems (two of the nine plants flowered.)

During the second individual *E. laevigata* plant measurements in July 2022, when plants had been growing in the environment for more than a year and a half, plant width and leaf length values were highest among plants in the Graminoid treatment plots (averages 29.7cm and 28.8cm and lowest among the vine plots (averages 15.4cm and 16.5cm) (p-value = 0.06 and p-value = 0.04 respectively) (Table 3.4) (Fig. 3.10). Additionally, there was much more flowering in 2022 with 9 plants flowering: eight first time bloomers and one blooming for the second time. One plant had three flowering stems, but their tops were predated by some mammal before measurements.

Table 3.3. Number of surviving individuals and percent change of outplanted *Echinacea laevigata* from March 2021 through July 2022 at Picture Creek Diabase Barrens.

Count of living plants	**Planted (Dec-20)**	**Mar-21**	**May-21**	**Jul-21**	**May-22**	**Jul-22**
	116	88	91	53	51	50
Change since last count	appeared	n/a	5	0	2	0
	disappeared	28	2	38	4	1
	% change	-24.14%	3.41%	-41.76%	-3.77%	-1.96%
% Change from planting		-24.14%	-21.55%	-54.31%	-56.03%	-56.90%
% Survival from planting		75.86%	78.45%	45.69%	43.97%	43.10%

Figure 3.8. The bright pink emerging vegetative bud of *Echinacea laevigata* in spring (left). A leafed out *Echinacea laevigata* seedling at Picture Creek Diabase Barrens with evidence consistent with predation by voles (right). The taproot is nearly entirely missing, eaten from the bottom upwards.

Table 3.4. Summary table for models testing for differences among *E. laevigata* planted with different life forms in year 2 post-planting (df=3,38 for all models).

Variable	Forbs	Graminoids	Intraspecific	Vines	F value	*p*-value
Plant height (cm)	21.7±2.6	22.4±2.3	18.7±2.7	15.3±3.2	1.3	0.29
Plant width (cm)	22.8±3.5	29.7±3.1	26.9±3.3	15.4±4.3	2.6	0.06
Number leaves (count)	6.2±1.0	7.1±0.9	7.2±0.9	5.1±1.3	0.7	0.53
Largest leaf length (cm)	25.3±2.7	28.8±2.5	23.8±2.6	16.5±3.4	3.0	0.04
Largest leaf width (cm)	4.3±0.5	4.9±0.5	4.0±0.5	2.9±0.6	2.1	0.12
Number of flowers (count)	0.07±0.1	0.29±0.1	0.28±0.1	0.13±0.2	0.5	0.67
Flowering stem height (cm)	85±6.7	87±6.2	77.5±4.3	81±6.7	0.6	0.63

