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**The Influence of Land Management on the Demography of the Pine Barren gentian
(*Gentiana autumnalis*) in New Jersey**

A Thesis

Submitted to the Faculty

of

Drexel University

by

Ryan R. Rebozo

in partial fulfillment of the

requirements for the degree

of

Doctor of Philosophy

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	ix
ABSTRACT	xi
CHAPTER 1: Introduction	1
Study Species	3
Study Sites	4
Study Overview and Objectives.....	8
Literature Cited	11
Chapter 2: The effects of prescribed burns and mowing on the demography of <i>Gentiana autumnalis</i> in the New Jersey Pine Barrens	15
Introduction.....	16
Materials and Methods.....	18
Results.....	23
Discussion	28
Literature Cited	35
Chapter 3: The effect of management practices (mowing and prescribed burning) on soil nutrients and mycorrhizal colonization of <i>Gentiana autumnalis</i>.....	49
Introduction.....	50
Materials and Methods.....	54
Results.....	59
Discussion	62
Literature Cited	69

Chapter 4: Negative effects of herbivory by <i>Endothenia hebesana</i> (verbena bud moth), <i>Chlorochlamys chloroleucaria</i> (black berry looper), and <i>Odocoileus virginianus</i> (white-tailed deer) on the rare <i>Gentiana autumnalis</i> (Pine Barren gentian) in New Jersey.....	81
Introduction.....	82
Materials and Methods.....	85
Results.....	89
Discussion.....	91
Literature Cited.....	94
Chapter 5: Insect visitation and seed set of the rare Pine Barren gentian (<i>Gentiana autumnalis</i>) under varying management regimes.....	103
Introduction.....	104
Materials and Methods.....	106
Results.....	113
Discussion.....	119
Literature Cited.....	126
Chapter 6: Thesis Summary and Future Directions	141
Introduction.....	141
Summary of Major Findings.....	142
Conservation Implications	145
Future Research Directions.....	147
Literature Cited.....	150
APPENDICES	151
Appendix A. Global and state element ranks as defined by NatureServe	151
Appendix B. Associate plant species encounter at each site	152
VITA.....	156

LIST OF TABLES

Table 2.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)....	39
Table 2.2: Population areal size, spring census density and percentage of seedlings and flowering plants at each site during 2011, 2012, and 2013. (ND=No Data, NS= Not Sampled)	40
Table 2.3: Average seed set for <i>Gentiana autumnalis</i> per fruit by site (2011, 2012 and 2013; ND= No Data due to limited flowers present).....	41
Table 3.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)....	74
Table 3.2: Average percent mycorrhizal root colonization, available soil nutrients ($\mu\text{g/g}$) and pH by site (N.D. = No Data)	75
Table 3.3: Average available soil nutrients ($\mu\text{g/g}^{-1}$), pH, percent mycorrhizal root colonization, percent canopy openness, ground cover and species richness by management practice	76
Table 3.4: Average soil bulk density (g/cm^3) within and along the periphery of macroplot at each site.....	77
Table 3.5: Correlation coefficient matrix of site variables PO_4 , NH_4 , NO_3 , bulk density (Bulk Den.), pH, percent mycorrhizal root colonization (Fungal Colo.), percent canopy openness (Canopy Op.), Braun Blanquet ground cover (Ground Co.), species richness (Spp. Rich.)	78
Table 4.1: 2012 <i>Gentiana autumnalis</i> density, percentage of flowering plants, percentage of seedling plants (2013 data) and abundance estimate for eight study sites (Data were estimated from sampling quadrat counts)	97
Table 4.2: Average gentian fruit seed set and average seed capsule weight with standard deviations for study sites (DD = data deficient)	98
Table 4.3: The percentage of sampled plants that exhibited damage consistent with <i>Endothenia hebesana</i> and <i>Chloroclamys chloroleukaria</i> herbivory and their sum for the 2012 study year (DD = Data Deficient).....	98
Table 4.4: The percentage of <i>Gentiana autumnalis</i> plants in the sampling quadrats that exhibited signs of deer herbivory in 2012.....	99

Table 4.5: Density of heterospecific flowering plant species, total floral density (flowering gentian density + density of heterospecific flowering plants) and Simpson's index of diversity for flowering plants at each study site	99
Table 5.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)...	131
Table 5.2: Average weekly density (1 m ²) of heterospecific and conspecific flowers at each site in September – October (2011-2013).....	132
Table 5.3: Total insect visitation observation time by site	133
Table 5.4: Total site gentian nectar standing crop by week in 2013 (µl).....	133
Table 5.5: Average seed set for <i>Gentiana autumnalis</i> per fruit by site (2011, 2012 and 2013; N.D.= No Data).....	134

LIST OF FIGURES

Figure 2.1: Frequency of the number of <i>Gentiana autumnalis</i> plants recorded in sample quadrats (1 m ²) randomly placed in macroplots of all sites from 2011-2013 (All management regimes included; n=427, max= 75 plants)	42
Figure 2.2: Percentage <i>Gentiana autumnalis</i> seedlings and flowering plants in study populations pooled by management practice (2011-2013).....	43
Figure 2.3: Gentian fall census density prior to management (2011) and in two successive years (2012-2013; S.E. bars).....	44
Figure 2.4: Gentian fall census percentage of flowering plants in study populations prior to management (2011) and in two successive years (2012-2013; S.E. bars).....	45
Figure 2.5: Average gentian yearly mortality by life stage in managed (disturbed) and unmanaged sites (all sites; S.E. bars).....	46
Figure 2.6: Log transformed PCA grouped by management practice. Variables- area, density (Den), abundance (Abun), flowering % (Flower), vegetative % (Veg), seedling % (Seedling), seasonal mortality (Smort), yearly mortality (Ymort), seed set (SS), population growth rate (PopG). 59.1% of variation explained in two principal components.	47
Figure 3.1: Range of average site canopy openness percentage for burned, mowed and undisturbed sites (minimum, 25 th percentile, mean, 75 th percentile, maximum).....	79
Figure 3.2: Log transformed PCA grouped by management practice. Variables- PO ₄ , NH ₄ , NO ₃ , pH, mycorrhizal colonization (colo), and canopy openness (co). 53.3% of variation explained in two principal components	80
Figure 4.1: Number of flowering gentians encountered in sampling quadrats across all sites for the eight week flowering period (September-October 2012). Weekly flowering total is further divided by number of flowers encountered at each functional sex stage	100
Figure 4.2: The percentage of flowering plants at each functional stage (including damaged flowers) encountered in the sampling quadrats of all sites for each week (September-October 2012).....	101
Figure 4.3: The percentage of gentian flowers exhibiting damage from either <i>Endothenia hebesana</i> or <i>Chlorochlamys chloroleucaria</i> as a function of total floral density (<i>Gentiana autumnalis</i> floral density + heterospecific floral density) for each site (r ² = 0.87)	102

Figure 5.1: Average nectar volume (µl) for flowers in the functional staminate, transitional, and pistillate stages. (minimum, 25 th percentile, mean, 75 th percentile, maximum).....	135
Figure 5.2: The percentage of insect visits made to gentian flowers by insect group (<i>Bombus</i> , Coleoptera, Diptera other than Syrphidae, Hymenoptera other than <i>Bombus</i> , Lepidoptera, and Syrphidae) across all sites from 2011-2013.....	136
Figure 5.3: Relative abundance of insects encountered visiting gentian flowers and on macroplot Pollard walks across all sites from 2011-2013 (<i>Bombus</i> , Coleoptera, Diptera other than Syrphidae, Hymenoptera other than <i>Bombus</i> , Lepidoptera, and Syrphidae; S.E. Bars).....	137
Figure 5.4: Gentian fall census density prior to management (2011) and in two successive years (2012-2013; S.E. bars).....	138
Figure 5.5: Gentian fall census percentage of flowering plants in study populations prior to management (2011) and in two successive years (2012-2013; S.E. bars).....	139
Figure 5.6: Relative importance of gentian visit constancy, gentian floral density, and total site visitation rate of <i>Bombus</i> , Syrphidae, and all other insects on average gentian fruit seed set across all sites	140

Abstract

The Influence of Land Management on the Demography of the Pine Barren gentian (*Gentiana autumnalis*) in New Jersey

Ryan R. Rebozo

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Gentiana autumnalis L. (Pine Barren gentian) is a rare early-successional, fall flowering perennial that occurs in the New Jersey Pine Barrens. My study (2011-2013) focused on the influence of management practices on *G. autumnalis* demography, site conditions, and pollination. I used a repeated measures design to census select *G. autumnalis* populations that were mowed, prescribed burned, or left unmanaged for a minimum of six years. Sites that were actively managed either by mowing or prescribed burning typically had greater gentian densities, higher percentage of flowering individuals, greater fruit seed set, and mycorrhizal root colonization in *Gentiana autumnalis* than unmanaged sites. Actively managed sites also had fewer woody plant species, and greater canopy openness. Insect visitation rate to the flowers of *G. autumnalis* was negatively correlated with heteropecific inflorescence density. Visitors from the family Syrphidae accounted for the greatest percentage of visits to gentian flowers, yet total *Bombus* visitation explained the greatest variation in fruit seed set of all insect visitors. Total site visitation was greatest in actively managed sites and was positively correlated with fruit seed set. As the number of flowering plants increased in managed sites, average fruit seed set increased in spite of lower individual flower

visitation rates, suggesting an allee effect on female fitness. *Gentiana autumnalis* populations responded positively within two to six months of introduced management in terms of gentian density, percentage of flowering individuals, and average seed set per fruit. Plants added to the population after management were adult, often flowering individuals which suggests this species can remain dormant below ground for at least one year. The percentage of flowering plants at recently managed sites reverted back to pre-management levels 14-20 months after disturbance, suggesting that populations of *G. autumnalis* may benefit from repeated management. Threats to *G. autumnalis* include fire suppression, disturbance (e.g., mowing) during the flowering season, deer herbivory, and seed and fruit damage from caterpillar infestation. It is recommended that management practices aimed at the conservation of *G. autumnalis* focus on maintaining open-site conditions that promote the increased flowering and seedling recruitment that are essential for the long term viability of populations. Site management should be scheduled prior to the beginning of the growth period in April, or after seed set in late November in order to avoid negative impacts to *G. autumnalis* growth and reproduction. Maintenance of early-successional habitat will also benefit associate plant species, facilitate positive interactions with mycorrhizal fungi, and attract insect pollinators. My study is the first to examine the effects of disturbance on the life history and demography of *G. autumnalis* in New Jersey, and will be essential for how to best manage this enigmatic species that is symbolic of the New Jersey Pine Barrens.

Chapter 1: Introduction

As many habitats across the eastern United States transition into late successional forests, early successional plant and animal species face greater local extinction pressures (Motzkin and Foster 2002). Since European settlement, it is estimated that over 90% of grassland habitat in much of the northeastern United States has been lost (Noss et al. 1995). This loss of early successional habitat is the result of decreasing disturbance frequency (Lorimer and White 2003). Different forms of forest disturbance can influence early-successional species differently (Reier et al. 2005). In fire dependent ecosystems (e.g., New Jersey Pine Barrens), the absence of fire disturbance may result in the loss of many early-successional plant species (Leach and Givnish 1996). Natural disturbance by wildfire can maintain early successional sites by reducing the encroachment and establishment of late successional species (Motzkin and Foster 2002, Bond et al. 2005). Whereas, less intense “cool season” prescribed burns may not consume enough overstory cover necessary to promote early successional habitat (Brose and Lear 1998).

Fire-prone ecosystems make up an estimated 40% of earth’s land surface (Chapin et al. 2002). The New Jersey Pine Barrens (approximately 550,000 ha) is a fire dependent ecosystem where frequent fires create a mosaic landscape of habitats in varying stages of succession (Forman 1998, McCormick and Forman 1998). The New Jersey Pine Barrens is characterized by its sandy, acidic soils, and the dominance of pitch pine forests (Boyd 1991). The term Pine Barrens developed as a result of the landscape dominated by pitch pines (*Pinus rigida*) and the word “barren”, indicating impoverished soil that was poor for tillage crops (Wacker 1998). The New Jersey Pine Barrens have a

long historical record of both natural wildfire and the use of prescribed burns.

Throughout recorded history, extensive wildfires have been common in the Pine Barrens (Little 1998). Anthropogenic fires are thought to have originated with Native Americans and their attempts to thin the forest to facilitate hunting (Little 1998). During European settlement, anthropogenic fire was commonly used to control forest encroachment around agriculture and settlements, and often resulted in many areas being burned annually (Little 1946).

Prescribed burns are effective at reducing woody plant cover (Clark and Wilson 2001), yet their intensity and fire return intervals may deviate from historical cycles, and this may negatively impact early-successional species (Wilcove et al. 1998, Quintana-Ascencio et al. 2003). Active fire suppression in New Jersey began in the early 20th century, with prescribed burning as a method of fuel load reduction starting in the late 1920's (Little et al. 1948, Buell and Cantlon 1953). Where prescribed burning is not feasible, mowing is used as a form of mechanical disturbance aimed at reducing vegetation encroachment (Van Clef 2009). The use of mowing to maintain open canopy has been shown to support a higher species richness of plants than similar sites left to succession (Bakker 1989, Lepš 1999, Jutila and Grace 2002). With an estimated 11,000 acres of managed roadsides in the New Jersey Pine Barrens, mowing has the potential to maintain suitable habitat for many early-successional plant species (Collins et al. 1998; Van Clef 2009).

Study Species

The Pine Barren gentian (*Gentiana autumnalis*) is a fall flowering, herbaceous perennial in the family Gentianaceae (tribe Gentianeae) that is currently threatened by fire suppression throughout its range (Kartesz 2014). Of the 99 genera in the family Gentianaceae, *Gentiana* is the most diverse with 360 species (Struwe 2014). *Gentiana* is believed to have originated in nutrient poor alpine soil in the Himalayas and in Southern China (L. Struwe personal communication). Of the fifteen sections classified under *Gentiana*, *G. autumnalis* belongs to *Pneumonanthe* (Halda 1996). Of all the sections in *Gentiana*, *Pneumonanthe* is the only one to extensively diversify in the New World, with most of its diversity occurring in the eastern portion of the United States (Yuan et al. 1996).

Gentiana autumnalis is typically single-stemmed, 15-55cm in height, and phyllotaxically arranged with opposite linear leaves. Flowers are typically solitary, campanulate, glabrous, and blue or occasionally white or violet in color (Poster et al. 2015). In New Jersey, *G. autumnalis* flowers in the fall (September – October), and produces winged seeds (Poster et al. 2015). Morphologically, *G. autumnalis* is different from other closely related species in that it lacks bracts that subtend the flower and has larger corolla lobes in proportion to the inflorescence size (J. Pringle personal communication).

Populations of *Gentiana autumnalis* in New Jersey are disjunct from southern populations, which are located in Virginia, North Carolina, and South Carolina (Kartesz 2014). It is considered at a moderate risk of extinction (G3) throughout its range and

ranked extirpated (SX) in Delaware, imperiled (S2) in South Carolina, and critically imperiled (S1) in Virginia (NatureServe Explorer 2013). *Gentiana autumnalis* is considered rare (S3) in New Jersey where there are less than 50 known occurrences (State of New Jersey 1998). See Appendix 1 for definitions of symbols for species status. Paradoxically, it is not listed as endangered by the New Jersey Natural Heritage Program (New Jersey Natural Heritage Program 2010). However, because populations only occur in the Pine Barrens in the southern portion of the state, *G. autumnalis* is afforded some protection under the New Jersey Pinelands Comprehensive Management Plan (New Jersey Pinelands Commission 1980 and as amended in 2007). In New Jersey, *G. autumnalis* favors open habitats and moist sandy soil (Stone 1911; Harshberger 1916; Jenkins and Blades 1990). *Gentiana autumnalis* is the most globally threatened species of Gentianaceae extant in New Jersey (Poster et al. 2015). Other potential threats to this species include changes to hydrology, mowing during the growing season, and herbicide applications (Bien et al. 2009).

Study Sites

Study sites for this project were selected from known populations of *Gentiana autumnalis* in the New Jersey Pine Barrens (Figure 1). Populations were situated along roadsides and open meadows that bordered pitch pine upland and lowland habitats that were in varying degrees of forest succession (open, partial, and closed canopy; McCormick 1970). Sites were considered unmanaged (= undisturbed) if no management aimed at reducing vegetation, fuel load, or canopy cover was conducted within six years of the start of the study (2011). Sites were considered managed (= disturbed) if they were

mowed or prescribed burned less than six years of the start of the study. As a result of differences in population size, density, and areal size, not all populations were included in the demography, site condition, herbivory, or pollination analysis.

Franklin Parker Preserve (FPP) - UTM- 539050 E 4404259 N

The Franklin Parker Preserve is a 9,770 acre preserve that is comprised of post-agricultural bogs, Atlantic White Cedar swamps, and both upland and lowland pitch pine forests. The Franklin Parker Preserve population (118 m²) was located alongside a sand road that was regularly managed by roadside mowing and manual removal of saplings. *Gentiana autumnalis* at this site grew among ericaceous shrubs and under a partial canopy of a stand of mature pitch pine (*Pinus rigida*). This site was used in the demography, site condition, pollination, and herbivory studies.

Stockton College (Stockton) - UTM- 539028 E 4370377 N

The Stockton College population (250 m²) was located in a late-successional wooded lot approximately 160 m from an asphalt road. *Gentiana autumnalis* grew among an understory of ericaceous shrubs and under a closed canopy of *Pinus rigida*. The Stockton site was added to this study in 2012 and had not been managed in over six years. The Stockton population was used in the demography, site condition, pollination, and herbivory studies.

Atsion – UTM- 523389 E 4398526 N

The Atsion population (560 m²) was located within the Wharton State Forest and approximately 15 m from an asphalt road. Historically, this location supported a robust

population of *G. autumnalis*, particularly after a fire disturbance (T. Gordon, personal communication). As of the start of this study this site had not been managed in over six years. *Gentiana autumnalis* at this site grew alongside ericaceous shrubs and mature *Pinus rigida* canopy. The Atsion population was used in the demography, site conditions, and herbivory studies.

County Road 561 (561) – UTM- 528874 E 4378722 N

The 561 population (87 m²) was located within a residential area and alongside an asphalt road that was bordered on one side by a stand of mature *Pinus rigida*. This was an open site managed with roadside mowing from 2011 – 2013. This site had received mowing during the flowering season as late as 2011. This site was included in the demography, site conditions, and herbivory studies.

Warren Grove Gunnery Range

Six *G. autumnalis* populations were located at the Warren Grove Gunnery Range operated by the 177th Fighter Wing of the New Jersey Air National Guard. These populations varied in site conditions and management regime:

Lower Cabin (LC) – UTM- 548058 E 4397306 N

The Lower Cabin population (24 m²) was located in the buffer zone (non-active military training zone) and alongside a sand road. *Gentiana autumnalis* grew among a partial canopy of mature *Pinus rigida* in the periphery. The Lower Cabin site was annually mowed from 2011 through 2013 and disturbed by flooding events in 2012 and

2013 that eroded sand onto several sampling quadrats. This site was used in the demography, pollination, and herbivory studies.

Calico – UTM- 547618 E 4396293 N

The Calico population (251 m²) was located in the buffer zone alongside a sand road. *Gentiana autumnalis* at this site grew along the roadside under partial canopy due to mature *Pinus rigida* in the periphery. This site was added to the study in 2012 and underwent roadside mowing in 2012 and 2013. This site was included in the herbivory study.

Runway (RunM/RunB) – UTM- 550691 E 4395099 N

The Runway population (6300 m²) was located in an open field within the military training zone (impact zone) that was last managed in 2005. The study site was surrounded by a network of four sandy roads. To compare differences in management practices in March 2012 the 6,300 m² site was subdivided into 2100m² plots (210 m x 10 m) for treatments: a) disk, b) mowed (RunM), and c) prescribed burned (RunB). The Runway population, thought to be the largest *G. autumnalis* population in New Jersey, was used in the demography, site condition, pollination, and herbivory studies.

Sightline (Sight) – UTM- 551196 E 4394139 N

The Sightline population (46 m²) was located within the military training zone, approximately 14 m from a sand road. Now overgrown by shrubs and small statured *P. rigida*, the site was formerly actively managed as a treeless “sightline” that was used to guide incoming aircraft to ground targets. Following a prescribed burn in March 2012,

the areal size of the Sightline population increased to 1596m² in 2013. The Sightline population was used in the demography, site condition, pollination, and herbivory studies.

Sightline 2 (Sight2) – UTM- 551297 E 4394257 N

The Sightline 2 population (50 m²) was located within the military training zone. This site was added to the study in 2012, after a prescribed burn produced *Gentiana autumnalis* aboveground biomass. Prior to 2012, this site had not been managed for over six years and was a rare plant monitoring site where *Gentiana autumnalis* was not encountered. The *G. autumnalis* in this site grew among ericaceous shrubs and small statured *Pinus rigida*. This site was included in the demography, and site conditions studies.

North Pole Road (NPR) – UTM- 551285 E 4397187 N

The North Pole Road population (2.2 m²) was located alongside a sand road in the gunnery range buffer zone. This site was managed with roadside mowing from 2011 – 2013. This is an open site free from ericaceous shrubs and approximately 10 m from mature *Pinus rigida*. The North Pole Road population was included in the demography study.

Study Overview and Objectives

Demography data are lacking for many rare plant species in New Jersey. These data are essential for determining population trends over time. Assessing the population status of rare species is imperative for implementing long-term monitoring programs.

Prior to this study, no data existed on the effects of management practices on populations of *G. autumnalis* in New Jersey. Results from this study will be useful to resource managers when making conservation decisions for the management of *G. autumnalis* and populations of other rare, early-successional plant species.

I monitored and censused 10 *G. autumnalis* populations over three years (2011 – 2013). These data allowed me to analyse how different management practices influence the demography of *G. autumnalis* populations over time. In addition, my study examined the role that edaphic conditions and mutualistic relationships play in population dynamics. The growth and survivorship of plant populations are directly influenced by the properties of the soil in which they grow (Lukac and Godbold 2011). Soils function not only as a medium for growth, but are also a source of nutrients, water, and mutualistic interactions (Lukac and Godbold 2011). Of the estimated 90% of higher plant species that are associated with mycorrhizal fungi, two thirds are believed to form associations with arbuscular mycorrhizal fungi (AMF; Fitter and Moyersoen 1996). The symbiosis between AMF and their host plants is considered the most common underground symbiosis (Smith and Read 2008). In exchange for carbon from plant roots, AMF facilitate the delivery of inorganic nutrients into the plant's root system (Fitter 1985). In the presence of disturbance, some mycorrhizal fungi become influential in not only nutrient acquisition, but also the establishment of early successional plant species. For this reason, understanding the availability of nutrients and mutualistic associates in the soil is vital to understanding aboveground plant population dynamics (Bever et al. 2010).

Aboveground mutualism with insect pollinators is another important focal point when attempting to understand the demography of rare plant populations. The diversity, and phenology of the flowering plant community may influence pollinator diversity, floral constancy, and visitation rate (Bruckman and Campbell 2014). Small plant populations that are limited in abundance, density or even diversity of flowers may be limited in their ability to attract sufficient insect visits to be successfully pollinated due to limited rewards (Fischer and Matthies 1997, Fischer and Matthies 1998, Oostermeijer et al. 2000, Dauber et al. 2010).

The objectives of this study were to 1) characterize *G. autumnalis* sites both biotically and abiotically, 2) develop baseline population data for study sites in New Jersey, 3) monitor changes in the demography of populations over time and under varying management regimes, 4) identify the presence of arbuscular mycorrhizal fungi on the roots of *G. autumnalis*, 5) study the breeding and pollination system of *G. autumnalis*, 6) identify threats to the viability of populations in New Jersey, and 7) contribute to the development of management recommendations focused on using anthropogenic disturbance to develop suitable habitat for early-successional plant species.

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Chapter 2: The effects of prescribed burns and mowing on the demography of *Gentiana autumnalis* in the New Jersey Pine Barrens

Abstract

Gentiana autumnalis L. (Pine Barren gentian) is a rare, fall-flowering perennial that is endemic to pine barren habitat from New Jersey to South Carolina. This fire adapted, early successional species is at risk in New Jersey as a result of changes to hydrology, mowing during its growing season, herbicide applications, and fire suppression. On average, populations of *Gentiana autumnalis* have a limited proportion of reproductive individuals ($\bar{x} = 26.7\%$, $\pm 20.45\%$ of individuals in sampling quadrats) and seedlings ($\bar{x} = 2.4\%$, $\pm 2.6\%$ of individuals in sampling quadrats). The percentage of flowering individuals and site density are significantly larger at managed than unmanaged sites ($F_{(2,425)} = 12.59$ $p < 0.001$; $T_{(1,426)} = -4.28$ $p < 0.001$). Introducing disturbance in the form of prescribed burns and mowing prior to spring growth in previously undisturbed sites resulted in increased gentian densities, increased flowering and increased seed set ($F_{(1,52)} = 7.4$, $p < 0.001$) in the same year. Actively managed sites averaged significantly greater seed set and had greater population growth rates than sites that are left unmanaged. While disturbance is an important factor in creating habitat for early successional species, avoiding management during the flowering period will limit potential negative effects. Using prescription burns and roadside mowing to create and maintain early-successional habitat is paramount when planning for the conservation management of disturbance-dependant species such as the New Jersey Pine Barrens.

Introduction

Environmental disturbance can have either a positive or negative effect on plant community structure (Johnson and Miyanishi 2007). Certain forms of anthropogenic disturbance have been linked to lowered plant population growth rates and negative impacts on plant-animal interactions (Bruna and Oli 2005, Liu et al. 2013, Leal et al. 2014). On the other hand, many early-successional plant species require short disturbance intervals and become rare when these natural cycles are altered (Hodgson 1991, Pavlovic 1995). Wild fire is a form of disturbance that is capable of reducing plant biomass and altering successional stages (Bond et al. 2005), with potentially positive and negative impacts on plant species survival and fitness.

Fire-prone ecosystems make up an estimated 40% of earth's land surface (Chapin et al. 2002). The New Jersey Pine Barrens (approximately 550,000 ha) in the mid-Atlantic region of the eastern United States is a fire dependent ecosystem where frequent fires create a mosaic landscape of habitats in varying stages of succession (McCormick and Forman 1998, Forman 1998). Periodic burns reduce shrub cover, maintain an open canopy, and delay the encroachment of late successional species (Foreman 1998). The New Jersey Forest Fire Service has for the last 60+ years employed a wildfire suppression program and has conducted cold weather prescribed burns to control fuel loads (Buell and Cantlon 1953, Forman 1998). Alterations of natural fire cycles can be a major threat to the persistence of disturbance dependent species (Wilcove et al. 1998). Although prescribed burns are effective at reducing woody plant cover (Clark and Wilson 2001), their intensity and fire return intervals may deviate from historical cycles, which

may negatively impact early-successional species (Quintana-Ascencio et al. 2003). When intense, natural wild fires are suppressed, fuel loads increase and roadways experience greater vegetation encroachment (Pastro 2011). The New Jersey Department of Transportation conducts annual roadside mowing to control vegetation encroachment (Van Clef 2009). It is estimated that approximately 11,000 acres of early successional habitat occurs along roadsides in the New Jersey Pine Barrens (Van Clef 2009). Mowing that maintains open-sunny areas supports higher species richness of plants when compared to late successional sites (Bakker 1989, Lepš 1999, Jutila and Grace 2002). Thus, mowed roadsides represent a potential refuge for early successional plants and have the potential to be as important as fire in maintaining species diversity (Collins et al. 1998). The focus of this study was to examine the effects of prescribed burns and mowing on the demography of *Gentiana autumnalis* (Pine Barren gentian).

Gentiana autumnalis is a disturbance-dependent gentian that occurs in pine barren habitats from South Carolina to New Jersey (Halda 1996; Poster et al. 2015). It is considered at a moderate risk of extinction (G3) throughout its range and ranked extirpated (SX) in Delaware, imperiled (S2) in South Carolina, and critically imperiled (S1) in Virginia (NatureServe Explorer 2013). *Gentiana autumnalis* is considered rare (S3) in New Jersey where there are less than 50 known occurrences (State of New Jersey 1998). Paradoxically, it is not listed as endangered by the New Jersey Natural Heritage Program (New Jersey Natural Heritage Program 2010), however, because populations only occur in the Pine Barrens in the southern portion of the state, *G. autumnalis* is afforded some protection under the New Jersey Pinelands Comprehensive Management Plan (New Jersey Pinelands Commission 1980 and as amended in 2007). Potential

threats to this species include changes to hydrology, mowing during the growing season, herbicide applications, and fire suppression (Bien et al. 2009).

This is the first study to examine the influence of management practices on the life history and demography of *Gentiana autumnalis*. Identifying best management practices and protocols that mitigate negative effects of succession and promote survivorship, recruitment and fecundity will be essential for the conservation of *G. autumnalis* in New Jersey and throughout its range along the Atlantic coastal plain.

Materials and Methods

Gentiana autumnalis

Gentiana autumnalis is a showy herbaceous perennial in the Gentianaceae family (tribe Gentianeae) that flowers in the fall (Poster et al. 2015). This plant species is one of the last to flower each fall in the New Jersey Pine Barrens and because of this may act as an important food source for late season and over-wintering insect pollinators. In New Jersey, this disturbance-dependent species favors open habitats and moist sandy soil (Jenkins and Blades 1990; Harshberger 1916; Stone 1911). *Gentiana autumnalis* plants exist in four life stages: seeds, seedlings (first year plants limited to a basal rosette of leaves), vegetative plants (second year or older plants that do not produce flowers in the study year) and flowering individuals (second year or older plants that flower in the study year). The roots of *Gentiana autumnalis* are thick and fibrous, typically bifurcated with few fine roots (Rebozo personal observation). The dichotomously branching, fleshy rootstock is believed to be a fire-adapted trait. Solitary stems on mature plants can grow up to 15 to 55 cm in height and typically produce solitary flowers, but occasionally 2-3

flowers per stem (Poster et al. 2015). Multiple stemmed individuals are not uncommon in the species, with up to 15 stems emerging from a single root stock. Flowers are characteristically campanulate, protandrous, penta-merous, and with plicae in between the corolla lobes. The corollas are variable in color and pattern and are blue to purple (occasionally white or rose) with spots or streaks inside. *Gentiana autumnalis* is a well known and easily recognizable species, yet little is known about its life history.

Nomenclature follows Poster et al. (2015).

Study Sites

I identified nine study sites at different locations in the New Jersey Pine Barrens. Study sites were selected based on different disturbance type (mowed, burned), frequency of disturbance, and date of last disturbance (Table 2.1). Sites listed as unmanaged at the start of this study (2011) had been unmanaged for a minimum of six years (Table 2.1). Study sites were situated along roadsides and open meadows that bordered pitch pine upland and lowland habitat and included late successional sites with closed canopies (McCormick 1970). Except for two reference sites (Stockton and Atsion), the remaining seven sites were actively managed at least once over the study (2011-2013). Four sites (FPP, 561, NPR, LC) were mowed each study year. Runway had a combination of both mowing and burning in the 2012 study year. In March 2012, three previously unmanaged sites received their first disturbance (Sites RunM, RunB and Sightline; Table 2.1). Introduced burning gave rise to the 10th site of the study (Sightline 2). This site was added to the study in 2012, after a prescribed burn produced *Gentiana autumnalis* above

ground biomass. Prior to 2012, this site had not been managed for over six years and was a rare plant monitoring site where *Gentiana autumnalis* was not encountered.

Population Census

I used a repeated measures design to census each population over three successive years (2011-2013). I established a rectangular macroplot that encompassed all *G. autumnalis* plants at each study site (Elzinga et al. 1998). I censused plants within 20 randomly nested quadrats (1m²) in macroplots greater than 20m² (n=9). In macroplots less than 20m² (n=1), I censused all plants. Each censused plant was identified with an individually numbered aluminum tag (25 x 85mm) inserted into the ground near the base of the stem fastened with a 75mm stainless steel nail. Populations were censused during spring growth (May) and the fall during the peak flowering period (September). For each censused plant we recorded whether it was in a seedling, vegetative (non-seedling), or reproductive life stage. Before dehiscence (October/November), I randomly collected up to 19 seed capsules from each population. Number of seed capsules included in the seed set count were limited by the number of flowering plants available. I counted all seeds in individual fruit to estimate average seed set per flower. Collected seeds were broadcast at their collected location following the seed count.

Because of the afforded protections to this species, our study was a non-manipulative survey that was limited to measuring population data under existing field conditions (sensu Kerr et al. 2007). However, I gained permission to conduct a manipulative experiment at one study site (Runway). Essentially this was an opportunistic “natural experiment” (Baum and Worm 2009; Schank and Koehnle 2009)

that examined the pre- and post-effects of burning, mowing, and disking. I divided a portion of the Runway site into three 10m x 210m rectangular strips, one for each treatment (burning, disking, and mowing). Disking was used to separate the burned from the mowed treatments. Treatments were conducted in March of 2012 and analyzed in the same year (Table 2.2). Due to the restrictive limitations working with this species, we were unable to replicate or randomize treatments. The literature cites several examples of “opportunistic natural experiments” without replication (Kerr et al. 2007; Baum and Worm 2009; Schank and Koehnle 2009).

Associated Plant Ground Cover and Tree Canopy Cover Estimates

We inventoried all associated plant species within each macroplot at each site. We randomly selected ten quadrats within each macroplot that were not used in the census of gentians to estimate ground cover using the Braun-Blanquet method; 0: no individuals, T: <1% trace amount, 1: <5% cover, 2: 5-25% cover, 3: 26-50% cover, 4: 51-75% cover, 5: 76-100% cover (Wikum and Shanholtzer 1978). We measured canopy cover percentage using true-color fisheye photographs that were processed with Gap Light Analyzer (GLA) version 2.0 imaging software. Five canopy photographs were taken at each site using a Nikon Coolpix 995 camera and Nikon fisheye lens (FC-E8 0.21x). Canopy photographs were taken approximately at the same time of day and under similar cloud cover to limit variation in the calculation of canopy openness due to cloud cover.

Statistical Analysis

I estimated abundance of each population by multiplying the mean density per m² by the areal size of each study site macroplot (Elzinga et al. 1998). Abundance for sites NPR and Atsion were based on the total number of gentians at the site and were not the result of an extrapolated density. I calculated the annual and seasonal survivorship of tagged plants. Annual mortality was defined as an individual plant that was present in the previous year's census, but produced no above ground growth the following year. Seasonal mortality was defined as an individual plant that produced above ground growth during the spring census but was not visible or died-back by the fall census. Fecundity was reported as the average seed set per flower in each study site. I used analysis of variance ($\alpha=0.05$) to compare differences in abundance, survivability, recruitment, and fecundity among and within sites and among treatments. A Tukey HSD was used when there were significant differences among variables. An independent T-test ($\alpha=0.05$) was used to compare pooled data for disturbed and undisturbed sites, and a paired t-test to compare pre- and post-disturbance responses. I used the statistical program R for all analyses (R Core Team 2014).

I used a Lefkovitch matrix to predict the probability of an individual plant progressing from one life stage to another the following year (Elzinga et al. 1998). Sensitivity analysis was used to identify which lifestage transition accounted for the greatest influence on the calculated population growth rate (Caswell 2001). These analyses were done with matrices at stable stage distribution using the R package Rramas (de la Cruz 2014). The annual population growth rate was calculated for each site and for

each management practice. A principal component analysis (PCA) was used to identify the percentage of variation explained by demographic variables with an emphasis on variation as a result of land management practice.

Results

Site Variation

Study sites varied in their population structure (Table 2.2). Population areal size ranged from 2.2m² to approximately 6,300m² ($\bar{x} = 762.3\text{m}^2, \pm 1722$), estimated site abundance ranged from 10 plants to 25,000 plants ($\bar{x} = 2402 \pm 6572$), and site density in m² ranged from 0.02 plants per m² to 27.7 plants per m² ($\bar{x} = 6.5$ plants per m², ± 7.14). There was a negative relationship between the density of plants (m²) and the number of quadrats ($r^2 = 0.70$) with the class of 1-5 plants having the greatest frequency of occupied quadrats (Figure 2.1). Density of *Gentiana autumnalis* was greater in managed sites than in unmanaged sites ($T_{(1,426)} = 4.28$ $p < 0.001$) and density was greater in mowed sites than in those managed with prescribed burn ($T_{(1,425)} = 3.94$ $p < 0.05$). There was no significant ($p > 0.05$) influence of population area size on gentian density or abundance.

Vascular plant species richness in sites ranged from 8 to 91 species within a macroplot. Site canopy openness ranged from 25.1% to 80.3%. Managed sites differed significantly from unmanaged sites in having greater canopy openness ($F_{(2,38)} = 29.59$ $p < 0.001$; Figure 2.2). There was a positive relationship between canopy openness and vascular plant species richness in sites ($r^2 = 0.39$; $p < 0.05$) and a negative relationship between species richness of woody plants and flowering percentage of *G. autumnalis* (r^2

= 0.32; $p < 0.05$). Disturbed and undisturbed sites showed no significant difference in ground cover as estimated by the Braun-Blanquet method ($p > 0.05$; $\bar{x} = 3.6$ for all sites) or vascular plant species richness ($p > 0.05$). Five of the ten study sites had an established ericaceous shrub community that dominated the understory. Those sites without ericaceous shrubs exhibited a greater percentage of flowering gentian individuals than sites with ericaceous woody shrubs ($T_{(1,426)} = 13.16$ $p < 0.001$).

***Gentiana autumnalis* life stage classes**

Vegetative plants typically dominated gentian patches with only one site (561) where flowering plants made up a greater proportion of the population than vegetative individuals over the three study years. Disturbed sites had a greater percentage of flowering individuals compared to undisturbed sites ($F_{(2,425)} = 12.59$ $p < 0.001$; Figure 2.2). The percentage of flowering individuals within populations ranged from 0% to 96.9% of the individuals ($\bar{x} = 26.7\% \pm 20.45\%$ over all sites). Sixty percent of flowering individuals flowered in successive years while 40% reverted back to a vegetative state for at least one year before flowering again. Seedling establishment occurred in six of the ten sites surveyed. At those sites, the percentage of surveyed plants that were seedlings ranged from 1.8% to 8.6% ($\bar{x} = 2.4\% \pm 2.6\%$ over all sites, 1-10 individuals in the sampling quadrats; Table 2.2; Figure 2.2).

Introduced Disturbance

In March 2012, three previously unmanaged sites were either mowed or burned (Sites RunM, RunB and Sightline; Table 2.1). In each of those three sites (RunM = mow and RunB, Sightline = burn), gentian density and flowering percentage increased

significantly (RunM Density $F_{(1,38)} = 6.55$ $p < 0.05$, RunM Flowering $F_{(1,38)} = 9.1$ $p < 0.01$. RunB Density $F_{(1,38)} = 5.94$ $p < 0.05$, RunB Flowering $F_{(1,38)} = 56.49$ $p < 0.001$, Sightline Density $F_{(1,38)} = 4.14$ $p < .05$, Sightline Flowering $F_{(1,38)} = 65.91$ $p < 0.001$) in 2012 when compared to 2011 (Figures 2.3 and 2.4). Introducing disturbance to previously unmanaged sites produced increased densities and a higher flowering percentage within two to six months after disturbance. Patch size increased from 45.9m² to 1596m² following disturbance for burned site Sightline (Sites RunM and RunB were confined by sand roads). The prescribed burning in 2012 promoted above ground gentian biomass at a rare plant monitoring site where *Gentiana autumnalis* was not previously encountered. This previously dormant patch was included into the study in 2012 as site Sightline 2, and remained in the study through 2013. Of the sites subjected to new management, all showed significantly increased gentian density (+27% - +136%) following management (RunM Density $F_{(1,38)} = 6.55$ $p < 0.05$, RunB Density $F_{(1,38)} = 5.94$ $p < 0.05$, Sightline Density $F_{(1,38)} = 4.14$ $p < 0.05$). All sites except for RunB were able to maintain or increase post-management density an additional year removed from management (Figure 2.3). All four newly managed sites also had significantly increased percentages of flowering individuals in the population (+12.6% - +42.1%; RunM Flowering $F_{(1,38)} = 9.1$ $p < 0.01$, RunB Flowering $F_{(1,38)} = 56.49$ $p < 0.001$, Sightline Flowering $F_{(1,38)} = 65.91$ $p < 0.001$; Figure 2.4). The Sightline site which was also burned exhibited a similar increase in flowering percentage (+35.8%) to the RunB site. In 2013, a year removed from disturbance, no statistically significant difference in site density was recorded at any site where disturbance was introduced compared to 2012 ($p > 0.05$). In 2013 all four sites exhibited a reduction in the percentage of flowering

individuals (RunM $F_{(1,38)} = 11.94$ $p < 0.001$, RunB $F_{(1,38)} = 23.98$ $p < 0.001$, Sightline $F_{(1,38)} = 146.4$ $p < 0.001$, Sightline2 $F_{(1,38)} = 11.94$ $p < 0.001$) failed to maintain 2012 levels of flowering percentage with sites RunwayM and Sightline dropping to flowering levels below those recorded in 2011 (Figure 2.4).

Seed Set

On several occasions, collected seed capsules did not have their contents included in the seed set calculation due to the presence of granivorous caterpillars. Seed set counts for individual fruit ranged from 112 to 1371 seeds and averaged 779 seeds across all sites (302 – 1044 average fruit seed set by site; Table 2.3). Average seed set per fruit was significantly higher in managed sites ($\bar{x} = 850 \pm 286.9$) in all three study years (2011-2013) when compared to unmanaged sites ($\bar{x} = 594 \pm 281.1$; $F_{(1,202)} = 28.6$, $p < 0.001$). There was no significant difference ($p > 0.05$) in average seed set per fruit between mowed and burned sites. Similar to the increased flowering response to introduced disturbance, sites RunB, RunM and Sightline each experienced a significant increase in seed set ($F_{(1,75)} = 7.4$, $p < 0.001$) per fruit following disturbance and a significant reduction in seed set ($F_{(1,75)} = 5.3$ $p < 0.001$) to pre-disturbance levels one year removed from disturbance (Table 2.3). The presence of seedlings at a site was positively related to the number of flowering individuals ($r^2 = 0.88$) and total site seed set ($r^2 = 0.89$) but not average seed set per fruit ($r^2 = 0.14$) for the previous year.

Survivorship

Plant life stage and presence or absence of active management were both found to significantly ($F_{(2,1163)} = 8.87$ $p < 0.001$) influence mortality in *Gentiana autumnalis* using

a two way factorial ANOVA. Mortality was greater in the seedling life stage (29.2%) followed by vegetative (11.0%) and flowering stages (6.6%; $F_{(2,1166)} = 83.56$ $p < 0.001$; Figure 2.5). Mortality was greater for all three life stages in unmanaged sites throughout the duration of the study, significantly so in the vegetative (unmanaged mortality $\bar{x} = 21.85$, managed mortality $\bar{x} = 8.49\%$, $F_{(1,789)} = 334.5$ $p < 0.001$) and flowering stages (unmanaged mortality $\bar{x} = 12.90\%$, managed mortality $\bar{x} = 3.81\%$, $F_{(1,344)} = 72.46$ $p < 0.001$). There was no statistically significant difference in mortality for seedlings (unmanaged mortality $\bar{x} = 31.10\%$, managed mortality $\bar{x} = 27.7\%$, $p > 0.05$; Figure 2.5). Lefkovitch matrices for each site depicted a low transition rate from vegetative stage to flowering stage overall, but a greater percentage of vegetative plants transitioned into flowering individuals in managed sites (managed $\bar{x} = 17.7\%$, unmanaged $\bar{x} = 1.02\%$, $F_{(1,15)} = 15.52$ $p < 0.001$). Two of the three highest rates of vegetative to flowering stage transitions occurred the season following a prescribed burn.

Population Growth Rate and Principal Component Analysis

Population growth rate averaged over all sites was positive ($\bar{x} = 1.24 \pm 0.47$, unmanaged: $\bar{x} = 1.01 \pm 0.3$, mow: $\bar{x} = 1.22 \pm 0.39$, burn: $\bar{x} = 1.81 \pm 0.58$). Population growth rate was greatest in managed sites, particularly in sites that were burned earlier in the same year ($F_{(2,14)} = 4.73$ $p < 0.05$). Sensitivity analyses focused on transitions between *Gentiana autumnalis* life stages found that transitions of vegetative plants to flowering plants had the greatest positive impact on growth rate of all life stage transitions ($\bar{x} = .821 \pm .185$, $F_{(1,28)} = 5.58$ $p < 0.05$). A log transformed principal component analysis using area, density, abundance, flowering %, vegetative %, seedling

%, seasonal mortality, yearly mortality, seed set and population growth rate as factors explained 59.1% of variation in two principal components (Figure 2.6). Principal component one correlated with variables seed set (loading score = 0.51), and density (0.46) while the second principal component correlated strongest with yearly mortality (0.49), and percentage of seedlings in a population (0.47). Correlation circles in the principal component analysis isolated undisturbed sites as being negatively related to percentage of seedling, percentage of flowering individuals, seed set, density and abundance.

Discussion

My data suggests that populations of *Gentiana autumnalis* increased at a faster rate when managed under a disturbance regime than if left alone. The successful conservation of *G. autumnalis* and potentially other rare, early-successional species will depend on understanding how land management and anthropogenic disturbances influence population dynamics. Managing for the establishment and survivorship of seedlings and for the greatest number of flowering individuals will help improve the viability of populations (Menges and Dolan 1998). Seedlings of this species were not only found in low numbers in all study sites, but also experienced the greatest mortality of all life stages of *G. autumnalis*. As these populations stand, few plants are capable of flowering in any given year, and many do not flower in successive years suggesting that factors limiting flowering capacity such as water availability, canopy openness and soil nutrients are frequently encountered in *G. autumnalis* patches. In addition, *G. autumnalis* populations have few seedlings and high mortality once germinated, again suggesting

limiting factors at these study sites. Seedlings were positively related to both the number of flowering individuals and average seed set in the previous year making management for increased flowering even more important for improving the viability of *G. autumnalis* populations. It is also important to note that few vegetative individuals were recorded transitioning into the reproductive stage in the three study years and that 40% of recorded flowering individuals in the study reverted back to a vegetative state for at least one year. Sensitivity analyses revealed that improving on the percentage of vegetative plants that transition into flowering individuals will have the greatest influence on population growth. The low percentage of flowering and low levels of seedling establishment recorded in those sites increase the likelihood that *G. autumnalis* populations will decline or become extirpated and increases the urgency of developing and implementing appropriate management protocols.

My findings reflect those found in the marsh gentian, (*Gentiana pneumonanthe*) under conditions of disturbance. In Britain, the marsh gentian exists mainly in actively managed sites and is also rare due to habitat loss and limited disturbance. Recently burned *Gentiana pneumonanthe* sites have subsequently more prolific flowering when compared to unburned sites (Chapman and Rose 1982). The same study reports that higher soil temperature due to a more open canopy result in earlier and more abundant flowering. My study differed because marsh gentians experienced a gradual reduction over several years in flowering post fire, while *Gentiana autumnalis* exhibited reduced flowering already in the second year post-fire. The oligotrophic soil conditions that are typically associated with *G. autumnalis* habitat may not be rich enough in nutrients to support elevated levels of flowering for more than one year post burning. In a study of

the biennial *Gentianella germanica*, fecundity and population growth in both *in situ* and *ex situ* plots are lower in smaller patches than larger patches (Fischer and Matthies 1998). While the size of the available patch was not related to density in *Gentiana autumnalis*, increased plant densities followed both prescription burn and mowing management regimes. Managed sites overall had greater numbers of individual plants, a higher ratio of reproductive individuals, lower mortality, greater seed set and greater population growth rates when compared to unmanaged sites. Sites managed by prescribed burns or mowings were capable of maintaining greater canopy openness than unmanaged sites and those sites whose disturbance was capable of preventing the establishment of a woody shrub understory exhibited a greater percentage of adult plants flowering when compared to sites with an established ericaceous plant community. While the management of sites for greater canopy openness and reduced shrub competition appeared to improve the demography of *G. autumnalis* patches over unmanaged sites, limited seedling establishment remained an issue in all sites.

Recruitment in plant populations is limited by the availability of suitable microsites and/or seed production (Eriksson and Ehrlén 1992). Recruitment in *Gentiana pannonica* is not limited by seed production, but rather by the availability of early successional habitats (Erktova and Kosnar 2012). *Gentiana pannonica* populations in later successional habitats produce relatively high levels of seed but are still limited in their seedling recruitment (Erktova and Kosnar 2012). Site conditions appeared to be responsible for limited recruitment recorded for *Gentiana autumnalis*. Sites actively managed by prescribed burns and mows had maintained open canopy, but for many

unmanaged sites, competition with woody plants for nutrients and shading by the cover those plants provided appeared to limit seedling establishment for *Gentiana autumnalis*.

Site 561 averaged the greatest seed set per plant as well as the highest percentage of flowering plants in the population, yet no seedlings were recorded in the three survey years. This site was located along a paved roadway and until 2012 was mowed annually prior to fruit maturation. While the annual mowing was effective at maintaining an open canopy, preventing the establishment of woody plants and maximizing flowering and seed set, the timing of the disturbance may be the cause of no seedling establishment. This is one example of how disturbance alone is not enough to ensure a positive response in the early successional plant community. The form and timing of disturbance is critical when managing for population viability. Another mowed site, Lower Cabin, experienced a reduction in population size as a result of multiple washouts after heavy storms. While this site was also managed to maintain open canopy, its proximity to a sand road and the slope of the ground between the road and patch resulted in the washing of sand from the road way onto plants in the site. Successful management must take into account phenology and hydrology in addition to maintaining early successional conditions.

The timing of burns and burning intervals may also influence late-flowering plant response and population viability (Howe 1994, Chapman et al. 1989). A study focused on dormant versus growing seasons burns shows that when plots were burned early in the season, late-flowering plants had an opportunity to dominate ground cover (Howe 1994). When burns were conducted during the growing season, ground cover was more evenly split between early and late-flowering species (Howe 1994). While less ground cover did

not positively influence *G. autumnalis* in my study, it was beneficial in increasing seedling recruitment in another disturbance dependent species, *Salvia pratensis* (meadow sage), after active management (Hegland et al. 2001). Chapman et al. (1989) developed a fire interval model for *G. pneumonanthe* that incorporated associate plant species, seed production, flower production, survivorship and climate. Their model predictions estimated that most *G. pneumonanthe* populations will become extinct within 60 years of a single burn, but burn intervals of 13 years have an 80% likelihood of populations surviving 100 years and a 65% likelihood of populations surviving for 150 years (Chapman et al. 1989). Menges and Dolan (1998) also report that burning produces the greatest viability in population models. In my study, introducing disturbance to previously unmanaged sites resulted in significant increases in density, flowering and seed set within the same year that disturbance was introduced. While the increased density was maintained an additional year removed from disturbance, flowering and seed set dropped to pre-disturbance levels after one year for *G. autumnalis*. Repeated management will be necessary to maintain optimal conditions in these sites. The initial management of sites may benefit from repeated disturbance to limit competition with shrubs. Afterwards, emulating the historical natural wild fire cycle of 8-10 years in the New Jersey Pine Barrens may be effective in maintaining positive gentian population responses to management.

The increase in density did not appear to be the result of an activated seed bank, but rather the activation of dormant root stock. The majority of plants added to the population after introduced management events were adult flowering plants and not seedlings. This suggests that *Gentiana autumnalis* has the ability to remain dormant

without any above ground growth for at least a year until favorable conditions are met. Mortality reported in my study is based upon above ground biomass, and due to the limited duration of the study may not account for dormancy. The potential for dormant plants to persist in sites with suboptimal conditions suggests that the management of small populations may allow a greater number of plants to be positively impacted than would be recorded based on above ground growth. Similar dormancy of up to three years has been reported in *Gentiana pneumonanthe* by Oostermeijer et al. (1996).

The results of my study suggest that management in the form of a mow or prescribed burn is beneficial in improving density, flowering, and fecundity in this species. In particular, sites managed to maintain early succession and prevent the establishment of an ericaceous shrub community had populations with greater flowering percentages, even when compared to other managed sites. Successful management will plan around the phenology of this species to promote an increased number and ratios of seedlings and flowering individuals. With increased development and wildfire suppression, land managers are tasked with maintaining populations of rare early successional plant species. The findings of this study indicate that in order to maximize the viability of *Gentiana autumnalis* populations, they must exist in disturbed patches. What we learned about *Gentiana autumnalis*' response to management may extend beyond this species and may be used as a model for the management of other disturbance dependent plant species that are found in similar habitats. Similar studies focused on demography under varying management practices are needed for other rare plant species as little is known of the potential impacts of our management of forests and roadsides has on plant populations. A further understanding of the pollination system and the edaphic

conditions associated with each site will be needed to better understand the variables influencing changes in demography.