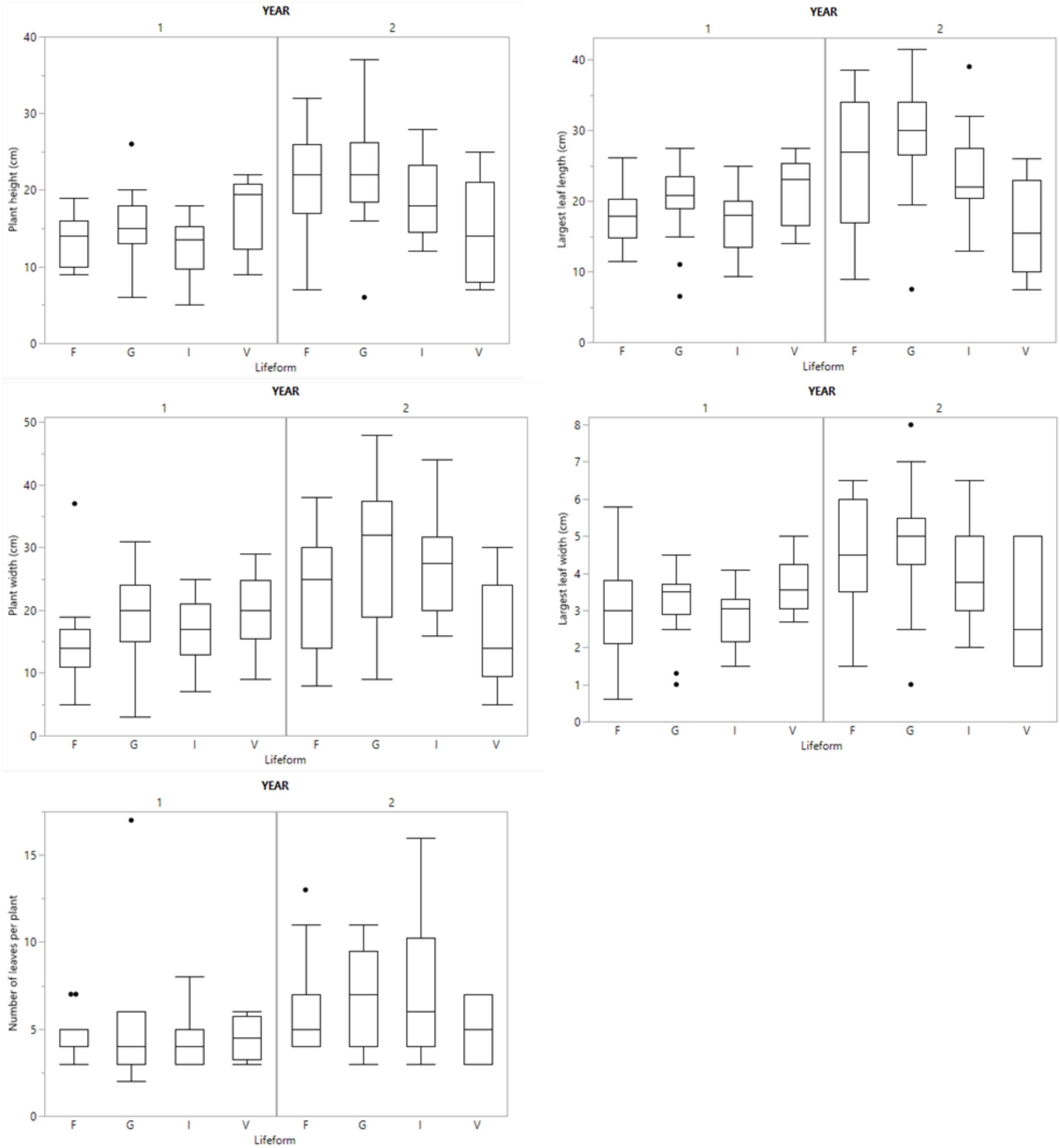

Figure 3.9. Comparisons of plant measurements by life form treatment (Forb (F), Graminoid (G), Intraspecific (I), Vine (V)) and year read left to right as plant height (not including flowering stems), largest leaf length (includes petiole), plant width (widest point to widest point without flattening plant), largest leaf width (at widest point on leaf), and number of leaves per plant.

Discussion

Neighboring Vegetation Composition

Plots in the natural population were centered over flowering *E. laevigata* plants, so that we could determine which species were growing directly adjacent to “successful” *E. laevigata* plants. Most plots contained a mixture of graminoids and forbs with few vines or woody plants. Plants neighboring *E. laevigata* changed in composition among the powerline and the forest glade plots. The powerline had higher richness and presence of other Piedmont Prairie species than the forest glade. This was expected since we know that the majority of Piedmont Prairie associates are heliophytes and primarily occur in open conditions (Stanley et al. 2019, Szakacs 2020).

The most important neighboring species, by relative cover, were largely native graminoids and forbs that we would expect to be found growing alongside *E. laevigata* in its natural community with the exception of the non-native *Lespedeza cuneata* (Stanley et al. 2019, Szakacs 2020). The forest glade had much less forb and graminoid cover, which indicates that it is less species rich than expected of the rich understory community characteristic in the Xeric Hardpan Forest Community (Schafale 2012). Two of our eight *E. laevigata* plots in the forest glade were located in the large *Marshallia legrandii* population there which may have exaggerated the importance of *M. legrandii* relative to other neighboring species; however, it still remained a major component of the forest glade understory composition where *E. laevigata* grew even if this had not occurred. A large portion of the neighboring species in the forest glade understory were woody tree saplings, shrubs, and vines.

Comparatively, life form cover in the experimental augmentation reflected our experimental design, with life form plots having higher cover of their respective life forms than present in the natural community. This was especially true for Graminoid and Vine plots while Forb plots remained comparable to many plots located in the powerline. The species composition in the experimental plots more closely resembled the powerline plant community than the forest glade (Fig. 3.4). The vast difference between the plant communities neighboring *E. laevigata* in the powerline and the forest glade echo the vast differences in light availability in those two environments.