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Tables

Table 2.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)

Site	2011	2012	2013
Franklin Parker Preserve (FPP)	Mow	Mow	Mow
Runway	Unmanaged-Reference	Mow/Burn/Disk	No Management
Lower Cabin (LC)	Mow	Mow	Mow
Atsion	Unmanaged-Reference	Unmanaged-Reference	Unmanaged-Reference
Stockton (Stock)	Not in study	Unmanaged-Reference	Unmanaged-Reference
561	Mow	Mow	Mow
Sightline (Sight)	Unmanaged-Reference	Burn	No Management
Sightline2 (sight2)	Not in Study	Burn	No Management
North Pole Road (NPR)	Mow	Mow	Mow

Table 2.2: Population areal size, spring census density and percentage of seedlings and flowering plants at each site during 2011, 2012, and 2013. (ND=No Data, NS= Not Sampled)

Site	Population Area m ²	Gentian Density m ² ('11/'12/'13)	Flowering Percentage ('11/'12/'13)	Seedling Percentage ('11/'12/'13)
Stockton (Stock)	250	ND / 2.0 / 2.1	ND / 13.0 / 0.0	ND / 0.0 / 0.0
Lower Cabin (LC)	23.6	10.4 / 8.7 / 6.8	35.0 / 30.0 / 36.3	3.0 / 5.0 / 1.8
N. Pole Rd. (NPR)	2.2	9.1 / 7.3 / 9.5	78.6 / 46.2 / 38.1	0.0 / 0.0 / 0.0
Rt. 561 (561)	87.1	6.3 / 9.8 / 6.4	77.8 / 83.1 / 96.9	0.0 / 0.0 / 0.0
Runway ('11)	6,300	5.3 / NS / NS	13.2 / NS / NS	1.9 / NS / NS
Runway Mow (RunM '12-'13)	2,100	NS/2.1/3.6	NS/25.8/9.8	NS/3.2/2.4
Runway Burn (RunB '12-'13)	2,100	NS/5.9/5.0	NS/55.3/22.1	NS/6.5/3.5
Franklin Parker Preserve (FPP)	117.5	27.7 / 19.5 / 21.0	9.0 / 27.8 / 27.0	0.0 / 3.1 / 2.1
Sightline (Sight)	45.8 ('11); 1596 ('12 and '13)	0.39 / 0.96 / 0.96	11.8 / 47.6 / 2.78	0.0 / 4.8 / 0.0
Sightline2 (Sight2)	50	ND / 1.9 / 3.1	ND / 14.3 / 3.8	ND / 8.6 / 5.8
Atsion	559.4	0.02 / 0.03 / 0.02	0.0 / 14.3 / 27.3	0.0 / 0.0 / 0.0

Table 2.3: Average seed set for *Gentiana autumnalis* per fruit by site (2011, 2012 and 2013; ND= No Data due to limited flowers present)

Site	Avg. Seed Set 2011	Avg. Seed Set 2012	Avg. Seed Set 2013
Stockton (Stock)	ND	499.7 $\pm$ 57.6 (n=7)	301.5 $\pm$ 118.5 (n=5)
Lower Cabin (LC)	889.4 $\pm$ 156.2 (n=10)	878.7 $\pm$ 296.5 (n=10)	864.0 $\pm$ 292.9 (n=9)
N. Pole Rd.(NPR)	715.0 $\pm$ 492.1 (n=3)	450.5 $\pm$ 174.6 (n=3)	ND
Rt. 561	783.4 $\pm$ 209.5 (n=10)	953.0 $\pm$ 411.2 (n=10)	908.0 $\pm$ 244.7 (n=19)
Runway	630.75 $\pm$ 280.3 (n=12)	ND	ND
Runway Mow (RunM)	ND	1044.3 $\pm$ 229.9 (n= 14)	617.6 $\pm$ 238.8 (n=12)
Runway Burn (RunB)	ND	978.6 $\pm$ 211.8 (n=14)	692.5 $\pm$ 379.3 (n=11)
Franklin Parker Preserve (FPP)	645.3 $\pm$ 197.8 (n=10)	944.7 $\pm$ 401.4 (n=12)	881.5 $\pm$ 248.2 (n=17)
Sightline (Sight)	ND	710.7 $\pm$ 220.5 (n=9)	354.3 $\pm$ 141.6 (n=4)
Sightline 2 (Sight2)	ND	428.0 $\pm$ 63.6 (n=2)	ND
Atsion	ND	ND	ND

Figures

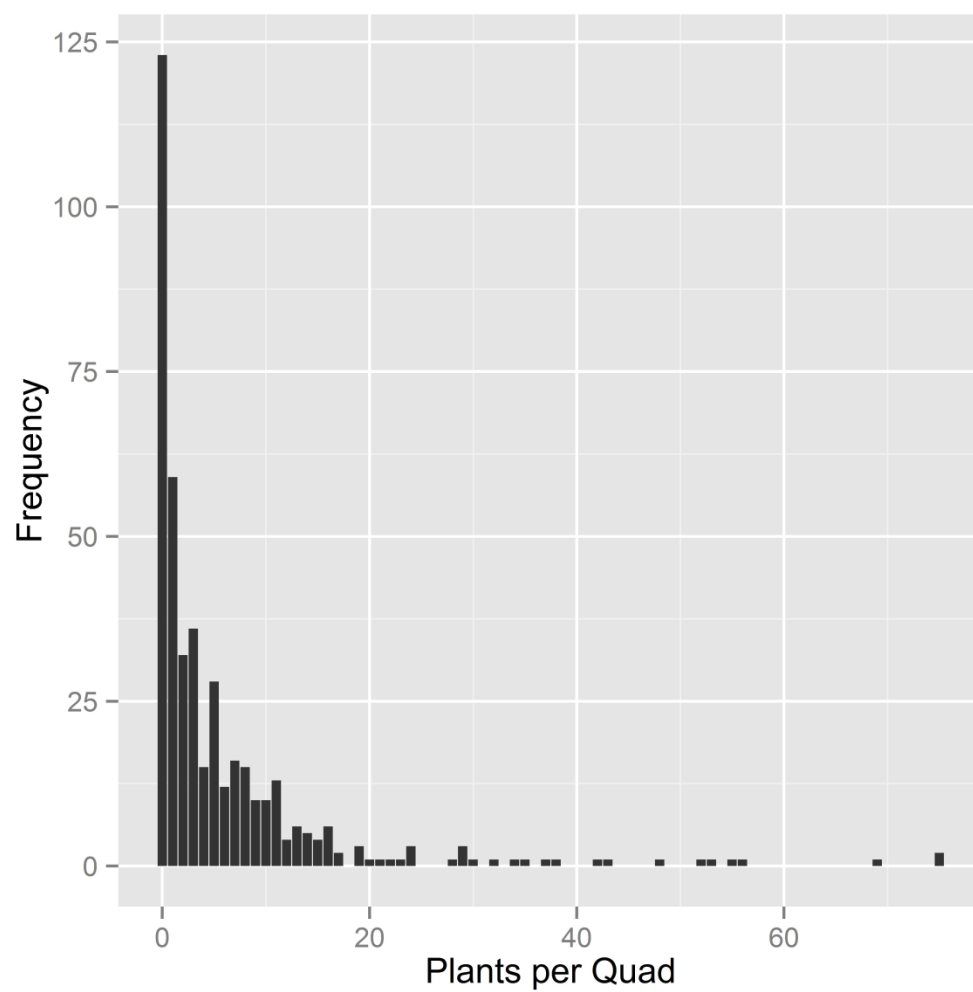


Figure 2.1: Frequency of the number of *Gentiana autumnalis* plants recorded in sample quadrats (1 m²) randomly placed in macroplots of all sites from 2011-2013 (All management regimes included; n=427, max= 75 plants)

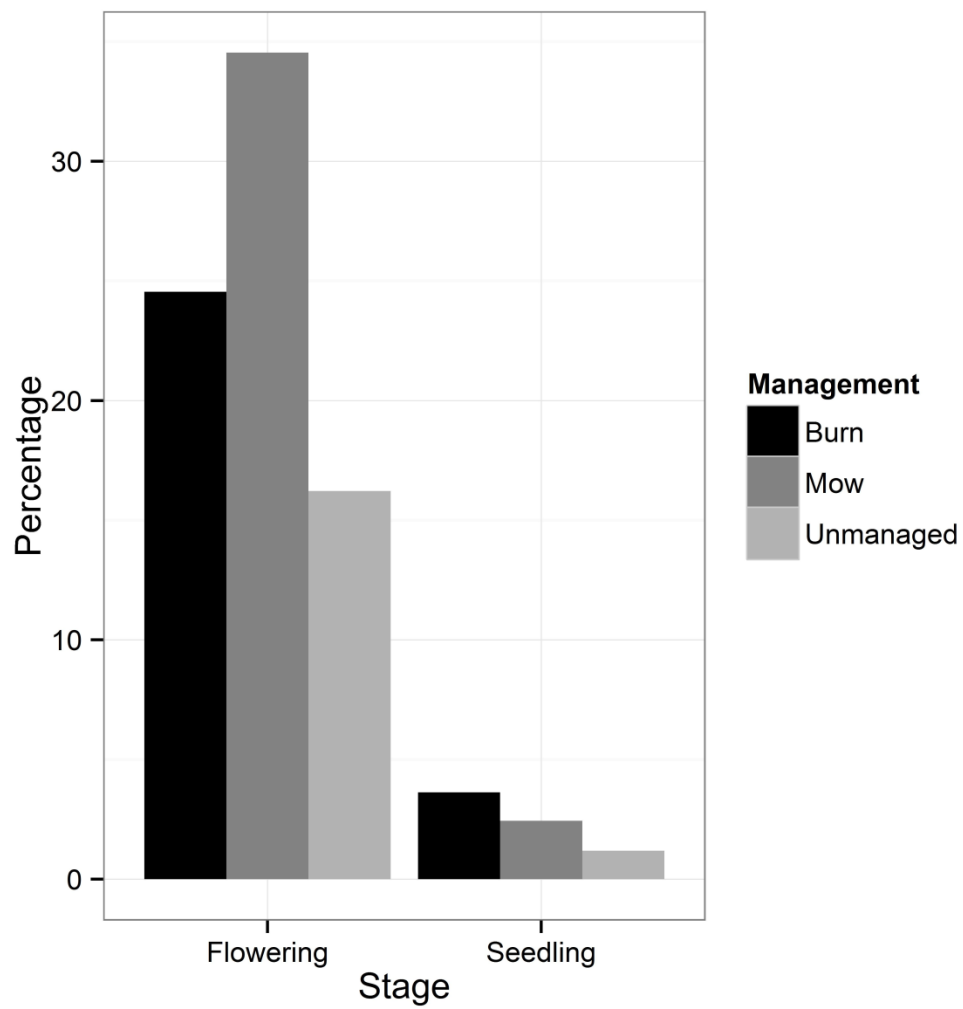


Figure 2.2: Percentage *Gentiana autumnalis* seedlings and flowering plants in study populations pooled by management practice (2011-2013)

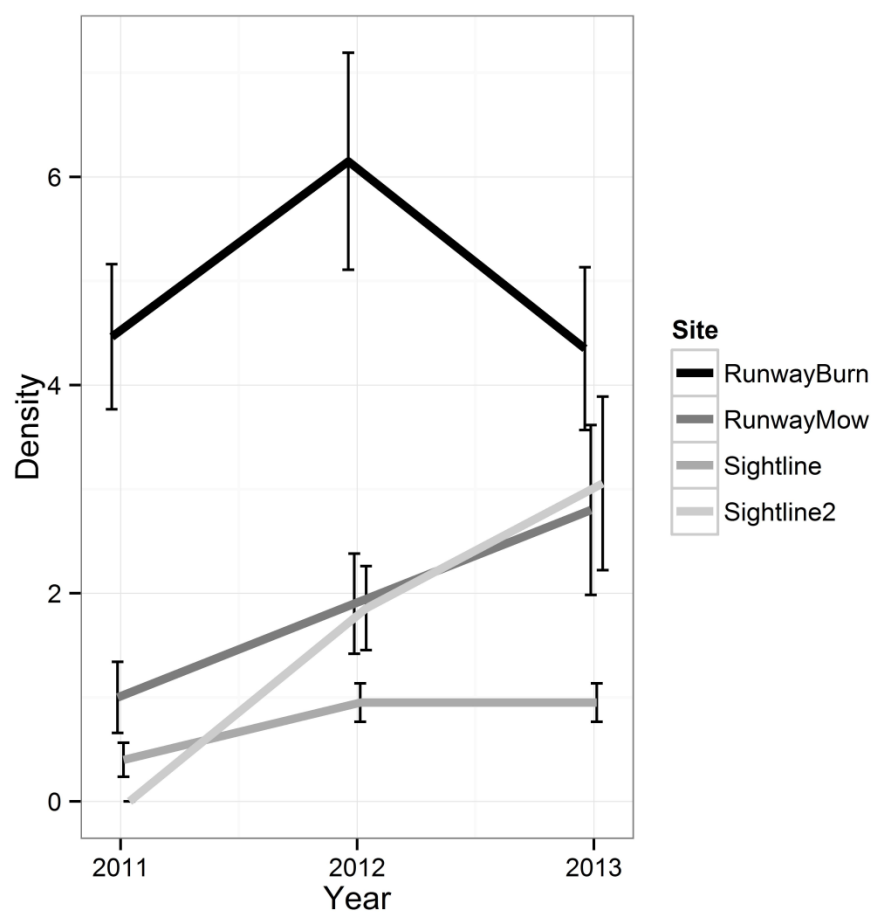


Figure 2.3: Gentian fall census density prior to management (2011) and in two successive years (2012-2013; S.E. bars)

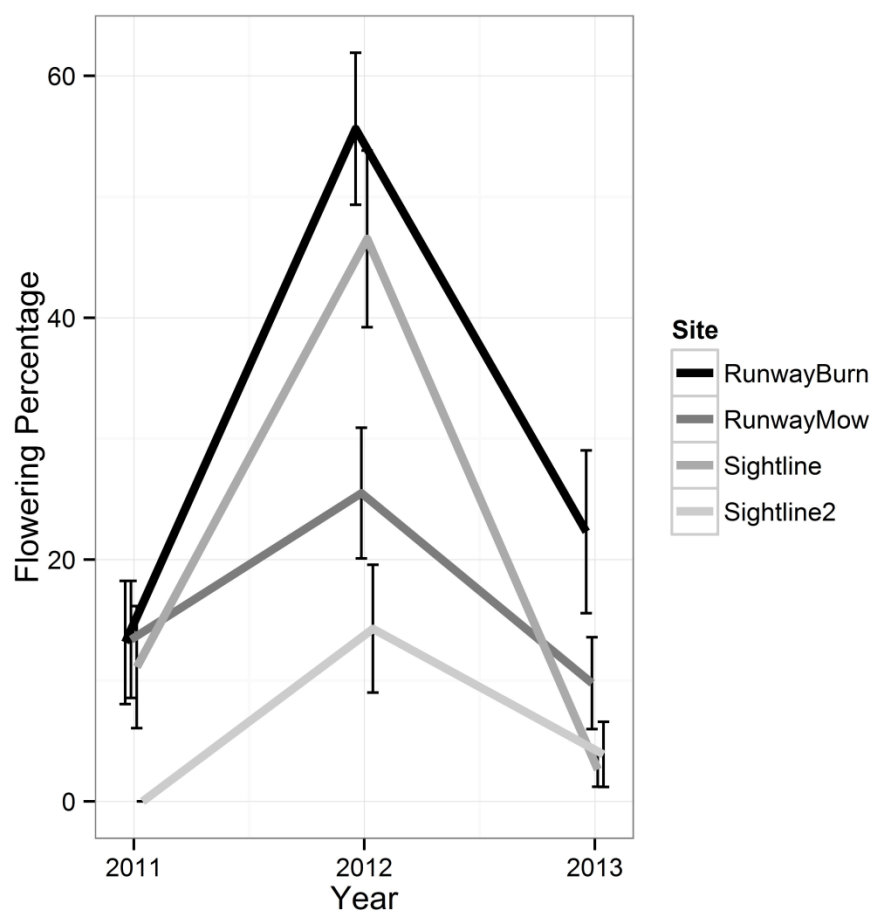


Figure 2.4: Gentian fall census percentage of flowering plants in study populations prior to management (2011) and in two successive years (2012-2013; S.E. bars).

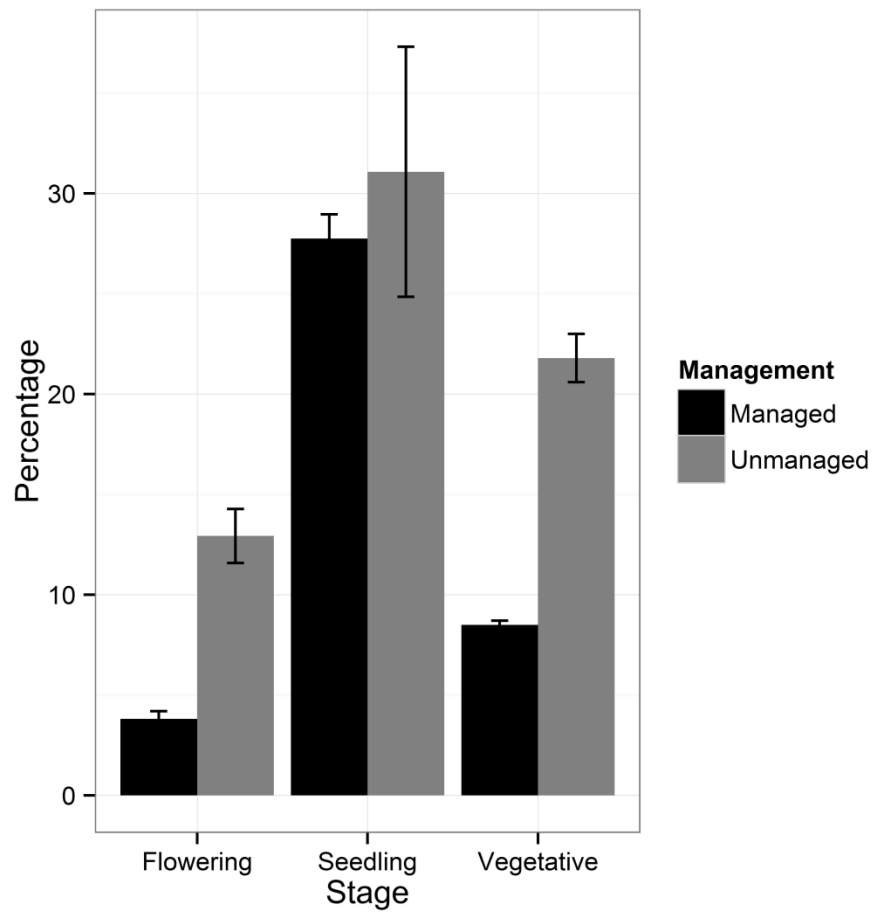


Figure 2.5: Average gentian yearly mortality by life stage in managed (disturbed) and unmanaged sites (all sites; S.E. bars)

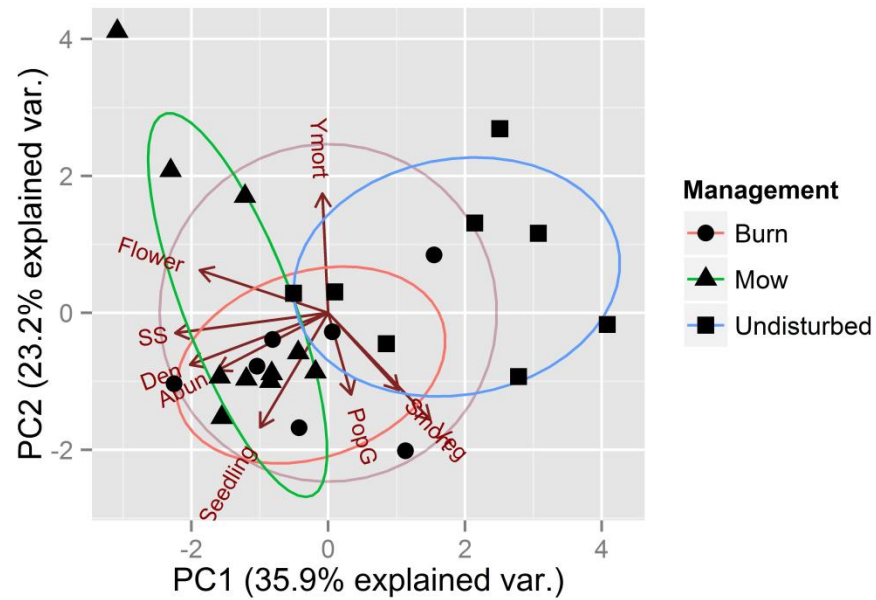


Figure 2.6: Log transformed PCA grouped by management practice. Variables- area, density (Den), abundance (Abun), flowering % (Flower), vegetative % (Veg), seedling % (Seedling), seasonal mortality (Smort), yearly mortality (Ymort), seed set (SS), population growth rate (PopG). 59.1% of variation explained in two principal components.

PCA Loading Scores

Variable	Principal Component 1	Principal Component 2
Abundance (Abun)	-0.37	-0.24
Gentian Density (Den)	-0.46	-0.21
Gentian Flowering % (Flower)	-0.42	0.18
Gentian Vegetative % (Veg)	0.34	-0.44
Gentian Seedling %(Seedling)	-0.22	-0.47
Seasonal Mortality (SMort)	0.23	-0.32
Yearly Mortality (YMort)		0.49
Seed Set (SS)	-0.51	
Yearly Population Growth Rate (PopG)		-0.33

Chapter 3: The effect of management practices (mowing and prescribed burning) on soil nutrients and mycorrhizal colonization of *Gentiana autumnalis*

Abstract

Understanding the edaphic conditions at sites where rare plant populations are found is important to the management and conservations of these species particularly in habitats that are disturbance dependent, acidic, or oligotrophic. The New Jersey Pine Barrens is a fire dependent ecosystem whose soil under natural conditions is both acidic and oligotrophic. The Pine Barren gentian (*Gentiana autumnalis*), is a fall flowering perennial that is rare in New Jersey, primarily due to fire suppression throughout its range. Previous work revealed that populations of *Gentiana autumnalis* that are actively managed have a higher density of plants, a greater percentage of flowering individuals, and a greater average seed set per fruit when compared to unmanaged sites. This study measured the variation in soil nutrients, pH, species richness, ground cover, canopy openness, and mycorrhizal colonization among *Gentiana autumnalis* patches in order to characterize the variation among these sites and to relate these site conditions to previously recorded demography data. Sites managed either with mowing or prescription burns had greater canopy openness ($p < 0.001$), mycorrhizal colonization ($p < 0.01$), and less woody plant species ($p < 0.05$) when compared to unmanaged sites. Gentian density was negatively correlated to NH_4 ($r = -0.67$) and NO_3 ($r = -0.73$) and positively correlated to degree of mycorrhizal colonization ($r = 0.64$) in the study sites. The percentage of flowering gentians in a given patch was negatively correlated with ground cover ($r = -0.76$), but positively correlated with pH ($r = 0.88$) while average seed

set per fruit was negatively correlated with PO_4 ($r = -0.75$) and positively correlated with bulk density ($r = 0.69$), canopy openness ($r = 0.64$) and site species richness (0.68).

Understanding the site conditions associated with robust plant populations is important when planning site management, particularly where rare plant species are involved.

Introduction

Understanding the relationship between edaphic conditions and disturbance regimes is important for the management and conservation of rare plant populations, particularly in habitats that are disturbance-dependent, acidic, or oligotrophic. The growth and survivorship of plant populations are directly influenced by the properties of the soil in which they grow (Lukac and Godbold 2011). Soils function not only as a medium for growth, but are also a source of nutrients, water, and mutualistic interactions (Lukac and Godbold 2011). For this reason, understanding the availability of nutrients and mutualistic associates in the soil is vital in understanding above ground plant population dynamics (Bever et al. 2010). Although nitrogen and phosphorus are essential nutrients for plant growth, in fire-prone oligotrophic ecosystems their availability and acquisition can be challenging. Tuininga and Dighton (2004) report that forest fires increase soil nutrients, yet these nutrients are less available to plants as a result of the interruption of mycorrhizal linkages (Tuininga and Dighton 2004). An increase in soil pH following a fire is also associated with a decrease in the capacity of acidophilic plants to uptake nutrients (Romanya et al. 1994). This becomes particularly important when plant populations are actively managed for conservation purposes. The focus of this study was to better understand the variation observed in population structure found in

populations of *Gentiana autumnalis* under different management regimes by measuring available soil nutrients and calculating root colonization by mycorrhizal fungi. These data were related to a concurrent study on the demography of *Gentiana autumnalis* that used the same populations, in order to better understand site to site variations (Chapter 2).

Of the estimated 90% of higher plant species that are associated with mycorrhizal fungi, two thirds are believed to form associations with arbuscular mycorrhizal fungi (AMF; Fitter and Moyersoen 1996). The symbiosis between AMF and their host plants is considered the most common underground symbiosis (Smith and Read 2008). In exchange for receiving carbon from plant roots, AMF facilitate the delivery of inorganic nutrients into the plant's root system (Fitter 1985). In the presence of disturbance, some mycorrhizal fungi become influential in not only nutrient acquisition, but also the establishment of early successional plant species. One observed influence of the increased uptake of inorganic nutrients in early successional plants is the promotion of seedling establishment (Smith and Read 2008). Study plots inoculated with mycorrhizal fungi have been shown to not only produce greater seed set when compared to non-mycorrhizal sites, but to also experience a greater level of seedling recruitment as well (Stanley et al. 1993). The increased levels of seedling recruitment caused by mycorrhizal colonization may then lead to greater plant densities in early successional fields (Koide and Dickie 2002). This interaction between mycorrhizal fungi and their plant hosts becomes particularly beneficial in plant species such as *Gentiana autumnalis* that persist in managed sites with low levels of seedling establishment (Rebozo et al. in prep). It is important to investigate how disturbance impacts mycorrhizal colonization under natural

field conditions for various plant species, as each may respond differently to these interactions (Moora et al. 2004).