Canopy openness

Unsurprisingly, we found that the flowering population in the powerline had significantly more canopy openness than the forest glade. In fact, canopy openness did not overlap at all, meaning that the *E. laevigata* flowering in these two environments are doing so under completely different light conditions. It is known from annual flower count surveys done by the NCPCP that the *E. laevigata* population in the forest glade increased in flowering after disturbance events such as thinning of the midstory and prescribed fire treatments. The difference in measured canopy openness between the powerline and the forest glade population indicates that the threshold canopy openness needed for *E. laevigata* to bloom is lower than what is found in the powerline and not a result of light gaps that are comparable to the powerline in the forest glade.

The lower canopy openness where *E. laevigata* was blooming in the forest glade environment ranging from 15% – 33%. This is important for conservation management of *E. laevigata* because it may allow a larger range of options for silviculture prescriptions when multiple use objectives are being considered. However, it must also be remembered that both the powerline and the forest glade locations on the site are the result of land use changes that altered the naturally occurring Xeric Hardpan Forest community that likely used to occur there (Stanley et al. 2019). In the restored woodland at Picture Creek, where the augmented plants are grown, canopy openness conditions are closer to the natural environment *E. laevigata* is thought to have inhabited prior to land use change. Canopy openness in this environment ranged from 34% – 85% making it more open than any of the forest glade plots and ranging from partial shade to the full canopy openness of the powerline. Additionally, repressed extant *E. laevigata* were observed blooming for the first time in parts of this woodland restoration during surveys by the NCPCP. That, coupled with the successful blooming in the augmentation, suggests that management for *E. laevigata* should likely target canopy openness that is in the mid to high ranges of where we found *E. laevigata* blooming. These higher canopy openness ranges would also support many Piedmont Prairie associate species that grow alongside *E. laevigata* (Szakacs 2020) even though *E. laevigata* may be able to linger in lower canopy openness levels like those seen in the forest glade population.

Evidence of Plasticity in *E. laevigata*

Researchers at Picture Creek have wondered how *E. laevigata* manages to persist in degraded habitat that has become overgrown with dense mesophytic trees and shrubs, as seen in the forest glade population on the site. During our vegetation cover measurements, we found evidence that *E. laevigata* has some plasticity in its growth habit that may be helping it survive the lower canopy openness in these areas. Total cover of *E. laevigata* was not significantly different between the plots found in the forest glade and the powerline. However, when cover was considered at heights from ground level to 1m, there were some significant differences between the two environments. *E. laevigata* plants in the powerline had more vegetative cover in the first quarter meter than the forest glade plants, while forest glade plants had more vegetative cover above half a meter than the powerline plants did. This confirmed observations during fieldwork that the forest glade plants were producing more cauline leaves on the plant than the ones in the powerline which tended to be squatter and basally disposed. It is not uncommon for plants to have plastic adaptations to different light conditions, often increasing leaf area in lower light environments to increase light capture (Niinemets 2010), but until genetic research is done to compare the plants in the two light environments, it will be unknown whether this adaptation is broad among the population or specific to the plants growing in each environment.

Additionally, in our experimental augmentation design, we found that *E. laevigata* had different leaf sizes, dependent on treatment. During the plant measurements in July 2022, when plants had been growing in the environment for more than a year and a half, plant width and leaf length values were highest among plants in the graminoid treatment plots (averages 29.7 cm and 28.8 cm) and lowest among the Vine plots (averages 15.4cm and 16.5 cm). Visually we could tell that the vines (typically *Muscadinia rotundifolia*) greatly overtopped the seedlings and cast more shade with their broad leaves than Forbs or Graminoids in their respective plots. Since the plants are still young, it is uncertain whether these leaf size differences are due to the plants adapting successfully to the light conditions caused by the surrounding vegetation or being affected by the surrounding vegetation, such that they did not have the resources to grow bigger. Although *E. laevigata* is a perennial herbaceous plant that regrows its leaves each year from energy stored in tap roots, there may be a certain tap root diameter that the plant has to reach to be competitive against surrounding vegetation. Future research could investigate the effect that tap root size has on the vigor of the plants and see how many years it typically takes to successfully flower under different conditions using the annual signatures described by Krings (2020) to solve these questions.