Previous research has indicated that mycorrhizal associations decrease with disturbance, but increase with reduced soil nutrients and lower pH (Egerton-Warburton and Jumpponen 2005). In the nutrient poor soils of the New Jersey Pine Barrens, forest fires increase available nutrients in the soil, but reduce ectomycorrhizal fungi abundance and diversity (Tuininga and Dighton 2004). This has also been documented post fire for trees existing in the New Jersey Pine Plains where a high fire frequency is experienced (Buchholtz and Gallagher 1982). While fire appears to be detrimental to mycorrhizae diversity, other types of disturbance such as roadside mowing may have less of an impact on mycorrhizal communities while still providing suitable habitat for *G. autumnalis*.

Mowing is often compared with prescribed burning as a management technique aimed at reducing vegetation encroachment (Eom et al. 1999; Howe 1999; Clark and Wilson 2001). These studies often demonstrate differential impacts on ecosystem processes that can have positive or negative consequences for rare plants (Kirkman et al. 1998). A better understanding of the environmental requirements for the sustainability of *G. autumnalis* populations is essential for developing a management plan for the species and could be applicable to other rare disturbance-dependent species in the New Jersey Pine Barrens.

Disturbance can influence nitrogen and phosphorous availability and its interactions with aboveground and belowground processes (Hobbs and Huenneke 1992). Prescribed burning and mowing often have similar impacts on light availability that can

be important for the management of early successional species (Howe 1999), but they may have dissimilar belowground influences, given that nutrient flushes are typically associated with prescribed burning (Wan et al. 2001), while mowing has less direct impact on belowground soil biota (Eom et al. 1999). The focus of this study was to examine the effect of these two management practices on soil nutrients and mycorrhizal colonization of *Gentiana autumnalis*, a rare perennial found in early successional habitats along the Atlantic Coastal Plain.

The influence of fire on soil nutrients is well-studied (Neary et al. 1999; Wan et al. 2001). Several studies show an increase in soil organic nitrogen following a burn due to increased ammonification and ash deposition, while phosphorus has a more varied response (Neary et al. 1999; Grogan et al. 2000; Wan et al. 2001). Less is known about the impact of prescribed burning on interactions between mycorrhizae, nutrient cycling, and plant populations (but see Anderson and Menges 1997). These data are particularly important when plant populations are actively managed for conservation objectives. Past studies comparing management techniques focused on aboveground factors such as changes in light availability and competitive biomass (Howe 1999; Clark and Wilson 2001). A greater appreciation of the role of belowground interactions in assessing anthropogenic disturbance regimes has recently emerged (Kardol and Wardle 2010), but little is still known about the belowground impacts of different management techniques, particularly for rare plants. Rare plants may have complex belowground relationships that can include negative responses to mycorrhizal colonization, particularly when faced with competition from invasive species (Klironomos 1999).

Materials and Methods

Study Species

Gentiana autumnalis is a fall flowering, showy herbaceous perennial in the Gentianeaceae family. This species is one of the latest flowering plant species in the New Jersey Pine Barrens and because of this it may act as an important food source for late season and over wintering insect pollinators. In New Jersey, this disturbance-dependent species favors open-moist sandy soil conditions (Jenkins and Blades 1990; Harshberger 1916; Stone 1911). Solitary stems on mature plants can grow to 30 to 60cm in height and typically support solitary flowers, although occasionally 2-3 flowers can be found. Although *G. autumnalis* flowers exhibit a high level of morphological variation, the flowers are characteristically protandrous, five-merous, and blue to purple (occasionally white). *Gentiana autumnalis* is popular among local amateur botanists and naturalists due to this floral variation, but little is known about the life history of this species.

Study Sites

I incorporated nine study sites of known gentian populations in the New Jersey Pine Barrens. Study sites were characterized by their disturbance type (mowed, burned), frequency of disturbance, and date of last disturbance (Table 3.1). All sites, with the exception of two reference sites (Stockton and Atsion), were actively managed at least once over the study (2011-2013). Three sites (FPP, 561, LC) were mowed each study year. The Runway site was subjected to both mowing and burning during the study period. In March 2012, three previously unmanaged sites received their first disturbance (Sites: RunM, RunB, and Sightline; Table 3.1). Introduced disturbance gave rise to the

tenth study site (Sightline 2) which produced no aboveground gentian growth prior to the 2012 burn. Sites used in this study were situated along road edges and open meadows that bordered pitch pine upland and lowland habitat and included late successional sites with closed canopies (McCormick 1970). Sites listed as unmanaged at the start of this study (2011) had been unmanaged for a minimum of six years (Table 3.1).

Data Collection

I used a repeated measures design to census each gentian population at each site from 2011 through 2013. I established a rectangular macroplot that encompassed all encountered *G. autumnalis* plants at each of the nine study sites (Elzinga et al. 1998). I established 20 randomly nested quadrats (1m²) within each macroplot in which all individual gentian plants were censused. In macroplots less than 20m² (n=1), I censused all gentian plants within the macroplot. Of the 10 study sites used in the demography study, nine were included for soil nutrient analysis, and eight used for fungal colonization (Table 3.2). Measurements of soil nutrients, pH, bulk density and canopy openness were taken in August 2012. Estimates of vegetation ground cover along with root harvest for measurements of percent fungal colonization were conducted in both 2012 and 2013. These soil and root analyses were conducted in conjunction with a demographic study of *Gentiana autumnalis* that spanned 2011 – 2013 (Rebozo et al. in prep).

Gentiana autumnalis is a rare species whose sparse and often small populations limited the opportunities for replicated, manipulative experiments. My study was a non-manipulative survey that was limited to measuring site data under existing field conditions (*sensu* Kerr et al. 2007). My manipulation at the Runway site was an

opportunistic “natural experiment” (Baum and Worm 2009; Schank and Koehnle 2009) that examined the pre- and post-effects of burning, mowing, and disking. I divided a portion of the Runway site into three 10m x 210m rectangular strips, one for each treatment (burning, disking, and mowing). Disking was used to separate the burned and mowed treatments. Treatments were conducted in March of 2012 and analyzed in the same year (Table 3.1). Due to the restricted limitations working with this species, I was unable to replicate or randomize treatments. There are several examples of “opportunistic natural experiments” without replication (Kerr et al. 2007; Baum and Worm 2009; Schank and Koehnle 2009).

Soil Analysis

I selected six randomly located quadrats within each macroplot for soil core extraction in addition to four soil cores taken outside of each gentian macroplot along its periphery to compare soils within and outside of the sampling area. Soil samples were taken in late August 2012, just prior to gentian flowering and after prescribed burning (March 2012). I collected soil samples for use in nutrient and pH analysis using 5.8 cm diameter standard percussion soil corer at a depth of 15 cm. I removed the organic horizon before collecting soil samples. Soil analysis was conducted at the Rutgers University Pinelands Field Station, where samples were processed within 48 hours of collection. I weighed samples to calculate bulk density, then had any fine roots and pebbles removed. Soil moisture content was determined by first weighing 10 gram subsamples, then drying the samples for 48 hours at 70°C before reweighing. Collected soil was manually homogenized prior to analysis.

I prepared ten gram aliquots for analysis using a 1.0 N KCl solution to extract nitrogen and a Bray (0.025 N HCl and 0.03 N NH₄F) solution to extract phosphorus (Allen, 1974). All extractions were done with 30 mL extractant:10 g f.w. soil. Extractions were maintained at 4°C until analyses were performed. Available NH₄ and NO₃ were analyzed using an Astoria Pacific AP3 autoanalyzer (Clackamas, OR). I measured available phosphorus colorimetrically using the ascorbic acid method (Allen 1974; American Public Health Association et al. 1985). I measured soil pH within each macroplot and site periphery using a YSI Ecosense pH100 meter in a solution of 5g of soil to 5ml of distilled water following pH of soil suspension methodology using 1:1 soil to water ratio (Soil Survey Division Staff 1993).

Mycorrhizal Colonization of Roots

Roots of *Gentiana autumnalis* used for analysis of mycorrhizal colonization were selected randomly from plants growing within the macroplot, but out of the sampling quadrats used for gentian surveys. I harvested ten plants from each site over two years (2012-2013). I harvested gentian roots in the field, wrapped them in moist paper towels and kept the roots on ice prior to root staining. The root staining process followed the methodology for non-pigmented roots explained in Phillips and Hayman (1970). I heated roots in 10% KOH solution at 90°C for one hour. I then rinsed the roots with dilute HCl before simmering in a lactic acid:glycerol:water (1:1:1 vol) solution containing 0.05% trypan blue. I determined the percentage of root colonization using the magnified intersections method (McGonnigle 1990). Fine roots were mounted in glycerin to a microscope slide and covered with 40x20mm cover slips. I used four slides of three root

subsamples for each root and placed along the long axis of the slide and viewed under 200x magnification. I made eight random perpendicular passes through the roots by moving the field of vision on the microscope. Eight passes of each root subsample totaled 96 intersections for each root. I recorded the presence of arbuscules, vesicles, spores, and hyphae at each intersection.

Associate Plant Species and Cover Estimates

I inventoried all associative plant species within each macroplot at each site in order to calculate species richness. I randomly selected ten quadrats within each macroplot to estimate ground cover using the Braun-Blanquet method; 0: no individuals, T: <1% trace amount, 1: <5% cover, 2: 5-25% cover, 3: 26-50% cover, 4: 51-75% cover, 5: 76-100% cover (Wikum and Shanholtzer 1978). I measured canopy cover percentage using true-color fisheye photographs that were processed with Gap Light Analyzer (GLA) version 2.0 imaging software. I took five canopy photographs at each site using a Nikon Coolpix 995 camera and Nikon fisheye lens (FC-E8 0.21x). Canopy photographs were taken at approximately the same time of day and under similar cloud cover to limit variation in the calculation of canopy openness due to cloud cover.

Statistical analysis

Paired T-tests ($\alpha=0.05$) compared mycorrhizal colonization percentage for 2012 and 2013 for each site, while Independent T-tests compared soil nutrient levels within the macroplot to soil nutrient levels in the periphery as well as the percentage of woody plant species in managed and unmanaged sites. Analysis of variance was used to compare site condition variables among sites and management treatments. Correlation coefficients

were calculated among site condition variables as well as between site conditions and demographic variables. A log transformed principal components analysis identified the percentage of variation explained by a subset of site variables with an emphasis on variation as a result of land management practice. I used R for all statistical analyses (R Core Team 2014).

Results

Gentiana autumnalis Roots

The roots of *Gentiana autumnalis* were thick and fibrous, typically bifurcated with few fine roots. The diameter of the “Y” shaped roots averaged 2.99mm (± 1.05 mm, Min = 1mm, Max = 5mm, n=34 roots), depth of the root system averaged 159.11mm (± 70.49 mm, Min = 59mm, Max = 311mm, n=26 plants), and the total length of root segments averaged 355.44 mm (± 210.24 mm, Min = 59mm, Max = 860mm, n= 18 plants). Harvested plants averaged 4.26 (± 5.27 , Min = 1, Max= 30, n=34) root branches, with multistemmed plants averaging more root branches than single stemmed plants (Multistemmed: 6.38 branches, ± 7.11 , n = 16 Single Stemmed: 2.39, ± 1.14 , n = 18; $F_{(1,32)} = 5.5$ p < 0.05). Root branching was also positively related to plant height ($r^2 = 0.49$). Plants with greater root branching also had a greater abundance of fine roots.

Canopy Openness, Ground Cover and Species Richness

Species richness in sites ranged from 8 to 91 species within the macroplot ($\bar{x} = 24.9$). Plant species commonly found in gentian macroplots include *Andropogon virginicus*, *Panicum virgatum*, *Comptonia peregrine*, *Gaylussacia baccata*, *Vaccinium*

pallidum, and *Lobelia nuttallii*. Unmanaged sites had a greater percentage of woody plants ($\bar{x} = 33\%$) when compared to managed sites ($\bar{x} = 22.2\%$; $T = 2.65$, $p < 0.05$). I considered sites that had 60% of their Braun Blanquet ground cover observations including wood shrubs as having an established woody understory. Sites whose ground cover was dominated by woody shrubs had significantly lower levels of gentian flowering from 2011-2013 ($\bar{x} = 14.3\%$) when compared to sites with no or limited woody shrub cover ($\bar{x} = 41.55\%$; $T = 13.2$, $p < 0.001$). Site canopy openness varied from 25.1% to 80.3%. Managed sites differed significantly in having greater canopy openness than unmanaged sites (Unmanaged $\bar{x} = 26.29\%$, Managed $\bar{x} = 59.2\%$; $F_{(2,38)} = 29.59$ $p < 0.001$; Figure 3.1). There was no significant difference in the canopy openness of mowed sites and sites managed with prescribed burns ($p > 0.05$). A weak positive correlation was found between canopy openness and species richness in sites ($r^2 = 0.39$) and a weak negative correlation exists between species richness of woody plants and flowering percentage ($r^2 = 0.32$). Managed and unmanaged sites showed no significant difference in herbaceous ground cover ($\bar{x} = 3.6$ for all sites).

Soil Nutrients and Fungal Colonization

Across all sites, soil PO_4 averaged $0.14 \mu\text{g/g}^{-1}$ (± 0.26 , $n=90$), NH_4 averaged $2.08 \mu\text{g/g}^{-1}$ (± 0.88 , $n=90$), NO_3 averaged $0.21 \mu\text{g/g}^{-1}$ (± 0.24 , $n=90$), bulk density averaged 1.36 g/cm^3 (± 0.35 , $n=90$), and pH averaged 4.2 (± 0.43 , $n=30$). Levels of available nutrients (PO_4 , NH_4 , NO_3) within study sites were compared to the levels of available nutrients in the periphery of the study sites. There were no significant differences ($p > 0.05$) between the level of soil nutrients within versus outside of the macroplot except for

three occasions, site 561 NO_3 ($T_{(1,5)} = 3.4$, $p < 0.05$), site FPP NO_3 ($T_{(1,5)} = 3.46$, $p < 0.05$), and site RunM NH_4 ($T_{(1,5)} = 3.1$, $p < 0.05$). The ratio of PO_4 , NH_4 , and NO_3 across all sites averaged 1:13.1:1.27. Variables NH_4 ($F_{(6,35)} = 8.58$, $p < 0.001$), and pH ($F_{(6,35)} = 89.71$, $p < 0.001$) were significantly different among study sites. Between managed and unmanaged sites, NO_3 (Unmanaged $\bar{x} = 0.27 \mu\text{g/g}^{-1}$, Managed $\bar{x} = 0.17 \mu\text{g/g}^{-1}$; $F_{(1,40)} = 4.65$, $p < 0.05$) was significantly greater in unmanaged sites. Soil pH was significantly greater in mowed sites (Unmanaged $\bar{x} = 4.03$, Mow $\bar{x} = 4.56$, Burn $\bar{x} = 4.19$; $F_{(2,39)} = 3.95$, $p < 0.05$) when compared to either unmanaged (Tukey HSD; $p < 0.01$) or burned sites (Tukey HSD; $p < 0.05$). Soil bulk density did not differ between samples from within the macroplot and samples taken from the periphery at each site ($p > 0.05$). Bulk density for unmanaged sites averaged 1.3 g/cm^3 , mowed sites averaged 1.44 g/cm^3 and burned sites averaged 1.34 g/cm^3 ($p > 0.05$; Table 3.4).

The percentage of gentian root colonized by mycorrhizal fungi averaged 85.2% (± 13.0 , $n = 80$) across all sites. No significant differences were found between mycorrhizal colonization percentages in 2012 and 2013 for individual sites, and thus percent mycorrhizal colonization for each year was pooled by site for further analysis (Table 3.3). Across all sites, arbuscules were present in 64.65% ($\pm 7.4\%$) of observed root intersections, vesicles were present in 6.91% ($\pm 3.1\%$) of intersections and spores were present in 7.03% ($\pm 4.62\%$) of intersections. Mycorrhizal colonization was significantly greater in managed sites than in unmanaged sites (Managed $\bar{x} = 74.67\%$, Unmanaged $\bar{x} = 86.16\%$; $F_{(1,40)} = 10.72$, $p < 0.01$). There was no significant difference in the percent colonization of mowed and burned sites ($p > 0.05$).

Density of *Gentiana autumnalis* in study sites for 2012 (Rebozo et al. in prep) were negatively correlated with NH_4 ($r = -0.67$) and NO_3 ($r = -0.73$) but positively correlated with fungal colonization ($r = 0.64$; Table 4). The percentage of flowering plants in gentian study site for 2012 (Rebozo et al. in prep) was negatively correlated with ground cover ($r = -0.76$) and positively correlated with pH ($r = 0.88$). Average floral seed set for gentian sites in 2012 (Rebozo et al. in prep) was negatively correlated with PO_4 ($r = -0.75$) but positively correlated with bulk density ($r = 0.69$), canopy openness ($r = 0.64$), and species richness ($r = 0.68$). The percentage of seedlings at these sites in 2012 was not strongly correlated with the site variables recorded. Site N:P ratio was positively correlated with fungal colonization ($r = 0.77$), species richness ($r = 0.49$), and floral seed set ($r = 0.51$), while being negatively correlated with ground cover ($r = -0.51$).

A log transformed principal component analysis using levels of PO_4 , NH_4 , NO_3 , pH, fungal colonization and canopy openness as factors explained 53.4% of variation in two principal components (Figure 3.2). Principal component one correlated with variables NH_4 (loading score = 0.63), and NO_3 (0.54) while the second principal component correlated strongest with canopy openness (0.6). Correlation circles in the principal component analysis isolated unmanaged sites as being negatively related to pH and canopy openness.

Discussion

There have been few studies on the influence of management practices on rare species and my study was the first to examine how management affects the relationship

between key belowground and aboveground processes for *Gentiana autumnalis*. There were significant differences between managed and unmanaged sites. Managed sites exhibited greater canopy openness, a lower percentage of woody plant species, and higher mycorrhizal colonization percentages. These characteristics were correlated with positive measures of demographic data from a related study conducted at the same sites (Rebozo et al. in prep). Both prescribed burns and mowing had similarly positive impacts, in contrast to a study of the rare species *Schwalbea americana* in which mowing was detrimental to populations (Kirkman et al. 1998). These data have important implications for the management of *Gentiana autumnalis* throughout the Atlantic Coastal Plain.

Plants in the genus *Gentiana* form associations with *Paris* type arbuscular mycorrhizal fungi, but little is known of natural levels of colonization and the influence of this mutualism on survivorship and reproduction (Sýkorová et al. 2003, Struwe et al. 2002). The high degree of fungal colonization at managed sites suggested that management practices did not interrupt mycorrhizal relationships, as has been found in other studies after fire disturbance (Tuininga and Dighton 2004; Neary et al. 1999). My results are similar to a study comparing management practices in tallgrass prairie, which also found no effect from prescribed fire or mowing on mycorrhizal colonization (Eom et al. 1999). Studies investigating a range of plant species responses to mycorrhizal inoculation usually find a high degree of variation in colonization (Anderson and Menges 1997; Wilson and Hartnett 1998). Few plants exhibit the high colonization rates found in *G. autumnalis*, but a study of Australian forbs found some of the most common species

(native and non-native) had the highest colonization rates (65%-80%; O'Connor et al. 2002).

The thick and fibrous roots of *G. autumnalis* are unique among closely related species (L. Struwe personal communication), and may increase mycorrhizal responsiveness, as is seen in other perennial plants with similar root architecture (Cook et al. 1988). *Gentiana autumnalis* has the ability to resprout from dormant root stock after a disturbance event (Rebozo pers. obs.) and root structure may play an important role in its ability to survive wild fires. The allocation of resources for storage in roots and post fire flowering are traits that have been associated with the tolerance of low soil fertility and fire disturbance (Kellman 1984, Brewer 1995). In a comparison of root biomass after burning or mowing, plants at burned sites had significantly greater root biomass and rooting depth while mowed sites had lower root biomass and depth (Kitchen et al. 2009). Plants may allocate more to roots due to nutrient flushes that often occur after burning, or the development of a more massive rootstock may be a fire-adapted trait that reduces plant mortality (Little 1979). This thick, bifurcated root architecture may improve the ability of *G. autumnalis* to compete after disturbance when both canopy openness and soil nutrients increase. Fire suppression on the other hand reduces the ability of fire adapted plant species to compete with later successional species (Brewer 2011). There was reduced reproductive output and increased mortality with lower levels of abundance and density in unmanaged compared to managed sites (Rebozo et al. in prep). However, there was no significant differences in mycorrhizal colonization between mowing and burning, suggesting that mowing is an acceptable form of management at sites where prescribed burning is not practical.

Low concentrations of soil nutrients are characteristic of the New Jersey Pine Barrens (Ehrenfeld et al. 1995). There were no significant differences in soil nutrients based on management practices in my study sites. This was in contrast to a study of tallgrass prairie in which mowing reduced soil N concentrations, particularly at sites that were also prescribed burned (Kitchen et al. 2009). Tallgrass prairie sites are generally richer in nutrients than sites in the New Jersey Pine Barrens. It could be that the biotic communities of the New Jersey Pine Barrens are more adapted to low-nutrient conditions that lead to increased ability to retain nutrients after disturbance. A study of gaps in the New Jersey Pine Barrens found no difference in soil nutrients between the gaps and the surrounding matrix (Ehrenfeld et al. 1995). Similarly, there were no significant differences in nutrients in the macroplot in this study when compared to areas in the periphery. Soil nutrients were overall weakly correlated with demographic variables, with the exceptions of negative correlation between gentian density and NH_4 and NO_3 and seed set with PO_4 . Positive correlations were found between site N:P ratio and site productivity in terms of gentian seed set, AMF colonization and plant species richness. Some of the more prolific gentian sites in terms of density and flowering percentage had lower than average levels of soil nutrients (ie: 561 and FPP). These results most likely reflect increased plant uptake during the sampling period, which corresponded to the onset of flowering and seed formation. These soil measurements were taken after a pulse in nutrients would have occurred and represent levels after leaching, microbial immobilization, and plant uptake, making it difficult to assess relationships between disturbance regime and soil nutrients. However, increased flowering and seed set in managed sites probably reflects increased access to nutrients after site management

alteration, either through greater nutrient availability or decreased competition from plant or microbial communities. Dramatic increases in post-fire flowering occurs in other fire-dependent ecosystems, including those harboring rare plants (Kirkman et al. 1998).

The removal of woody species sometimes has mixed effects on restoration objectives (Clark and Wilson 2001), but my study found a positive correlation between management and decreased woody cover, which in turn was correlated to increases in flowering plants and species richness (Rebozo et al. in prep). This correlation with seed set may be a result of greater floral advertisement to potential insect visitors. Shading had a significant impact on pollinator visitation in a study of an invasive shrub (McKinney and Goodell 2010). As a result of this positive response, management plans for *G. autumnalis* should include regular reduction of shrub cover. The positive correlation between percent fungal colonization and pH may be good indicators of gentian population stability as they are also positively correlated with density and flowering percentage respectively. Site 561 was found to have the highest average pH (5.35) of all study sites included. This is atypical of pine barren soil and is the result of this site being situated along a paved county road where road material and runoff may have amended the soil. This site, though small in size (87m²), maintained a high density of gentians ($\bar{x}$ = 7.5 per m²) and the highest flowering percentage of any study site ($\bar{x}$ = 85.9%; Rebozo et al. in prep). While *Gentiana autumnalis* does not appear to be negatively affected by the amended soil along route 561, this site was the only study site in which non-native plants were recorded, potentially increasing competition with *G. autumnalis* and other natives. However, the high mycorrhizal colonization rate we

observed may enable *G. autumnalis* to maintain populations at sites with non-natives, as was observed for a rare plant in another study (O'Connor et al. 2002).

This study demonstrated that management practices impact both belowground processes and *G. autumnalis* demographics. However, more work is needed to elucidate the key factors regulating *G. autumnalis* populations in order to develop sound management plans throughout its geographic distribution. A further study into the dynamics between mycorrhizal fungi and *Gentiana autumnalis* may include a comparison of the diversity of fungi on gentian roots to the potential inoculum available in the soil and other species root stocks. Controlled experiments (Sykorva 2003) reveal that manual inoculation of *Gentiana* roots with mycorrhizae is unsuccessful, but colonization due to the “nurse effect” involving surrounding plants is successful. Arbuscular mycorrhizal fungi (AMF) are important not only to the order in which species colonize these early successional sites, but also in determining the composition of plant species in disturbed sites (Gange et al. 1993). The distribution of AMF to heterospecific species through the nurse effect (Carillo-Garcia 1999) may play an important role in developing the early successional plant community and in maintaining plant species in disturbance-dependent systems.

It is often difficult to link post-disturbance soil characteristics to plant performance, particularly when there is a large gap between the disturbance event and key phenological events. To better understand how land management influences the edaphic conditions of these sites, soil measurements should be taken prior to disturbance, immediately after disturbance, and again during the flowering season. This would give a

better understanding as to how the availability of soil nutrients changes in a growing season, whereas this study used soil nutrients as a way to characterize general nutrient availability at different sites.

The principal components analysis used for the collected site condition variables showed a clear separation between the undisturbed sites and disturbed sites in the biplot. The disturbed sites exhibited greater canopy openness, higher pH, and greater mycorrhizal colonization. These are the same factors that correlate to differences in demographic characteristics between sites (Rebozo et al. in prep). In my study of *Gentiana autumnalis* populations, the actively managed sites were most productive in terms of density, abundance, flowering and seed set (Rebozo et al. in prep). In this study I found that these managed sites, both mowed and burned, differed from unmanaged sites in having greater canopy openness, less woody plant competition, and greater fungal colonization. Managing for early successional conditions through mechanical removal of plants or prescription burns where applicable has been shown to be beneficial to populations of *Gentiana autumnalis* and may very well be beneficial to many other early successional pine barren species.