Soil Moisture

Soil moisture fluctuated over the course of the growing season in the natural population. There was no significant difference between the average growing season soil moisture measurements between the powerline and the forest glade. However, comparing soil moisture on each measurement day revealed that on the day of the June measurement, the powerline had significantly more soil moisture than the forest glade. While the later measurement days did not show a difference between the two environments, more intensive soil moisture measurements would be needed to determine whether this moisture disparity happened often enough to affect the growth and success of *E. laevigata.*

Average soil moisture over the season did not significantly differ between the natural population plots and the experimental augmentation plots. There were significant differences during specific measurement days between the three locations in June and mid-July. However, the ranking of highest and lowest soil moisture percentages was different for both measurements, meaning there was no discernable trend in soil moisture difference between the powerline, the forest glade, and the experimental site. More in-depth soil moisture monitoring would need to be done to determine whether the changes in soil moisture would affect growth of *E. laevigata*. Since average soil moisture does not differ significantly over the season, it is possible that any fluctuations across the site balance each other out.

Augmentation Success and Survival

According to best management guidelines observed by the North Carolina Plant Conservation Program (NCPCP), in order to be a good augmentation, the augmentation needs to establish and maintain a viable population that requires a minimal amount of management intervention, uses an experimental design approach, and establishes a monitoring protocol and schedule for both short- and long-term objectives (NCPCP 2005). Our augmentation helps meet these guidelines, and it contributes to the recovery guidelines set out in the 1995 *E. laevigata* recovery plan (U.S. Fish and Wildlife 1995), by establishing a population in the restored woodland area on Picture Creek in an experimental design. Implementation of the established monitoring schedule for the first two years as well as the long term monitoring plans will determine whether this new augmented population is viable long term.

The initial survival of planted *E. laevigata* was very high, with nearly 78% of plant surviving winter and early spring of 2021. In the May 2021 count, 80% of the missing and dead plants were from block 3 where there was evidence of what would later be determined to be likely vole predation, with plants replaced by an underground hole and eaten from the tap root upward. Just two months later, survival had decreased by 42% and no surviving plants were found in blocks 3 and 4. The majority of dead and missing plants had evidence consistent with vole damage. Vole predation had been observed at other augmentations and was noted to occur less often with rocky soil (Pat and Herb Amyx, NCPCP volunteers, personal communication). This combined with the dramatic loss in our first year of the augmentation at Picture Creek, suggests that vole predation is a concern for the success of planted *E. laevigata*. Voles may also play a role in the dynamics of natural *E. laevigata* demographics on the site.

Fortunately, plant survivorship remeasured the following year in blocks 1 and 2 (May 2022), showed little change from the previous measurement with 51 of the original 116 plants surviving. Again, as seen in July 2022 measurements, survival from previous measurements was still stable with 50 plants remaining. We revisited Blocks 3 and 4 in 2022 to see if any plants had returned and found that just one plant survived vole predation in Block 4.

The stable survival across all the treatments, excluding those potentially affected by voles, supports that *E. laevigata* is a robust species when planted from germinated seedlings (Krings 2020). The presence of 9 flowering plants in 2022 shows that the augmented seedlings are reaching maturity and potentially able to produce seed. Due to their proximity to extant natural *E. laevigata* in the powerline, flowers in the augmentation can be easily pollinated by pollinators from the powerline population.

Conclusion

We found that flowering *E. laevigata* plants at Picture Creek grew in a diverse mixture of graminoids and forbs that differed in cover and composition between the powerline and the forest glade. *E. laevigata* displayed signs of plasticity in growth in the two different environments with plants in the powerline covering more area close to the ground than forest glade plants and the forest glade plants covering more area over half a meter high than the powerline plants.

Seedlings planted in vine-dominated experimental plots had shorter leaves than those growing in the forb- and graminoid-dominated experimental plots. We also found that plots that were clipped prior to planting had higher survival and flowered earlier than plots that were not clipped. This indicates that, during augmentation, planting seedlings among graminoids and forbs and clipping competing vegetation prior to planting may increase the success of planted *E. laevigata*.

Future research will expand the plot design and continue to measure the effects of vegetation competition on *E. laevigata*. Managers of natural populations of *E. laevigata* should continue using thinning of the canopy and midstory along with introducing or maintaining fire to promote the mid to high levels of canopy openness related to increased flowering of *E. laevigata*.

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