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Tables

Table 3.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)

Site	2011	2012	2013
Franklin Parker Preserve (FPP)	Mow	Mow	Mow
Runway	Unmanaged-Reference	Mow/Burn/Disk	No Management
Lower Cabin (LC)	Mow	Mow	Mow
Atsion	Unmanaged-Reference	Unmanaged-Reference	Unmanaged-Reference
Stockton (Stock)	Not in study	Unmanaged-Reference	Unmanaged-Reference
561	Mow	Mow	Mow
Sightline (Sight)	Unmanaged-Reference	Burn	No Management
Sightline2 (sight2)	Not in Study	Burn	No Management
North Pole Road (NPR)	Mow	Mow	Mow

Table 3.2: Average percent mycorrhizal root colonization, available soil nutrients ($\mu\text{g/g}$) and pH by site (N.D. = No Data)

Site	Average Fungal Colonization 2012-2013 (n=10)	Average Available NO_3 2012 (n=6)	Average Available NH_4 2012 (n=6)	Average Available PO_4 2012 (n=6)	Average Soil pH 2012 (n=3)
RunM	$78.3\% \pm 13.0\%$	0.25 ± 0.09	4.79 ± 0.57	0.013 ± 0.011	4.15 ± 0.06
RunB	$81.75\% \pm 11.76\%$	0.31 ± 0.12	4.26 ± 0.75	0.016 ± 0.015	4.36 ± 0.2
Sight	$91.16\% \pm 10.74\%$	0.24 ± 0.07	4.09 ± 1.77	0.018 ± 0.012	4.19 ± 0.02
Sight2	$82.43\% \pm 15.8\%$	0.25 ± 0.09	2.47 ± 0.64	0.021 ± 0.018	4.04 ± 0.04
561	$89.75\% \pm 11.19\%$	0.23 ± 0.05	2.66 ± 1.16	0.017 ± 0.009	5.35 ± 0.11
FPP	$93.63\% \pm 6.38\%$	0.15 ± 0.04	1.62 ± 0.42	0.021 ± 0.012	4.12 ± 0.01
LC	$89.81\% \pm 5.59\%$	N.D.	N.D.	N.D.	4.02 ± 0.14
Stock	$74.57\% \pm 15.52\%$	0.24 ± 0.08	3.09 ± 0.89	0.024 ± 0.02	4.12 ± 0.25
Atsion	N.D.	0.23 ± 0.03	2.17 ± 0.36	0.021 ± 0.014	3.93 ± 0.06

Table 3.3: Average available soil nutrients ($\mu\text{g/g}^{-1}$), pH, percent mycorrhizal root colonization, percent canopy openness, ground cover and species richness by management practice

Manage	Avg. PO4	Avg. NH4	Avg. NOx	Avg. pH	Avg. Colo.	Avg. Canopy Openness	Avg. Ground Cover	Avg. Spp. Rich.
Unmanaged	0.13 $\pm$ 0.12 n=12	1.66 $\pm$ 0.49 n=12	0.26 $\pm$ 0.41 n=12	4.03 $\pm$ 0.18 n=6	74.6% $\pm$ 4.41% n= 10	26.3% $\pm$ 3.42% n=10	3.3 $\pm$ 0.73 n=12	24.5 $\pm$ 4.7 n=2
Mowed	0.09 $\pm$ 0.07 n=18	1.91 $\pm$ 0.95 n=18	0.17 $\pm$ 0.14 n=18	4.54 $\pm$ 0.59 n=9	87.7% $\pm$ 8.57% n=35	58.8% $\pm$ 16.8% n=14	3.5 $\pm$ 0.94 n=40	51.7 $\pm$ 33.2 n=4
Burned	0.22 $\pm$ 0.55 n=18	2.22 $\pm$ 0.83 n=18	1.7 $\pm$ 0.06 n=18	4.2 $\pm$ 0.17 n=9	85.7% $\pm$ 8.38% n=35	61.5% $\pm$ 14.7% n=13	3.6 $\pm$ 0.44 n=30	42 $\pm$ 43.3 n=3
Managed (Pooled)	0.16 $\pm$ 0.39 n=36	2.06 $\pm$ 0.89 n=36	0.17 $\pm$ 0.11 n=36	4.36 $\pm$ 0.46 n=18	86.7% $\pm$ 8.48% n=70	60.2% $\pm$ 15.6% n=27	3.54 $\pm$ 0.72 n=70	47.5 $\pm$ 34.9 n=7

Table 3.4: Average soil bulk density (g/cm^3) within and along the periphery of macroplot at each site

Site	Average Bulk Density g/cm^3 (n=6)	Average Periphery Bulk Density (n=4;5 for rows 1 and 2)
Runway Mow	1.51 ± 0.15	1.58 ± 0.18
Runway Burn	1.61 ± 0.10	1.56 ± 0.35
Sightline	0.98 ± 0.35	1.15 ± 0.33
Sightline 2	1.12 ± 0.48	0.96 ± 0.49
561	1.21 ± 0.30	1.39 ± 0.10
FPP	1.59 ± 0.28	1.48 ± 0.15
Stockton	1.21 ± 0.38	1.49 ± 0.36
Atsion	1.38 ± 0.35	1.28 ± 0.33

Table 3.5: Correlation coefficient matrix of site variables PO₄, NH₄, NO₃, bulk density (Bulk Den.), pH, percent mycorrhizal root colonization (Fungal Colo.), percent canopy openness (Canopy Op.), Braun Blanquet ground cover (Ground Co.), species richness (Spp. Rich.)

Site Variables	PO ₄	NH ₄	NO ₃	Bulk Den.	pH	Fungal Colo.	Canopy Op.	Ground Co.	Spp. Rich.
PO ₄	—	-.71	-.36	-.33	-.24	-.04	-.83	.62	-.48
NH ₄	-.71	—	.73	.06	-.12	-.44	.62	-.48	.33
NO ₃	-.36	.73	—	-.06	.01	-.59	.46	-.59	.05
Bulk Den.	-.33	.06	-.06	—	-.1	-.08	.62	-.01	.93
pH	-.24	-.12	.01	-.1	—	.32	-.12	-.76	-.31
Fungal Colo.	-.04	-.44	-.59	-.08	.32	—	-.11	.14	-.14
Canopy Op.	-.83	.62	.46	.62	-.12	-.11	—	-.35	.76
Ground Co.	.62	-.48	-.59	-.01	-.76	.14	-.35	—	.05
Spp. Rich.	-.48	.22	.05	.93	-.31	-.14	.76	.05	—

Figures

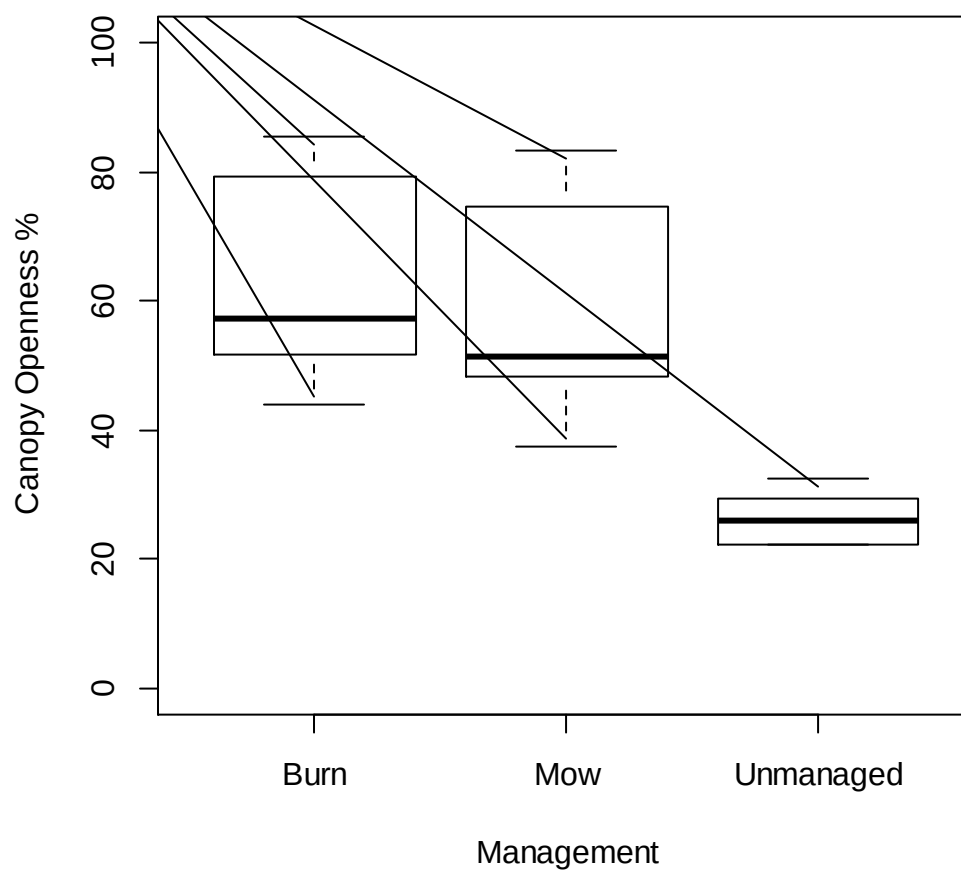


Figure 3.1: Range of average site canopy openness percentage for burned, mowed and undisturbed sites (minimum, 25th percentile, mean, 75th percentile, maximum)

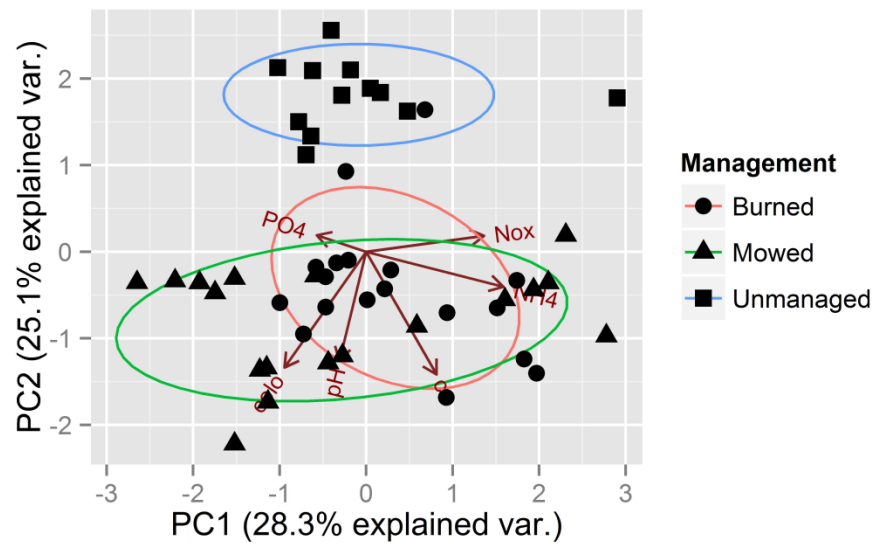


Figure 3.2: Log transformed PCA grouped by management practice. Variables- PO₄, NH₄, NO₃, pH, mycorrhizal colonization (colo), and canopy openness (co). 53.3% of variation explained in two principal components

Loading Scores

Variable	Principal Component 1	Principal Component 2
PO ₄	-0.23	
NH ₄	0.63	-0.17
NO ₃ (Nox)	0.54	
pH	-0.12	-0.53
Fungal Colonization % (colo)	-0.37	-0.56
Canopy Openness (co)	0.32	-0.60

Chapter 4: Negative effects of herbivory by *Endothenia hebesana* (verbena bud moth), *Chlorochlamys chloroleucaria* (black berry looper), and *Odocoileus virginianus* (white-tailed deer) on the rare *Gentiana autumnalis* (Pine Barren gentian) in New Jersey

Abstract

I report here the first known records of *Gentiana autumnalis* L. (Pine Barren gentian, Gentianaceae) as a host plant species for the Lepidoptera species *Endothenia hebesana* (Tortricidae) and *Chlorochlamys chloroleucaria* (Geometridae). Surveys in the Pine Barrens of New Jersey of flowering and fruiting plants and their seed set revealed the presence of *C. chloroleucaria* feeding on reproductive structures of *G. autumnalis*, and *E. hebesana* feeding on *G. autumnalis* seeds. The percentage of flowers with damage from either of these species ranged from 0.0% to 80.1% ($\bar{x} = 23.7\%$, ± 27.4) per population ($n = 8$), and was positively correlated ($r^2 = 0.87$) to total floral density. Additionally, deer herbivory occurred in all but one study site with 2.1 - 34% of plants grazed. The Pine Barren gentian is a rare early successional species whose populations in New Jersey are threatened by fire suppression, herbicide application, and roadside mowing. Seedling establishment is of particular concern for this perennial species as most study sites had no seedlings recorded in 2013. Insect and deer herbivory were previously unknown, but significant threats to the ability of this rare species to set seed and persist. Further conservation management of *Gentiana autumnalis* should take the reported levels of moth infestation into account when assessing and monitoring populations.

Introduction

I conducted a population study of *Gentiana autumnalis* at eight locations in the New Jersey Pine Barrens. Each site had a different aged disturbance event (fire, mowing). This study was part of a larger, more comprehensive look at the influence of different disturbance regimes on pollinator visits, plant density, and reproductive success. *Gentiana* population structure at these sites is reported in Table 4.1. Although impacts to *G. autumnalis* from roadside mowing (during the growing season), herbicide applications, and fire suppression (Bien et al. 2009) occur, our study is the first to report the effect of caterpillars of the moths *Endothenia hebesana* and *C. chloroleucaria* herbivory on *G. autumnalis*.

Gentiana autumnalis L. (Pine Barren gentian) is a fall-flowering herbaceous perennial that occurs in early successional pine barren habitat from New Jersey to South Carolina (Kartesz 2014). Globally, *Gentiana autumnalis* is considered a regional endemic and is classified as G3 indicating that it is at moderate risk of extinction (NatureServe Explorer 2013). However, this species is now extirpated in Delaware and Maryland, considered imperiled (S2) in South Carolina, and critically imperiled (S1) in Virginia (NatureServe Explorer 2013). In New Jersey it is considered rare (S3) with less than 50 known occurrences (State of New Jersey 1998). Although it is not listed as endangered by the New Jersey Natural Heritage Program (New Jersey Natural Heritage Program 2010), it is afforded protection under the New Jersey Pinelands Comprehensive Management Plan (New Jersey Pinelands Commission 1980 and as amended in 2007)

because its distribution is restricted within the state to the Pine Barrens of southern New Jersey.

Gentiana autumnalis typically occurs in open-sunny, moist, early successional habitat (Halda 1996). In New Jersey, this species is a disturbance-dependent plant that responds positively after low intensity fires and reduction of canopy cover (Rebozo et al. in prep). However, certain types of disturbance such as roadside mowing during the growing season, herbicide applications, and fire suppression have negative impacts on the viability of *G. autumnalis* populations (Bien et al. 2009). In addition, little is known about the potential impacts to *G. autumnalis* as a result of herbivory by mammals or insects.

Gentiana autumnalis typically produces solitary flowers but may rarely produce 2 or 3 flowers per stem. Flowers in this species are protandrous, developing first as functionally staminate (anthers release pollen) then transitioning into a functionally pistillate stage (stigma being receptive). Fruits in this species are capsules that extend beyond the corolla as a result of ovary stalk elongation. Once the capsule dehisces, winged seeds are released and dispersed as the plant sways in the wind (Pringle 1967). Average plant seed set in sites that had populations that produced seeds ranged from 450 to 1090 seeds (Table 4.2). The ability of this plant to maximize seed set is a function of growing conditions, and insect visitation.

Endothenia hebesana (Walker), Verbena Bud moth, is a leaf roller moth in the family Tortricidae that is found throughout the eastern United States (Miller 1983).

Endothenia hebesana is native and common in southern New Jersey. Caterpillars of *E.*

hebesana are known to feed on the seeds of their host plants, which include at least 10 plant families (Brown et al. 2008). Of the known host plants for *Endothenia hebesana*, only one previous record in the genus *Gentiana* exists, *Gentiana saponaria* (Windus and Snow 1993). In addition to *Gentiana saponaria*, other rare plants that host *E. hebesana* larvae are *Frasera caroliniensis* and *Gentianopsis crinite* (Gentianaceae), *Physostegia virginiana* and *Teucrium canadense* (Lamiaceae), *Agalinis auriculata* (Orobanchaceae), and *Sarracenia purpurea* (Sarraceniaceae; Brown et al. 2008).

Chlorochlamys chloroleucaria (Guenée), the Black Berry Looper, is a moth in the Geometridae family. This widespread species is found from Canada in the north to southern United States (Florida to Texas) and it has at least two generations each year in the southern states (Wagner et al. 2001). This species is native and common in southern New Jersey. Caterpillars can be found from May to November and is often associated with the fruits of Asteraceae (the sunflower family) and the leaves of plants such as dogbane (*Apocynum cannabinum*, Apocynaceae) and *Comptonia peregrina* (Myricaceae; Wagner et al. 2001). This species may be unique among eastern Geometridae moths in its ability to consume fleshy fruits (Wagner 2005). Known host families for this species include Apocynaceae, Asteraceae, Caryophyllaceae, Myricaceae, Rhamnaceae and Rosaceae (Keeler and Balogh 2003). Ex situ experiments have demonstrated that larvae of this species prefer feeding on pollen and doing so may influence their development (Guillet et al. 1995).

White-tailed deer (*Odocoileus virginianus*) abundance in New Jersey exceeds 111,000 individuals with densities as high as 44 deer per km² in some areas (NJDEP

2011). Plant diversity in the state is negatively correlated with white-tailed deer density (NJDEP 2011). White-tailed deer are generalist herbivores and can feed on vegetative, floral and fruiting parts of many plants (Maslo and Wehman 2013). Dense deer populations may have strong, negative effects on plant communities in New Jersey (Maslo and Wehman 2013).

Materials and Methods

***Gentiana autumnalis* census**

I used eight study sites at different locations in the New Jersey Pine Barrens that were part of a larger study of the demography of *Gentiana autumnalis*. Study sites were situated along roadsides and open meadows that bordered pitch pine upland and lowland habitat and included late successional sites with closed canopies. I carried out a census of *Gentiana autumnalis* at each site biannually (May, September; 2011 – 2013) as part of a larger demography study (Rebozo et al. in prep). I established a macroplot encompassing the extent of *G. autumnalis* plants within each site in which 20 random 1x1m quadrats were used for sampling following the methodology of Elzinga et al. (1998). I made a complete life stage census within each quadrat (seedling, vegetative, or reproductive), and issued a numbered tag (Gempler's aluminum plant tags) to each plant to ensure that individual plants could be identified during revisits to measure survivorship and recruitment rates through successive years (Rebozo et al. in prep). I recorded flower phenology weekly from September through October at each site by noting the functional sex stage (staminate or pistillate) or overall reproductive stage (bud, transitional, seed set) of each flower. Flowers were transitional if the receding anthers no longer contained

pollen and the stigma was not yet receptive. I also recorded gentian seedlings as a percentage of the total gentian population for each site. Seedlings were counted as any first year plant limited to a basal rosette of leaves and too small to flower in the study year. I randomly collected one seed capsule from 7 - 19 plants at each site (contingent on population size and number of available seed capsules) post flowering to compare differences in seed-set among sites. On several occasions, I could not include data from collected seed capsules in the seed set calculation due to the presence of granivorous caterpillars. Average floral seed set and masses of total floral seed set were measured as part of a larger pollination and fecundity study. I opened collected seed capsules, and weighed their contents before counting all seeds in order to record seed set mass and number of seeds per capsule. I was careful not to harvest too many capsules due to the rare status of *G. autumnalis*, and only collected seed capsules from patches whose total seed set was robust enough to support a harvest. Seed set numbers and levels of insect infestation reported here are those from the 2012 field season.

I calculated the density of associated plants with flowering phenology overlapping with *Gentiana autumnalis* using the random quadrat method. Total flowering density was reported as the sum of gentian flower density at a site along with the density of all other flowers from any other flowering species encountered in the sampling quadrats. I calculated Simpson's diversity index for each macroplot using species of plants flowering during *G. autumnalis* peak flowering (Simpson 1949). Reported percentages of seedlings and flowering individuals are those plants at each life stage that survived to the fall census and that were not subjected to herbivory. Numbers of established seedlings and the percentage of the population they made up for the 2013 season are reported here and

was recorded using the same quadrat methodology described above.

***Gentiana autumnalis* damage estimation**

During gentian flowering I examined each plant for visible signs of larval feeding (damage, frass, and silk). I recorded the number of flowers with either noticeable caterpillar damage or the presence of *Chlorochlamys chloroleucaria* larvae for infestation calculations. For estimation of *Endothenia hebesana* damage I recorded number of larva or damaged seed capsules encountered among sampled gentian capsules. Total caterpillar damage was recorded as flowers of *G. autumnalis* that had damage consistent with either *E. hebesana* or *C. chloroleucaria* feeding in the sampling quadrats. *Endothenia hebsana* damage was identified by either encountering a feeding caterpillar within a seed capsule, or identifying an emptied seed capsule with silk and an exit hole chewed through the side of the fruit. *Chloroclamys chloroleucaria* damage was identified by encountering the caterpillar feeding within the flower, encountering a chrysalis within the flower, or identifying signs of feeding on the reproductive structures of the flower typically with frass and silk present. Flowers that experienced extensive *Chloroclamys chloroleucaria* damage were typically encountered as a closed corolla held in part by silk. Infestation estimates were based on plants with noticeable tissue damage. Because initial larval feeding can occur without producing noticeable damage, I expect that the reported infestation may be an underestimation of the actual levels of herbivory per population.

During the biannual censuses, I inspected *Gentiana autumnalis* plants within sampling quadrats for visible signs of deer herbivory. Deer herbivory during both the spring and fall censuses affected both flowers and vegetative structures. I recorded the

percentage of all censused plants at each study site that showed signs of deer herbivory.

Insect Identification

Two *Endothenia hebsana* larvae were reared to adulthood using *G. autumnalis* plant tissue as a food source. Adult moth genitalic preparations were confirmed as *Endothenia hebsana* by John W. Brown of the US Department of Agriculture Systematic Entomology Laboratory (Beltsville, Maryland).

After two unsuccessful attempts were made to rear caterpillars of *Chlorochlamys chloroleucaria* to maturity, I attempted DNA barcoding methodology for species identification (Hausmann 2011). I ground up a chrysalis and had its DNA extracted following the Qiagen DNeasy Animal Tissue Spin Column Protocol (Qiagen 2006). After extraction, all mitochondrial DNA was amplified by PCR at Rutgers University, using a standardized protocol developed in Karl Kjer's laboratory at Rutgers University and carried out by Julianne McLaughlin of Rutgers University. 10 pmols of each of the Folmer primers, or HCO 2198 and LCO 1490, and 22 μ L of PCR water were added to PureTaq™ Ready-To-Go™ PCR beads (GE Healthcare), to make a 25 μ L solution. The PCR program was as follows: initial heating at 94°C for three minutes, 30 cycles were conducted, each cycle with 95°C at 30s, 50°C at 45s, and 72°C at 2 min. At the end of the PCR the reaction was first kept at 4°C then at -20°C. Following the completed PCR, a gel electrophoresis test was conducted on the amplified material. After the gel electrophoresis test, the sample was sequenced by GENEWIZ (South Plainfield, New Jersey) using sequencing in both directions. The sequences files were then imported into a plasmid editing program ApE. The sequences were converted into FASTA format

using the program TextWrangler, and submitted for identification to BOLD database (Ratnasingham and Hebert 2007).

Analysis of variance compared differences in plant density and percentage of flowering plants among sites as well as deer herbivory in managed and unmanaged sites. Linear regression compared insect damage with density and diversity of plants. I used the statistical program R for all analyses (R Core Team 2014).

Results

Readings from the DNA barcoding were clear and required almost no editing. The sequence was 100% identical to one *Chlorochlamys chloroleucaria* found in Tennessee. Plant censuses revealed that patch density and the percentage of flowering plants were significantly different among *Gentiana autumnalis* sites ($p < 0.05$). The percentage of adult *G. autumnalis* plants flowering at the eight study sites ranged from 2.8% to 96.9% ($\bar{x} = 36.5\%$, ± 35.3). Total density of flowering plants (All plants flowering in either September or October in a site including *G. autumnalis*) at each site was 0.004 per m² to 13.5 flowering plants per m². Spring and fall patch surveys revealed that five of the eight study sites did not have any seedlings in 2013, and in the three sites in which seedlings occurred (Lower Cabin, FPP, Runway) they were just 1.8%, 2.1% and 3.2% of the total population (Table 4.1). Three functional flowering stages were identified in the flowering phenology surveys, staminate, pistillate and transitional (i.e., stage between staminate and pistillate in which flowers no longer have pollen present but whose stigma is not yet receptive). Flowering in *G. autumnalis* populations was staggered, with peak flowering at all sites occurring in the third week of September (Figure 4.1). Open

flowers in the study sites were mostly in the male phase from the onset of flowering to the second week of October when female phase flowers made up a larger proportion of the population (Figure 4.2).

Noticeable floral damage first occurred in phenology surveys in the third week of September, peaking at the end of the flowering season in the fourth week of October (Figure 4.2). I found individual *Endothenia hebesana* caterpillars in seed capsules from 3 of the 8 study sites (Table 4.3). In sites where *E. hebesana* larvae were present, damage ranged between 20%-33% ($\bar{x} = 10.8\%$, $\pm 14.1\%$; over all study sites including those without noticeable damage) of seed capsules (Table 4.3). *Endothenia hebesana* larvae fed on both seeds and the adjacent plant tissue.

Chlorochlamys chloroleucaria caterpillars were first observed in the second week of September with noticeable floral damage present in the third week of September. These caterpillars fed on the reproductive structures of *G. autumnalis* during both staminate and pistillate phases of the flowers. *Chlorochlamys chloroleucaria* damage was present in seven of the eight sites studied (Table 4.3). Floral damage at these sites ranged between 5.5%- 46.8% ($\bar{x} = 13.8\%$, $\pm 16.1\%$) of flowering plants.

Total caterpillar damage from *Endothenia hebesana* and *Chlorochlamys chloroleucaria* ranged from 0.0% to 80.1% ($\bar{x} = 23.7\%$, $\pm 27.4\%$) of all *G. autumnalis* flowers at a study sites. Linear regression revealed a positive relationship between total floral density and total caterpillar damage per site ($r^2 = 0.87$). The relationship between flowering plant diversity and total caterpillar damage by site was not significantly significant.

Deer herbivory in 2012 (Table 4.4) ranged none to 34.4% of plants damaged in

the sample quadrats ($\bar{x} = 9.41\%$, $\pm 10.21\%$). Other than site Stockton, no site had greater than 16% deer herbivory in 2012. Sites that were actively managed either through mowing or prescribed burning experienced less deer herbivory when compared to unmanaged sites ($F_{(2,318)} = 17.1$, $p < 0.001$).

Discussion

Low seedling establishment at the study sites may be attributed to low population abundance, low flowering percentages and insect herbivory both of flowers and seeds. The protandrous flowering exhibited by *G. autumnalis* requires staggered blooming times to ensure the presence of both male and female flowers at any given time. With a strict flowering phenology along with the often reduced population size and reduced flowering percentages recorded in my surveys, many patches of *G. autumnalis* were limited in their reproductive potential prior to insect visitation.

Many *Gentiana autumnalis* plants that flowered were damaged by both *Endothenia hebesana* and *Chlorochlamys chloroleucaria* larvae. Seven of the eight sites sampled had signs of *C. chloroleucaria* (Table 4.3), whereas three of the eight study sites sampled showed signs of *E. hebesana* damage (one site had no visible sign of any moth damage to flowering gentians; Table 4.3). Up to 100% seed predation by *Endothenia hebesana* has been reported in individual fruit of *Agalinis auriculata* (Mulvaney et al. 2006). Similarly, population infestation levels of 89% in *Agalinis auriculata* and 90% in *Gentiana saponaria* occur (Mulvaney et al. 2006, Windus and Snow 1993). While *E. hebesana* damage at my study sites was not as extreme as those reported by Mulvaney et al. (2006) and Windus and Snow (1993), total insect damage for one study site (561)

approached such figures (80.1% total moth damage site 561 Table 4.3). Keeler and Balogh (2003) report an average of 0.12 *C. chloroleucaria* larvae per individual plant in their sampling of 253 plants of *Eriogonum alatum*. Their reported level of *C. chloroleucaria* presence lies within the range reported in my study.

The strong positive relationship between total insect damage and total floral density indicated that insect damage was a greater problem in sites with prolific flowering as opposed to sites with few flowering plants. Sites with few flowering plants were limited in their capacity to maximize pollinator visitation and reproductive output making any additional insect damage further limiting to their total seed set. Sites with a greater density of flowering plants on the other hand would be expected to have increased pollinator visitation and seed set, yet have a greater percentage of their flowers targeted by *Endothenia hebsana* and *Chlorochlamys chloroleucaria*. Similarly, larger populations of *Gentiana cruciata* had a greater proportion of their fruit damaged by insects when compared to smaller populations with less flowering plants (Kéry et al. 2001). By damaging up to 80% of flowers in what would otherwise be viable and prolific populations, insect damage from these two common, native moth species has the potential to seriously impact reproductive output and viability in this already rare plant species.

Plants in the genus *Gentiana* contain many bitter compounds (Jensen and Schripsema 2002), and are typically considered unpalatable to mammals (Smit et al. 2006). In spite of the unpalatable compounds, deer herbivory of gentians was recorded in seven of the eight study sites used. Deer herbivory in this species differed from that of

the two insects in that it occurred throughout the growing season and affected all of the life stages (seedling, vegetative, reproductive). Deer herbivory was not significantly related to flowering density, gentian abundance or flowering diversity, but rather was most likely influenced by the density of deer at a given site. Two sites experienced deer herbivory on over 10% of their population, both of which were sites that had high levels of deer activity (deer sightings, scat and tracks). While most sites had negligible deer damage, site Stockton (34.3% deer herbivory; Table 4.4) had the potential for serious population damage and mortality due to deer grazing. The Stockton site was a wooded lot among residential development. The high levels of recorded deer herbivory at this site may be a result of high local deer density and limited grazing locations. Deer density has a strong influence on the magnitude of impact these herbivores can have on plant populations (Russell et al. 2001).

Floral damage from *E. hebesana* and *C. chloroleucaria* and deer herbivory had the potential to negatively impact most plants in a gentian population. Since many of these sites had few flowering plants, any damage to reproductive individuals severely limited the reproductive potential of the population. Insect herbivores negatively impact seed production, and both seedling emergence and establishment in populations of *Liatrus cylindracea* (Kelly and Dyer 2002). The frequency and extent of herbivory may contribute to the rarity of plant species locally (Schemske et al. 1994). Herbivory from insects and deer is a greater threat to *G. autumnalis* than previously expected. Additional long term studies will be needed to determine levels of damage over time and the resulting impact to the demography of this rare species. Future management practices for this species should take into account both negative and positive species interactions.

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Tables

Table 4.1: 2012 *Gentiana autumnalis* density, percentage of flowering plants, percentage of seedling plants (2013 data) and abundance estimate for eight study sites (Data were estimated from sampling quadrat counts)

Site	Density (plants/m ²)	Flowering %	Seedling %	Abundance
Calico	5.2	85.7	0	57
Atsion	0.3	27.3	0	16
Sightline	1.0	2.8	0	1,531
561	6.4	96.9	0	557
Franklin Parker Preserve	21.0	27.0	2.1	2,467
Lower Cabin	6.8	36.3	1.8	160
Runway	4.3	18.3	3.2	25,956
Stockton	2.1	13.0	0	55

Table 4.2: Average gentian fruit seed set and average seed capsule weight with standard deviations for study sites (DD = data deficient)

Site	Average Seed Set (# of Seeds per flower; 2012)	Average Seed Set Weight Per Flower (mg; 2012)
Calico	724.9 $\pm$ 254.5 n=10	23.5 $\pm$ 11.8
Atsion	DD	DD
Sightline	710.7 $\pm$ 220.5 n=9	39.0 $\pm$ 21.9
561	953.0 $\pm$ 411.2 n=10	36.4 $\pm$ 25.7
FPP	944.7 $\pm$ 401.4 n=12	55.5 $\pm$ 18.8
LC	878.7 $\pm$ 296.5 n=10	43.7 $\pm$ 19.3
Runway	1004.9 $\pm$ 226.5 n=28	45.3 $\pm$ 19.4
Stockton	499.7 $\pm$ 57.6 n=7	22.3 $\pm$ 4.1

Table 4.3: The percentage of sampled plants that exhibited damage consistent with *Endothenia hebesana* and *Chloroclamys chloroleukaria* herbivory and their sum for the 2012 study year (DD = Data Deficient)

Site	<i>C. chloroleucaria</i> damage	<i>E. hebesana</i> damage	Total Insect Damage
Calico	5.5%	0.0	5.5%
Atsion	25.0%	DD	25%
Sightline	0.0%	0.0%	0.0%
561	46.8%	33.3%	80.1%
FPP	20.5%	20.0%	40.5%
LC	2.95%	0.0	2.95%
Runway	7.07%	22.5%	29.57%
Stockton	2.5%	0.0	2.5%

Table 4.4: The percentage of *Gentiana autumnalis* plants in the sampling quadrats that exhibited signs of deer herbivory in 2012

Site	Deer Herbivory % 2012
Calico	0
Atsion	12.5
Sightline	16.0
561	4.1
FPP	2.1
Lower Cabin	2.3
Runway	4.9
Stockton	34.3
Sightline 2	5.4

Table 4.5: Density of heterospecific flowering plant species, total floral density (flowering gentian density + density of heterospecific flowering plants) and Simpson's index of diversity for flowering plants at each study site

Site	Peak Associative Flower Density per m ²	Total Floral Density per m ²	Simpson's Index of Diversity (Flower Diversity)
Calico	0	0.19	0
Atsion	0	0.004	0
Sightline	0.02	0.027	0.401
561	7.3	13.5	0.645
FPP	3	8.47	0.387
LC	0	1.025	0
Runway	1.5	2.075	0.619
Stockton	0.024	0.174	0.5

Figures

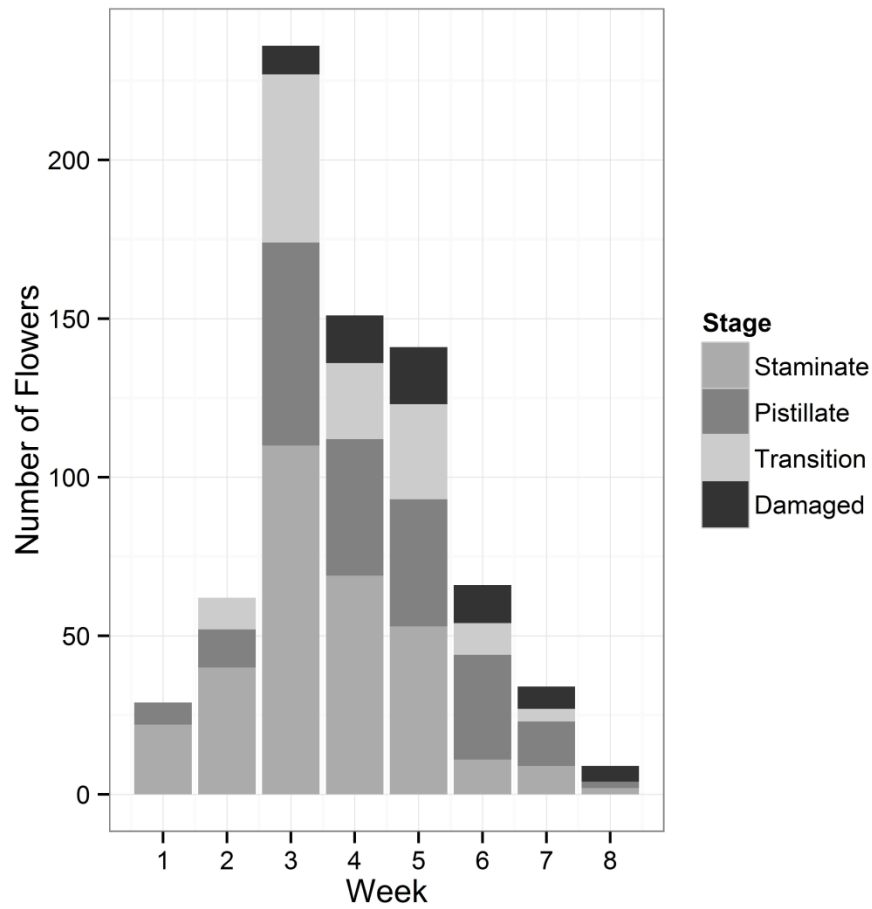


Figure 4.1: Number of flowering gentians encountered in sampling quadrats across all sites for the eight week flowering period (September-October 2012). Weekly flowering total is further divided by number of flowers encountered at each functional sex stage.

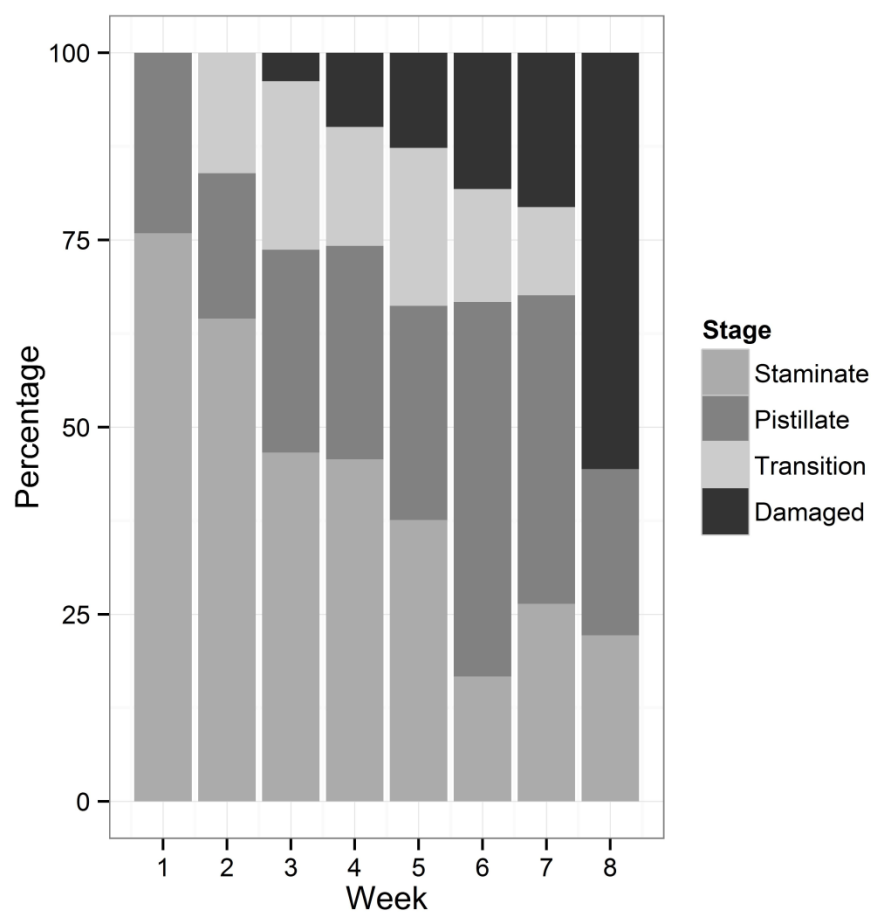


Figure 4.2: The percentage of flowering plants at each functional stage (including damaged flowers) encountered in the sampling quadrats of all sites for each week (September-October 2012)

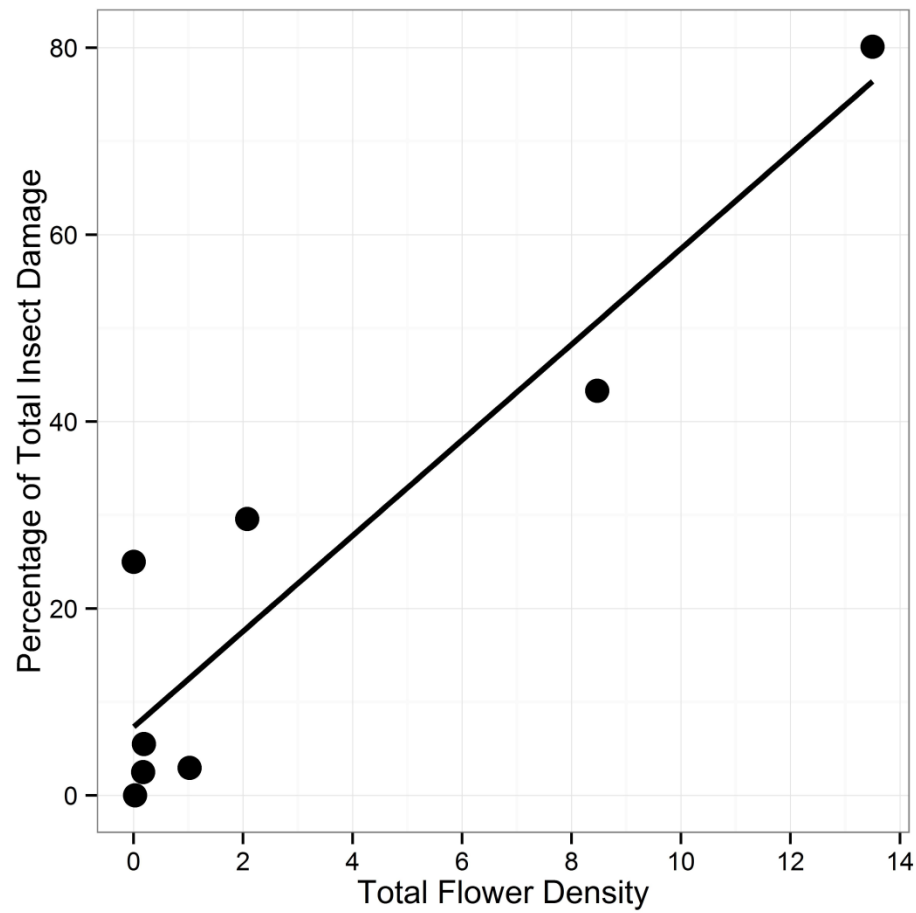


Figure 4.3: The percentage of gentian flowers exhibiting damage from either *Endothenia hebesana* or *Chlorochlamys chloroleucaria* as a function of total floral density (*Gentiana autumnalis* floral density + heterospecific floral density) for each site ($r^2 = 0.87$)

Chapter 5: Insect visitation and seed set of the rare Pine Barren gentian (*Gentiana autumnalis*) under varying management regimes.

Abstract

Understanding the pollination system of rare plants that occur in low numbers is of particular importance especially when developing a conservation management plan. *Gentiana autumnalis* L. (Pine Barren gentian) is a fall flowering perennial that is rare in New Jersey in part because of widespread fire suppression. *Gentiana autumnalis* has a protandrous and asynchronous flowering strategy that may impact insect visitation rate and reproductive success in sparse populations. My study (2011-2013) examined the influence of management practices (mowing and prescribed burning) on *G. autumnalis* insect visitation rate and demography. On average, populations of *Gentiana autumnalis* have few reproductive individuals ($\bar{x} = 26.7\%$, $\pm 20.45\%$ of individuals in populations) and seedlings ($\bar{x} = 2.4\%$, $\pm 2.6\%$ of individuals in populations). The protandrous and asynchronous nature of *Gentiana autumnalis*' flowering in which the number of flowering plants peaks three weeks after initial flowering makes the potential reproductive success of this species limited in small populations. This study recorded insect visitation from 2011-2013 in populations of *Gentiana autumnalis* that varied in management regime, patch size, and abundance of plants. Diptera accounted for 79.4% of all visits to gentian flowers with Syrphidae accounting for 45.5% of total insect visitation. *Gentiana autumnalis* flowers offer little in the way of nectar rewards to attract insects, averaging 0.21 μl at an average of 20.9% sugar concentration. Gentian peak flowering and peak nectar standing crop were an average of four weeks earlier than peak insect visitation in the populations used in this study. Insect visitation was negatively

correlated to the density of flowers of heterospecific plant species ($r = 0.48$) in the study sites. Seed set in this species averaged 779 seeds per fruit and was not found to be significantly influenced by floral insect visitation rate ($p > 0.05$). Hand pollination experiments typically resulted in lower seed set than open pollinated flower, but flowers hand pollinated with pollen from outside the population produced significantly greater ($p < 0.05$) fruit seed set than those hand pollinated with pollen from within the same population. Overall, floral constancy, and *Bombus* total site visitation rate explained most of the variation in *G. autumnalis* fruit seed set. Sites managed by either prescribed burns or mowing produced greater average fruit seed set than sites that were unmanaged, independent of population size ($p < 0.001$). Introducing disturbance to previously unmanaged sites resulted in a decrease in average floral visitation yet an increase in average fruit seed set suggesting a mate finding allee effect for female fitness in these populations. Populations of *Gentiana autumnalis* may be pollen and resource limited based on time spent in the pistillate phase, limited ability to flower in successive years and variation in seed set.

Introduction

Successful reproduction in plant populations is essential to their long term persistence (Kwak and Bekker 2006). The pollinator diversity, floral constancy and visitation rate experienced by a plant population is influenced by the surrounding flowering plant community (Bruckman and Campbell 2014). Small plant populations are at a higher risk due to a lower abundance of flowers (Fischer and Matthies 1997, Fischer and Matthies 1998, Ghazoul 2005, Dauber et al. 2010). The limited number of flowers in

small populations lack the floral rewards needed to maximize insect visitation (Oostermeijer et al. 2000). Floral advertisement at a given site and the resulting “pollination market” are a function of the density and diversity of flowering plants at a site and influence pollinator behavior (Cohen and Shmida 1993). Patchy environments contain resources which are limited in abundance and increase both the foraging time and energy expended for foragers to reach resources (MacArthur and Pianka 1966). Flowering plants often depend on many different pollinator species to meet their reproductive needs, and these pollinators in turn rely on a diversity of plant species for their nutritional needs (Jordano et al. 2006). When pollinator visitation is reduced or ineffective, seed set may become limited in sites. The composition and abundance of the co-flowering plant community, reduced population sizes, pollinator loss and habitat fragmentation are potential causes of pollen limitation (Knight et al. 2005). In 63% of plant populations studied, reproduction is pollen limited and seed production could be increased by increasing quantity and/or quality of pollination (Knight et al. 2005).

Populations of *Gentiana autumnalis* are typically of small size and low density (Rebozo et al. in prep). In addition, at many sites a small percentage of individuals flowers in any year. Small reproductive population size coupled with asynchronous protandry limits the number of available mates in the population (Spira and Pollack 1986). Populations of *G. autumnalis* have few seedlings and seed limitation may be one of the causal factors. Concurrent studies have examined the site conditions associated with gentian sites and the demography of populations in relation to land management practices (Rebozo et al. in prep). This study will expand on the demography of this

species under varying land management practices by focusing on insect visitation, relative insect abundance, standing nectar crop and seed set in these same study sites.

Management that promotes the growth of herbaceous plants by reducing canopy cover (e.g., burning, mechanical disturbance) is positively correlated with pollinator abundance (Campbell et al. 2007). Prescribed burning and roadside mowing were found to positively influence *G. autumnalis* plant density and the percentage of flowering plants in populations (Rebozo et al. in prep). Understanding how these management practices influence flower visitation and seed set when compared to later successional sites will improve our understanding of reproduction in this species. Considerations should be made to understand how a given management regime influences pollinators in addition to the rare plant population of interest. Traditionally, most plant conservation practices have been focused on preserving the proper habitat type for a given plant species and have not taken into account the complex structure of the pollination community (Kearns 2003). More recently, a focus on establishing pollinator habitat near farmed landscapes has drawn attention to the need to preserve plant pollinator interactions (Wratten et al. 2012).

Materials and Methods

Study Species

Gentiana autumnalis is a well known and easily recognizable species, yet little is known about the life history of this species. *Gentiana autumnalis* (Gentianeaceae, tribe Gentianeae) is a showy herbaceous fall-flowering perennial (Poster et al. 2015). This species is one of the last to flower each fall in the New Jersey Pine Barrens and may be

an important food source for late season and over wintering insect pollinators. In New Jersey, this disturbance-dependent species favors open- habitats with moist sandy soil conditions (Jenkins and Blades 1990; Harshberger 1916; Stone 1911). Solitary stems on mature plants can grow up to 15 to 55cm in height. Inflorescences typically consist of solitary flowers but can be occasionally two to three flowers (Poster et al. 2015).

Individual plants can have up to 15 stems from a single root stock. Flowers are characteristically protandrous, campanulate, pentamerous, and with plicae in between the corolla lobes. Nomenclature follows Poster et al. 2015.

Study Sites

I used six study sites at different locations in the New Jersey Pine Barrens that were part of a larger concurrent study of the demography of *G. autumnalis*. Study sites were situated along road edges and open meadows that bordered pitch pine upland and lowland habitat and included late successional sites with closed canopies (McCormick 1970). Sites listed as unmanaged at the start of this study (2011) had been unmanaged for a minimum of six years (Table 5.1). Disturbance type (mowed, burned), frequency of disturbance, and date of last disturbance (Table 5.1) were used to characterize each site. In March 2012, three previously unmanaged sites received their first disturbance (Sites RunM, RunB, and Sightline).

Flower Development and Flowering Phenology

I documented the developmental stage weekly at each site during the flowering seasons of 2011-2013 by recording the functional gender (staminate or pistillate) or overall reproductive stage (bud, transitional, seed set) of each flower in twenty randomly

placed 1m² quadrats within the macroplot. Flowers were considered transitional if the remnant anthers no longer contained pollen and the stigma was not yet receptive. Total flowering density is reported as the sum of gentian flower density at a site along with the density of all other inflorescences from any other flowering species encountered in the sampling quadrats during gentian flowering.

Insect Visitation

Insect visitation observations were phytocentric and involved either human observations or time lapse cameras to record insect visitation throughout the flowering period at all study sites. I used PlotWatchers™ cameras (Day 6 Outdoors, Columbus, Georgia) programmed to take pictures every three seconds and power off in the absence of daylight. These cameras were placed at one location for two consecutive days before being transported to a new site or moved to another individual plant. Human observations involved an observer stationed in one place within 2m of the focal flower and recorded the visiting insect, and the subsequent floral visit made by the same insect. An insect visit was recorded if there was contact with a stamen or stigma. After a visit was recorded the observer while remaining in place, recorded whether the visiting insect subsequently visited another gentian flower, the flower of another plant species, or left the line of sight. I considered subsequent visits to another gentian flower floral constancy and were used to later calculate the percentage of visits that result in gentian fidelity. Camera observations recorded those same data with the exception of gentian fidelity. Insects were collected with the use of an aerial net for later identification. I separated observations into two time periods: morning (Sunrise-12:00pm), and afternoon (12:01-

Sunset). I did not include single observations that extended into both observation periods when calculating visitation by time period but were retained for calculation of visitation by site and floral sex stage. The analysis of visitation by flower sex stage did not include transitional flowers, and only included video observations where flower sex was confirmed prior to and after footage was taken. Observations took place at wind speeds below 4 m/s and in the absence of precipitation to ensure observed insect foraging was not biased towards insects capable of better maneuvering in inclement weather conditions.

Insect Abundance

I estimated relative insect visitor abundance using timed Pollard walks that consisted of walking the macroplot boundaries for 10 minute sampling periods (Pollard 1977). These Pollard walks focused on insects from the four orders identified on flowers of *Gentiana autumnalis*; Diptera, Hymenoptera, Lepidoptera, and Coleoptera. I recorded all insects encountered from these four orders within the survey period. I used an area extending 3m in front of and at either side of the observer to identify insects (Pollard 1977). I collected any insects that were not identified by eye with the use of an aerial net (Pollard 1977). I surveyed sites on days where an observer was present for visitation observations. I calculated relative insect abundance for each week and used these data to identify any differences in relative species abundance among sites that influenced visitation data. I then compared the relative abundance of insects encountered during Pollard walks to the relative abundance of insects encountered at gentian flowers to

determine if certain insects visit gentian flowers disproportionally more or less than their relative abundance to other insects at the study site.

Standing Nectar Crop

I collected nectar volume and measured sugar concentrations in 2013 from sixty gentian flowers with the use of 5 μ l microcapillary pipettes (Kimble, Vineland, NJ) and a sugar refractometer (Bellingham and Stanley 0-50 Brix low volume refractometer; Corbet 2003). I performed collections randomly from flowers in all three stages (staminate, transitional, pistillate) in sampling quadrats that were not included in pollination observation or controlled pollination experiments. I conducted collections at a minimum of 24 hours after rainfall to prevent dilution of sugar concentrations and mis-estimation of nectar volume. I recorded sugar concentrations with an accuracy of 0.5%. I used these measurements coupled with flowering and phenology data to estimate site nectar standing crop by extrapolating the average nectar content by the number of flowers at each gender stage for each week and each study site. I measured nectar replenishment two ($n = 40$ flowers) and four hours ($n = 20$ flowers) after all available nectar was removed from gentian flowers. Flowers were isolated from insect visitation with the use of 180 fine mesh polyester mosquito netting (Coghlan's mosquito netting) between nectar extractions.

Breeding System

I investigated the breeding system of *Gentiana autumnalis* including autonomous self pollination, and self-compatibility. I tested for autonomous self-pollination and for self-compatibility, comparing fruit seed set following hand self- and cross pollinations. I

enclosed twelve flowering plants prior to anthesis in 180 fine mesh polyester mosquito netting (Coghlan's mosquito netting) anchored to the ground with landscaping staples, in order to exclude insect visitors and test for autonomous self-pollination. The plant remained isolated from potential pollinators until the corolla closed indicating initiation of fruit development. I then collected seed capsules prior to dehiscence to record seed set. To test for self compatibility, I emasculated fifteen flowers in the staminate phase and pollinated with self-pollen when the flower was in the pistillate stage. Between the emasculation and pollination, the flower was isolated from pollinators by mosquito netting, and the collected anthers were maintained at 4°C. Cross-pollinated flowers were treated identically. I cross pollinated 25 flowers with pollen donors from the same population and 25 flowers with pollen donors from an outside gentian population to test for an effect of outside population outcrossing on seed set. I deposited pollen on receptive stigmas by rubbing the entire pollen load of a donor anther onto the surface (Marshall and Ellstrand 1985, Sobrevila 1989). After pollination, I bagged plants to prevent further pollen deposition.

Seed Set

In October and November 2011-2013, I randomly collected one seed capsule from 7-19 plants with dehiscent capsules each year at each site (contingent on population size and number of available seed capsules) to compare differences in seed-set among sites. The number of seed capsules included in the seed set count were limited by the number of flowering plants available. On several occasions, collected seed capsules were not included in the count due to the presence of granivorous caterpillars. I measured the

length and width of seed capsules, removed the fruit wall, and weighed their dried contents (Mettler Toldeo PR502; 0.5 mg precision), and counted all seeds to estimate average seed set per flower. I returned collected seeds to their collected location and broadcasted randomly following the seed count.

Data Analysis

Observation results were used to calculate insect visitation rate for each patch. I calculated visitation rate as the number of visits per number of flowers per time spent observing to produce a rate of visitation per flower per minute (Dauber 2010). I then multiplied the visitation rate per minute by 60 to calculate visitation rate per hour. I standardized my observation data into 10 minute intervals for analysis. I calculated total site visitation by multiplying the average floral visitation rate by the number of flowers available in the population, and calculated total seed set by multiplying the average number of seeds per fruit by the number of flowers in the population. I used one way analysis of variance ($\alpha=0.05$) to test for significant differences among sites, while t-tests ($\alpha=0.05$) were used to compare variation between sex stages, times of day and pre/post disturbance changes. I used Tukey HSD for post-hoc analysis. I used a multiple regression analysis to test for correlation among insect visitation rate, insect visitor evenness, insect visitor constancy, and gentian floral density with average fruit seed set. I calculated the relative importance of gentian floral constancy, gentian floral density, and total site visitation by *Bombus*, Syrphidae, and other insects on average fruit seed set across all sites by using the metric lmg (percentage of total r^2 explained by each variable;

Lindeman et al. 1980, Grömping 2007). I used the R statistical program for all statistical analyses (R Core Team 2014).

Results

Flowering Phenology

Flowers of *Gentiana autumnalis* are nyctinastic, closing prior to the onset of dusk and under significant cloud cover. Flowering phenology surveys revealed that this species is protandrous, with flowers beginning in a functionally staminate phase and transitioning into a functionally pistillate phase. Three flowering stages were identified in the flowering phenology surveys, staminate, pistillate and transitional (stage between staminate and pistillate in which flowers no longer have pollen present but whose stigma is not yet receptive). Flowering in *G. autumnalis* was asynchronous at each patch, with peak flowering at all sites occurring in the third week of September. Patches were male dominated from the onset of flowering to the second week of October. Average weekly density of heterospecific flowers for September and October ranged from 0 flowers per m² to 2.8 flowers ($\bar{x} = 0.32 \pm 0.54$; Table 5.2). No flowers of associated plant species were recorded after the first week of October at any of the study sites. Flowers remained in the staminate phase for an average of 5.8 days (± 0.47 days), transitional stage for 3.4 days (± 0.51 days) and pistillate stage for 14.3 days (± 3.22 days).

Visitation Rate

Total insect observation time (sum of physical observations and recorded observations) per site from 2011-2013 ranged from 47 to 58 hours (Table 5.3). The

variation in observation time was a result of discarded observations due to weather, or technical issues. Insects that visited gentian flowers and made contact with either stamens or the stigma typically did so sternotribically. Insect visitation rate by site ranged from 0.79 to 5.6 visits per flower per hour ($\bar{x} = 2.19 \pm 2.47$ visits per hour). Across all study sites, visitation rate was significantly greater in the last three weeks of October (2.97 visits per hour ± 1.05) when compared to the first five weeks of gentian flowering (0.95 visits per hour ± 0.36 ; $T_{(1,1338)} = 3.28$ $p < 0.001$). No flowers from associative plant species were recorded in any of the sampling plots from the second week of October to the completion of gentian seed set in the three study years. Average insect visitation rate on gentian flowers was negatively correlated with the density of co-flowering plants available at the time of observation ($r = -0.48$; $p < 0.001$). Morning visitation (sunrise to 12:00pm; 2.69 per hour) was significantly greater than afternoon insect visitation (12:01pm to sunset; 1.95 per hour; $T_{(1,1360)} = 4.9$; $p < 0.001$). Staminate flowers had a greater average visitation rate (3.58 visits per hour) over all sites when compared to pistillate flowers (2.11 per hour; $T_{(1,623)} = 5.11$; $p < 0.001$). Insect visitation rate was significantly greater in sites managed by either mowing and prescription burn ($\bar{x} = 2.46$ visits per hour) when compared to unmanaged sites ($\bar{x} = 1.54$ visits per hour; $T_{(1,1498)} = 8.29$; $p < 0.001$). Similarly, total site visitation was greater in managed sites when compared to unmanaged sites ($T_{(1,1498)} = 11.43$; $p < 0.001$). *Bombus* showed the greatest gentian flower fidelity of the insect visitors ($\bar{x} = 87.5\%$; $F_{(5,87)} = 3.7$; $p < 0.05$). Floral constancy was positively related to average fruit set set ($r^2 = 0.52$).

Nectar Standing Crop

Nectar sugar concentrations did not differ significantly ($p > 0.05$) between staminate and pistillate flowers, with staminate flowers averaging 20% ($\pm 2.7\%$) sugar and pistillate flowers averaging 21.5% ($\pm 3.1\%$). No nectar replenishment was recorded within two hours of nectar depletion, and only 5% of sampled flowers produced extractable nectar in four hours. Nectar volume was significantly greater in the staminate stage ($\bar{x} = 0.28 \mu\text{l}$) than in either the pistillate ($\bar{x} = 0.17 \mu\text{l}$) or transitional ($\bar{x} = 0.17 \mu\text{l}$) stages ($F_{(2, 82)} = 9.1$; $p < 0.05$). Nectar standing crop was greatest between the third and fourth week of September for each site ($p < 0.05$; Table 5.4). These weeks coincide with peak flowering and a nearly even distribution of staminate and pistillate flowers.

Insect Diversity

Across all sites, flies from the family Syrphidae accounted for 45.5% of all insect visits (2011-2013). Other Diptera accounted for 33.9% of all insect visits to gentian flowers with the genus *Bombus* comprising 4.1% of visits, other Hymenoptera 3.7%, Lepidoptera 6.7% and Coleoptera 6.1% (2011-2013). The percentage of visits to gentian flowers by insects in the family Syrphidae ranged from 8% to 75.5% ($\bar{x} = 45.5\%$; $\pm 21.04\%$) and visits by insects in the genus *Bombus* ranged from 0% to 12.1% ($\bar{x} = 4.1\%$; $\pm 4.33\%$) among sites (2011-2013). Relative insect visitor abundance at gentian flowers was greater than site abundance for Syrphidae, *Bombus* and other diptera ($p > 0.05$), with relative site abundance of Lepidoptera ($F_{(1,47)} = 6.2$; $p < 0.05$) and Coleoptera significantly greater than that observed on gentian flowers ($F_{(1,47)} = 9.3$; $p < 0.05$). Insect visitor diversity did not differ significantly ($p > 0.05$) between staminate and pistillate stages.

Open Pollination Seed Set

Average fruit seed set across all sites was 779 (± 308) seeds per capsule (seed set by site is reported in Table 5.5). Average seed set per fruit was found to be significantly higher in managed sites ($\bar{x} = 898.9 \pm 286.9$) in all three study years (2011-2013) when compared to unmanaged sites ($\bar{x} = 546.6 \pm 281.2$; $T_{(1,202)} = 5.67$, $p < 0.001$). Individual fruits contained from 112 to 1371 seeds ($\bar{x} = 779 \pm 308.5$). Weight of seeds per fruit averaged 43.7 mg across all sites (± 21.0 mg; 56.1 μg per seed) and did not significantly differ between managed and unmanaged sites ($p > 0.05$). Both average number of seeds per fruit ($r^2 = 0.39$; $p < 0.001$) and average weight of seeds per fruit ($r^2 = 0.50$; $p < 0.001$) were positively related with the density of gentian flowers per site. The presence of seedlings at a site was found to be positively related to the number of flowering plants ($r^2 = 0.88$, $p < 0.001$) and total site seed set ($r^2 = 0.89$; $p < 0.001$) but not average seed set per flower ($r^2 = 0.14$; $p > 0.05$). Average fruit seed set per site was not strongly correlated with average floral visitation. An analysis of the relative importance of gentian floral constancy, gentian floral density, and total site visitation of *Bombus*, Syrphidae, and all other insect visitors on average fruit seed set across all sites showed that these variables explained 64.27% of the model. Floral constancy explained 49.04% of the cumulative r^2 , while gentian floral density accounted for 14.72%, *Bombus* total site visitation 18.83%, Syrphidae total site visitation 6.2%, and all other insect total site visitation 11.21% (Figure 6).

Breeding System

Flowers isolated from pollinators and left without pollen deposition did not fully develop fruit and aborted seed set. Hand pollination experiments overall produced lower levels of seed set than encountered in open pollinated plants. Flowers that had their own preserved pollen deposited on a receptive stigma averaged 208.73 seeds per capsule (± 117.19). Flowers with pollen introduced from within site pollen donors averaged 485.94 seeds per capsule (± 234.79), while flowers with pollen introduced from outside populations averaged 528.27 seeds per capsule (± 348.68 ; $F_{(2,62)} = 2.12$, $p < 0.05$). Seed set from all outcrossed flowers averaged 503.19 seeds per capsule (± 281.1). The manual deposition of pollen on a receptive stigma resulted in the closure of the corolla within the same day.

Introduced Disturbance

In March 2012, three previously unmanaged sites received their first disturbance (Sites RunM, RunB and Sightline). In each of these three sites (one mow and two burns), gentian density and flowering percentage increased significantly (RunM Density $F_{(1,38)} = 6.55$ $p < 0.05$, RunM Flowering $F_{(1,38)} = 9.1$ $p < 0.01$. RunB Density $F_{(1,38)} = 5.94$ $p < 0.05$, RunB Flowering $F_{(1,38)} = 56.49$ $p < 0.001$, Sightline Density $F_{(1,38)} = 4.14$ $p < .05$, Sightline Flowering $F_{(1,38)} = 65.91$ $p < 0.001$) in 2012 when compared to 2011 (Figures 4 & 5). The observed increases in density and flowering percentage occurred in the same year as the introduced disturbance. Patch size increased from 45.9m² to 1596m² following disturbance for burned site Sightline (Sites RunM and RunB were confined by sand roads). All sites except for RunB were able to maintain or increase post disturbance

density an in 2013, a full year removed from management (Figure 4). The percentage of flowering plants in the population at all three sites also significantly increased (+12.6% - +42.1%) (RunM flowering $F_{(1,38)} = 9.1$ $p < 0.01$, RunB flowering $F_{(1,38)} = 56.49$ $p < 0.001$, Sightline flowering $F_{(1,38)} = 65.91$ $p < 0.001$; Figure 5). The Sightline site which was also burned exhibited a similar increase in flowering percentage (+35.8%) to the RunwayB site. In 2013, a year removed from management, we did not record any statistically significant difference in site density compared to 2012 levels at any site where management was introduced ($p > 0.05$). In 2013 all three sites exhibited a reduction in the percentage of flowering individuals (RunM $F_{(1,38)} = 11.94$ $p < 0.001$, RunB $F_{(1,38)} = 23.98$ $p < 0.001$, Sightline $F_{(1,38)} = 146.4$ $p < 0.001$) and failed to maintain 2012 levels of flowering percentage with sites RunwayMow and Sightline dropping to flowering levels below those recorded in 2011 (Figure 5). Similar to the flowering response to introduced management, sites RunB, RunM each experienced a significant increase in seed set ($F_{(1,75)} = 7.4$, $p < 0.001$) per fruit following management and a significant reduction in seed set ($F_{(1,75)} = 5.3$, $p < 0.001$) to pre-disturbance levels one year removed from management. Visitation rate per flower for these two sites was significantly reduced following the management event (RunB $T_{(1, 253)} = 3.76$, $p < 0.01$; RunM $T_{(1, 213)} = 3.24$, $p < 0.01$), but total site visitation rate increased significantly following disturbance (RunB + 217%, $T_{(1,253)} = 9.3$, $p < 0.001$; RunM + 82%, $T_{(1,213)} = 2.21$, $p < 0.05$). We could not compare visitation rate and seed set pre and post disturbance for site Sightline as only one plant flowered in that site during the 2011 field season and was subjected to herbivory early in the flowering period.

Discussion

Many studies have examined the possibility that differences in reproductive traits may explain some cases of plant rarity (Godefroid et al. 2014; Lavergne et al. 2004). My study suggests that several factors related to reproduction could contribute to weak or limited population growth in *G. autumnalis*. The protandrous nature of *G. autumnalis* makes the potential reproductive success of this species limited in small populations (Fischer and Matthies 1997, Fischer and Matthies 1998, Dauber et al. 2010). A concurrent study focused on the demography of several populations of *Gentiana autumnalis* in New Jersey found that many sites had few plants and that on average across all sites 26.7% of plants flowered in a given year (Rebozo et al. in prep). With most plants producing solitary flowers, the number of potential mates at many of these sites is low. For these reasons, many populations of *G. autumnalis* in New Jersey may be pollen limited. The closure of the corolla within a day of ample pollen deposition as shown in the hand pollination experiments is in stark contrast to the 14 days pistillate flowers remained open on average in open pollination conditions, indicating that floral longevity may be influenced by pollen deposition (Henning and Weigend 2012). Due to the long-lived nature of these flowers, nyctinasty in this species may function as a way to protect reproductive investment until ample pollen deposition is achieved, as has been seen in another protandrous gentian species (Bynum and Smith 2001)..

The nectar standing crop in individual *Gentiana autumnalis* flowers was typically a fraction of a μl , but may not necessarily be indicative of the total sugar reward available to insect visitors (see Manetas and Petropoulou 2000). The greater insect visitation

recorded in morning hours compared to afternoon visits may be explained in part by nectar replenishment rates in gentian flowers that in most cases did not produce measurable nectar in four hours. A similar result was documented in a study of an endemic island population isolated from related plants on the mainland (Pérez-Bañón et al. 2003). Early visits may deplete nectar stores and limit the available rewards later in the day. Greater nectar production in the staminate phase of protandrous flowers has been documented in the literature and is believed to be to be a result of the need for additional visits to maximize male fecundity when compared to female stage fecundity (Varga et al. 2013, Carlson 2007). The greater visitation recorded in the staminate stage as opposed to the pistillate stage is likely attributed to the greater volumes of nectar in the male stage, and the availability of pollen as a reward. Total gentian visitation (functionally staminate and pistillate flowers) increased after flowering from heterospecific species commenced, but insects appeared to preferentially visit the staminate flowers as indicated by the greater visitation rate experienced by staminate flowers when compared to pistillate flowers. The greatest gentian nectar standing crop levels did not coincide with peak insect visitation, as this occurred only after competition from heterospecific species ceased. In the sites used for this study, the transition to a pistillate dominated population occurred on or near the second week of October which coincides with the point at which flowers of heterospecific plant species were no longer recorded in random sampling surveys. The reduction in rewards to potential pollinators at this time of year as a result of limited flowering from other plant species may have led to the increase in total gentian visitation. Anecdotally, insects appeared to preferentially visit the flowers of other plant species than those of *G. autumnalis*.

The breeding system of *Gentiana autumnalis* shares some similarities and several differences with that of other *Gentiana* species. Studies into the breeding system of *Gentiana pneumonanthe* and *Gentiana andrewsii* found that their flowers were protandrous, and self compatible, though opportunities for self pollination in the field were limited (Petanidou et al. 1995, Costelloe 1988). The often solitary flowers of *Gentiana autumnalis* limit the total seed set produced in a site when compared to other rare gentian species such as *Gentiana lutea* that produces up to 8,000 seeds per plant as a result of multiple fruits (Kerry et al. 2000). Like *G. autumnalis*, *G. pneumonanthe* flowers closed soon after hand pollination, but flower longevity under open pollination conditions was much shorter than *G. autumnalis*. Flowers of *G. pneumonanthe* remained in the staminate stage for an average of 3.9 days and in the pistillate stage for just 1.3 days on average (Petanidou et al. 1995). Although nectar volume in *G. pneumonanthe* (0.03 – 0.17 μ l) and sugar concentration in both *G. pneumonanthe* (9-39%) and *G. andrewsii* ($\bar{x}$ = 19.1%) were similar to those recorded in *G. autumnalis*, both *G. pneumonanthe* and *G. andrewsii* have a bumble bee dominated pollination system (Petanidou et al. 1995, Costelloe 1988). Melittophily has been recorded in other species of *Gentiana* and is believed to be the ancestral trait for Gentianaceae (Pojar 1974, Spira and Pollack 1986, Meszaros et al. 2002). Syrphid flies were recorded visiting flowers of *G. pneumonanthe* but were regarded as being poor pollen vectors and were even observed acting as pollen thieves, feeding on pollen deposited on stigmas (Petanidou et al. 1995). The effectiveness and efficiency of syrphid flies as pollinators of *Gentiana autumnalis* as compared to bumble bees is not known, but syrphid flies have been recently been recognized as important pollinators in many different environments (Kearns 1992; Larson

et al. 2001; Woodcock et al. 2014). While generally considered less efficient than bees, in some cases pollen loads on flies were equivalent to or greater than those of solitary bees (Kearns 1992). It is not unusual for protandrous flowers to be frequently visited by syrphid flies, particularly under isolated conditions (Lazaro et al. 2005; McKinney and Goodall 2010, Stpiczyńska et al. 2015).

Flowers of *G. autumnalis* follow the bee pollinated syndrome (Endress 1994), though there typically exists a poor correlation between floral morphology and pollination syndrome in Gentianaceae (Albert and Struwe 2002). Floral visitors and pollinators are primarily bees for other species of *Gentiana* that have been studied, including *G. pneumonanthe* (Petanidou et al. 1995; Spira et al. 1986). It is not clear why syrphid flies are the main visitors to *G. autumnalis* flowers, particularly since extensive records of flight periods for a wide range of bee species in New York indicate that many species would still be active into the fall (Giles and Ascher 2006). Possible explanations for the predominance of Diptera visits may be due to their foraging distances (Lysenkov 2014), energy requirements (Heinrich 1975), or their use of open flowers for basking (Heinrich and Pantle 1975).

While Diptera accounted for the greatest percentage of visits to gentian flowers, visits by *Bombus* explained more of the variation in average fruit seed set than visitation from all other recorded insects. This may be a result of *Bombus* displaying the greatest floral constancy of all recorded visitors, and gentian floral constancy having the strongest relationship with average fruit seed set of the measured variables. While further studies will be needed to elucidate the effectiveness and efficiency of *G. autumnalis* pollination

by *Bombus* and other insects, the constancy displayed by *Bombus* visitors alone may make them more effective at depositing conspecific pollen than Diptera.

When attempting to understand rare plant population dynamics, total site seed set in addition to individual plant fitness is of interest. I found lower floral visitation rates post introduced management, yet higher average fruit seed set at these sites. The increased number of flowering plants at sites with introduced disturbance coupled with presumably similar levels of pollinator abundance resulted in greater insect activity at the population level, but lower individual flower visitation rates. The resulting increase in average fruit seed set following the increase in number of available flowers suggests a mate finding Allee effect for female fitness in these populations (Gascoigne et al. 2009). This greater visitation had a strong relationship with the total seed set of the site. This is particularly important for understanding the potential benefits of managing for increased flowering at *G. autumnalis* sites.

In order to have a more accurate depiction of the role visitation rate plays on individual fruit seed set, all insect visits to the flowers in which seeds are counted must be recorded. Incorporating additional methods of manual pollen deposition in the future may be beneficial in determining maximum seed set in this species (Kearns and Inouye 1993). Young and Young outline several explanations as to why hand pollinated flowers may produce less seeds than outcrossed individuals, including damage to female tissue, single donor pollen may be less effective than multiple donors, and low pollen viability (Young and Young 1992). Outcrossed pollen produced significantly greater seed set than the selfing treatment. While hand crossed flowers typically produced fewer seeds on

average than open pollinated flowers, flowers hand crossed with pollen from outside of the population produced greater seed set on average than flowers hand crossed with pollen from within the population. Populations of *G. autumnalis*, particularly smaller populations, may suffer to a degree from inbreeding. Introducing pollen from distant populations would then be of higher quality and result in greater seed set. A population genetics study would be needed to understand the levels on genetic diversity present in these variable *G. autumnalis* populations.

Understanding how differences in population density influence seed set in rare species is important in their conservation (Metcalf and Kunin 2006). Introduced disturbance by both mowing and prescription burns had positive effects on the density of gentians, the percentage of flowering plants in a population and average fruit seed set. Insect visitation rate was typically higher in managed sites, though two sites where disturbance was introduced experienced a subsequent reduction in insect visitation per flower, but greater total site visitation. This is particularly important to note for future management of *G. autumnalis* populations as total site visitation resulted in greater total site seed set. A field and manipulative experiment found that insect visitation was higher for plants in light gaps than those shaded by canopy (Kilkenny and Galloway 2008). In a study of a flower typically found along forest edges, location influenced nectar production (higher in sunny locations) and visitor identity but not necessarily visitation rate; shaded flowers were visited almost exclusively by Dipterans and exposed flowers by bees (Nocentini et al. 2013). Furthermore, in a study of calcareous grasslands, habitat heterogeneity was an important predictor of hoverfly diversity, which may influence reproductive success for many plants that are pollinated by hoverflies in these habitats

(Meyer et al. 2009). While not all disturbance events result in a positive change in reproductive success (Trant et al. 2010), the correct timing and form of disturbance can be beneficial to early successional species such as *G. autumnalis*. In the case of *G. autumnalis*, managing for increased gentian floral density and reducing competition with other species, particularly later successional species, may improve the reproductive success of a population.

Traditionally, studies of rare plant demography have been concerned with the identification of sensitive life stages; there has been little attention given to mutualistic interactions in the management of rare plants (Schemske et al. 1994). In a disturbance-dependent environment such as the New Jersey Pine Barrens, high site stochasticity may lead to complex interactions that can be challenging to disentangle. In the case of rare plants, it is critical to elucidate the key mechanisms driving populations in order to develop effective management plans. Threats to *Gentiana autumnalis* populations persist in the form of fire suppression, mowing during flowering, herbivory, and granivory. The formulation of land management guidelines that incorporate knowledge of the flowering phenology and pollination system of *Gentiana autumnalis* may lead to increased demographic success for a species that is considered synonymous with the New Jersey Pine Barrens.

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Tables

Table 5.1: Site Management Regimes 2011-2013 (Unmanaged-Reference= No management in over 6 years, No Management= No Management in the study year)

Site	2011	2012	2013
Franklin Parker Preserve (FPP)	Mow	Mow	Mow
Runway	Unmanaged-Reference	Mow/Burn	No Management
Lower Cabin (LC)	Mow	Mow	Burn
Stockton (Stock)	Not in study	Unmanaged-Reference	Unmanaged-Reference
Sightline (Sight)	Unmanaged-Reference	Burn	No Management

Table 5.2: Average weekly density (1 m^2) of heterospecific and conspecific flowers at each site in September – October (2011-2013)

Site		FPP	RunM	RunB	LC	Sight	Stock
1st week of September	Co-flower density	1.5	2.1	1	0.1	1.75	0.3
	Gentian density	0.2	0	0	0.25	0	0
	Total density	1.7	2.1	1	0.35	1.75	0.3
2nd week of September	Co-flower density	0.19	1.25	0.5	0	1.5	0.3
	Gentian density	0.4	0.3	0.55	0.2	0	0
	Total density	0.59	1.55	1.05	0.2	1.5	0.3
3rd week of September	Co-flower density	0.15	0.4	0.35	0	1.65	0.15
	Gentian density	2.65	0.75	0.75	1.7	0.13	0.13
	Total density	2.8	1.15	1.1	1.7	1.78	0.28
4th week of September	Co-flower density	0.15	0.3	0.1	0	0.5	0.15
	Gentian density	2.55	0.25	1.5	0.73	0.22	0.18
	Total density	2.7	0.55	1.6	0.73	0.72	0.33
1st week of October	Co-flower density	0.05	0.5	0.2	0	0.1	0
	Gentian density	2.25	0.2	0.65	1.2	0.2	0.12
	Total density	2.3	0.7	0.85	1.2	0.3	0.12
2nd week of October	Co-flower density	0	0	0	0	0	0
	Gentian density	1.61	0.2	0.35	0.35	0.16	0.12
	Total density	1.61	0.2	0.35	0.35	0.16	0.12
3rd week of October	Co-flower density	0	0	0	0	0	0
	Gentian density	0.8	0.05	0.2	0.2	0.13	0.08
	Total density	0.8	0.05	0.2	0.2	0.13	0.08
4th week of October	Co-flower density	0	0	0	0	0	0
	Gentian density	0.2	0.05	0.15	0	0	0.05
	Total density	0.2	0.05	0.15	0	0	0.05

Table 5.3: Total insect visitation observation time by site

Site	Observation Time (Hours/Minutes)
Franklin Parker Preserve (FPP)	49 hrs 30 min
Stockton	58 hrs 20 min
Lower Cabin	51 hrs 10 min
Sightline	47 hrs 10 min
Runway (Mow)	53 hrs 40min
Runway (Burn)	49 hrs 10min

Table 5.4: Total site gentian nectar standing crop by week in 2013 (µl)

Week	FPP	RunM	RunB	LC	Sight	Stock
1	5.9	0	0	1.2	0	0
2	10.6	176.4	277.2	2.1	0	0
3	67.8	360.2	342.3	14.2	4.5	2.1
4	65.2	112.4	620.6	6.7	7.9	2.5
5	41.9	83.0	272.0	9.8	7.2	1.2
6	18.6	35.7	118.7	1.4	6.0	1.5
7	6.0	17.9	17.9	0.9	4.4	1.0
8	0	0	17.9	0	0	0.7

Table 5.5: Average seed set for *Gentiana autumnalis* per fruit by site (2011, 2012 and 2013; N.D.= No Data)

Site	Avg. Seed Set 2011	Avg. Seed Set 2012	Avg. Seed Set 2013
Stockton (Stock)	N.D.	499.7 $\pm$ 57.6 (n=7)	301.5 $\pm$ 118.5 (n=5)
Lower Cabin (LC)	889.4 $\pm$ 156.2 (n=10)	878.7 $\pm$ 296.5 (n=10)	864.0 $\pm$ 292.9 (n=9)
Runway	630.8 $\pm$ 280.3 (n=12)	N.D.	N.D.
Runway Mow (RunM)	N.D.	1044.3 $\pm$ 229.9 (n= 14)	617.6 $\pm$ 238.8 (n=12)
Runway Burn (RunB)	N.D.	978.6 $\pm$ 211.8 (n=14)	692.5 $\pm$ 379.3 (n=11)
Franklin Parker Preserve (FPP)	645.3 $\pm$ 197.8 (n=10)	944.7 $\pm$ 401.4 (n=12)	881.5 $\pm$ 248.2 (n=17)
Sightline (Sight)	N.D.	710.7 $\pm$ 220.5 (n=9)	354.3 $\pm$ 141.6 (n=4)

Figures

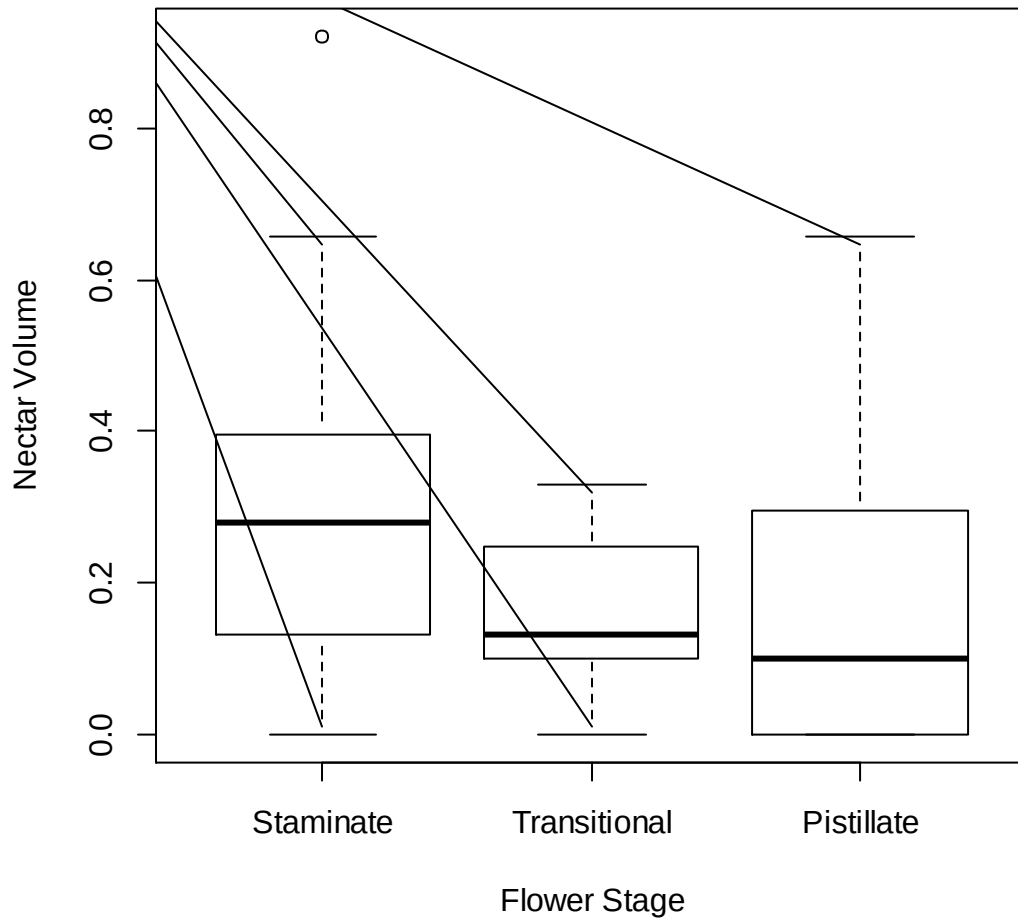


Figure 5.1: Average nectar volume (µl) for flowers in the functional staminate, transitional, and pistillate stages. (minimum, 25th percentile, mean, 75th percentile, maximum)

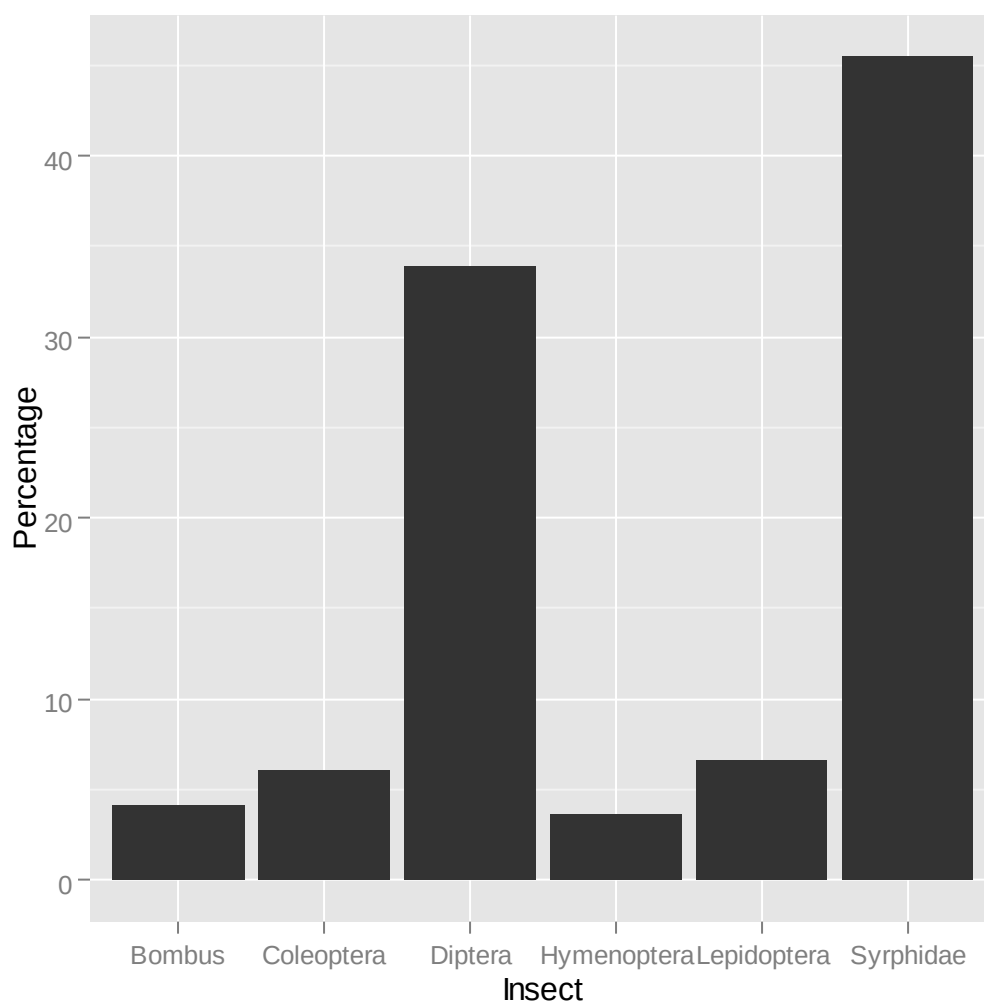


Figure 5.2: The percentage of insect visits made to gentian flowers by insect group (*Bombus*, Coleoptera, Diptera other than Syrphidae, Hymenoptera other than *Bombus*, Lepidoptera, and Syrphidae) across all sites from 2011-2013

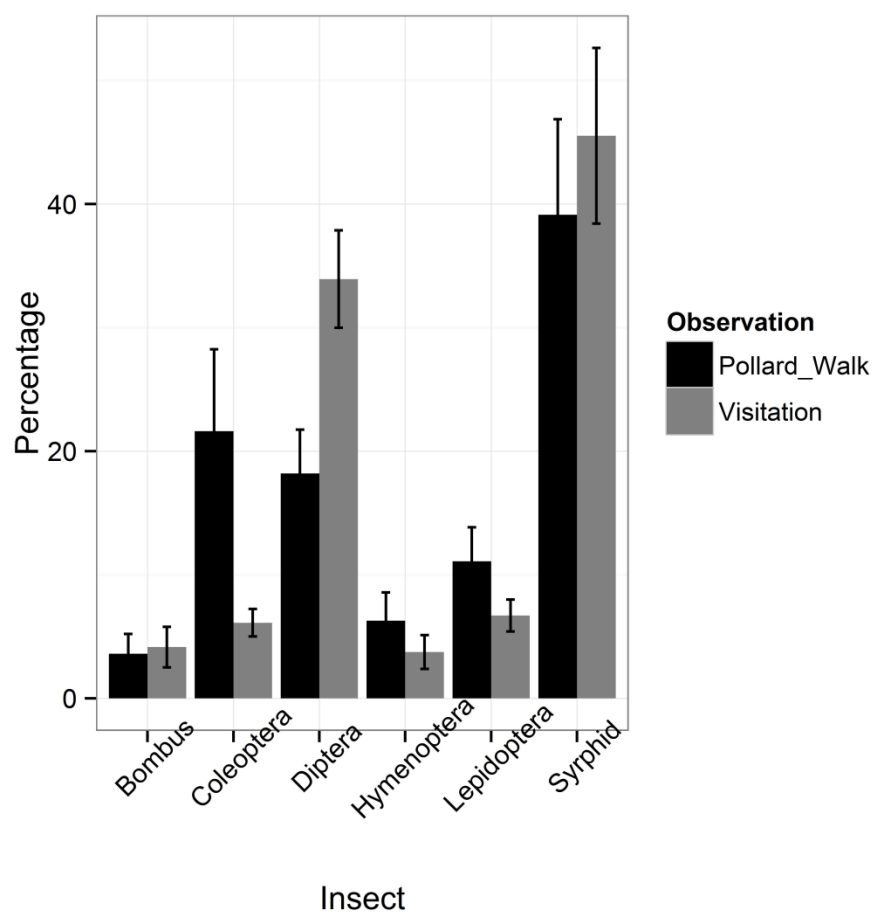


Figure 5.3: Relative abundance of insects encountered visiting gentian flowers and on macroplot Pollard walks across all sites from 2011-2013 (*Bombus*, Coleoptera, Diptera other than Syrphidae, Hymenoptera other than *Bombus*, Lepidoptera, and Syrphidae; S.E. Bars)

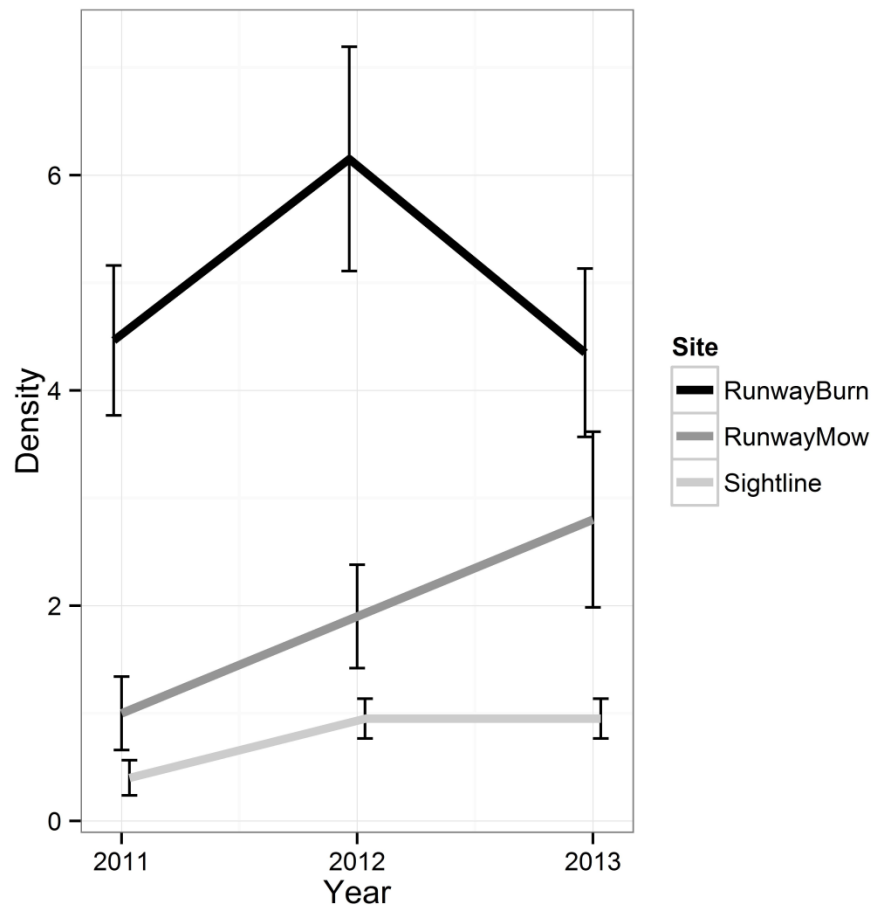


Figure 5.4: Gentian fall census density prior to disturbance (2011) and in two successive years (2012-2013; S.E. bars)

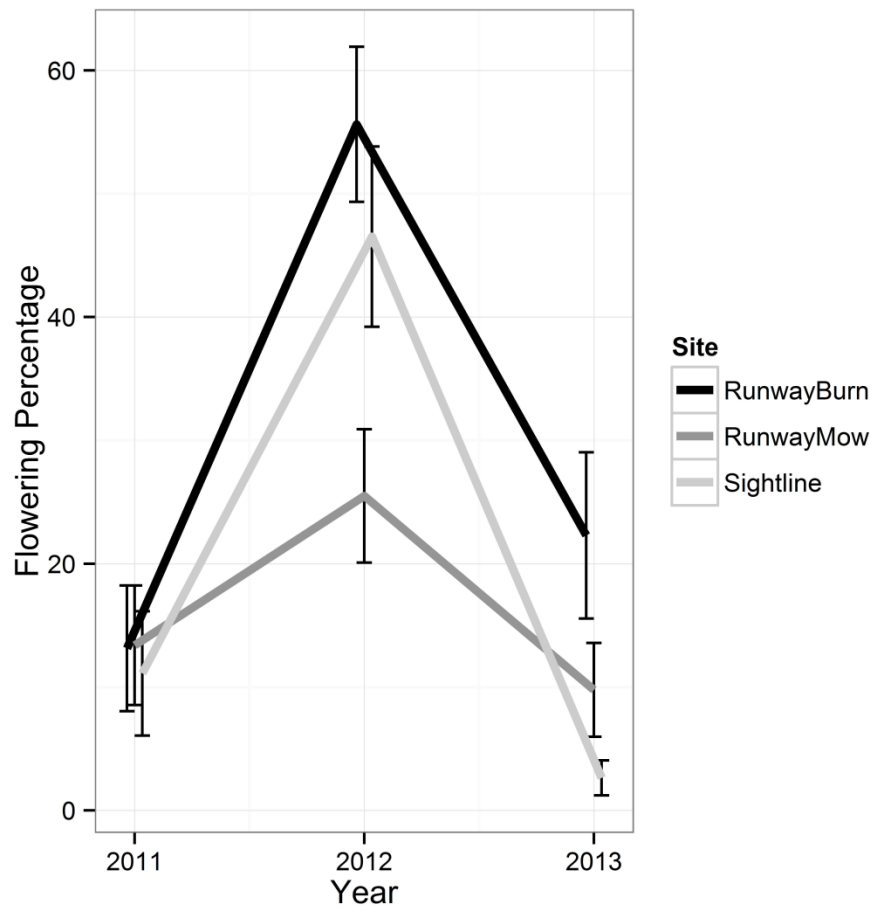


Figure 5.5: Fall census percentage of flowering plants of *Gentiana autumnalis* prior to disturbance (2011) and in two successive years (2012-2013; S.E. bars).

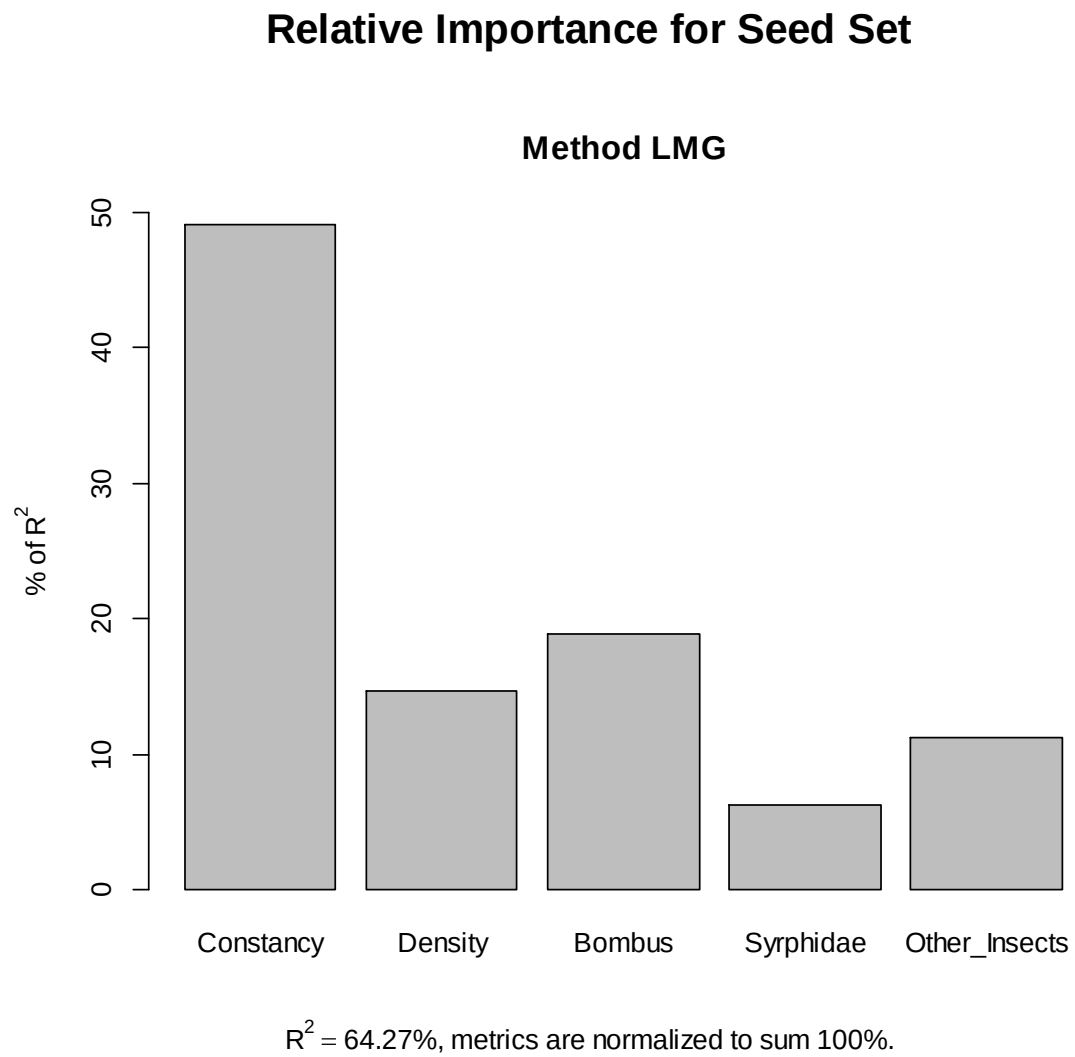


Figure 5.6: Relative importance of gentian visit constancy, gentian floral density, and total site visitation rate of *Bombus*, Syrphidae, and all other insects on average gentian fruit seed set across all sites.

Chapter 6: Thesis Summary and Future Directions

Introduction

Due to increased development, the Pine Barrens of New Jersey has undergone widespread wild-fire fire suppression for nearly a century (Little et al. 1948, Buell and Cantlon 1953, Forman 1998). The result of this fire suppression is a lack of early-successional sites and increased rarity of the plant species associated with natural disturbance. With proper management, it is possible to successfully reintroduce disturbance to long undisturbed sites for the promotion of plant diversity and disturbance-dependent species fitness (Billeter et al. 2007). The timing, frequency, and intensity of disturbance will be dependent on an understanding of the life history of the targeted species. Studies on the population dynamics of rare plants needed to inform these decisions are often lacking (Franks et al. 2009). Fire suppression is a main factor in the decline of *Gentiana autumnalis* outside of public lands (Nature Serve 2014).

I used a repeated measures design to census *Gentiana autumnalis* populations under varying management practices in the New Jersey Pine Barrens. Compiling these demographic data provides baseline population data for previously unstudied *G. autumnalis* sites. In addition to demography data, data on herbivory, mycorrhizal root colonization, fall soil nutrient pools, insect visitation, self-compatibility, flowering phenology, and seed set were collected for these study sites. These findings can contribute vital demographic, site condition, and mutualism data to inform management decisions that will enhance both *G. autumnalis* populations and early-successional sites.

Summary of Major Findings

The major findings of the previous chapters are summarized as follows:

1) Chapter 2: The effects of prescribed burns and mowing on the demography of *Gentiana autumnalis* in the New Jersey Pine Barrens.

Populations of *Gentiana autumnalis* were found in both early successional and late successional sites. Populations typically have few flowering individuals and seedlings. Sites managed through prescribed burning or mowing had significantly greater gentian densities, average fruit seed set, plant survivorship, and percentage of flowering plants than unmanaged sites. Previously unmanaged sites that had disturbance introduced during the study period increased in density of gentians, the percentage of flowering individuals, and average fruit seed set within the same year as the disturbance event. Average fruit seed set and the percentage of flowering individuals in a population returned to pre-disturbance levels the year after a disturbance event, indicating that repeated management may be necessary in some sites. *Gentiana autumnalis* was found to be able to remain dormant and produce no aboveground biomass for at least one year. Sensitivity analyses focused on transitions between *G. autumnalis* life stages found that transitions of vegetative plants to flowering plants (Disturbed $\bar{x} = 17.7\%$, Undisturbed $\bar{x} = 1.02\%$) had the greatest positive impact on growth rate of all life stage transitions. Population growth rate was also greater in managed sites than unmanaged sites ($\bar{x} = 1.24 \pm 0.47$, undisturbed: $\bar{x} = 1.01 \pm 0.3$, mow: $\bar{x} = 1.22 \pm 0.39$, burn: $\bar{x} = 1.81 \pm 0.58$).

2) Chapter 3: The effect of management practices (mowing and prescribed burning) on soil nutrients and mycorrhizal colonization of *Gentiana autumnalis*.

Sites dominated by woody shrub cover had a lower percentage of flowering plants than those sites with few or no woody shrubs. Arbuscular mycorrhizal fungal colonization was present in unusually high levels in the roots of *Gentiana autumnalis* with over 80% of root observations identifying the presence of hyphae. Actively managed sites had greater mycorrhizal colonization percentages and canopy openness than unmanaged sites. Mycorrhizal colonization, canopy openness, and soil pH were all positively correlated with gentian density and flowering percentage and may serve as indicators of productive gentian populations. Soil nutrients were overall weakly correlated to gentian demographic variables and may not be strong indicators of gentian population performance.

3) Chapter 4: Negative effects of herbivory by *Endothenia hebesana* (Verbena bud moth), *Chlorochlamys chloroleucaria* (Black berry looper), and *Odocoileus virginianus* (White-tailed deer) on the rare *Gentiana autumnalis* (Pine Barren gentian) in New Jersey.

The flowers of *Gentiana autumnalis* are protandrous and their emergence in a population is staggered. Peak flowering occurs in the third week of September, when noticeable insect damage was first recorded. Flowering populations of *G. autumnalis* experience herbivory and granivory from two temporally separated

moth species. Herbivory from *Chlorochlamys chloroleucaria* caterpillars occurs during flowering and targets the reproductive structures of the flower. Granivory from *Endothenia hebesana* caterpillars occurs within the fruit post flowering. We recorded up to 80% of flowers in a population of *G. autumnalis* damaged by either of these two moth species. The percentage of flowers damaged by these two moth species was positively related to total floral density with a site ($r^2 = 0.87$). Deer herbivory occurred throughout the growing season and targeted both flowers and vegetative plant material. We recorded damage due to deer herbivory for up to 34% of gentian plants in a study site. High rates of herbivory may be linked to high local deer density and limited foraging area.

4) Chapter 5: Insect visitation and seed set of the rare Pine Barren gentian (*Gentiana autumnalis*) under varying disturbance regimes.

Hand pollination experiments overall produced lower levels of seed set than encountered in open pollinated plants. Flowers that had their own preserved pollen deposited on a receptive stigma averaged 208.73 seeds per capsule (± 117.19). Flowers with pollen introduced from within site pollen donors averaged 485.94 seeds per capsule (± 234.79), while flowers with pollen introduced from outside populations averaged 528.27 seeds per capsule (± 348.68 ; $F_{(2,62)} = 2.12$, $p < 0.05$). Insect visitation to the flowers of *Gentiana autumnalis* was dominated by Diptera, which in total accounted for 79.4% of all visits. Insects in the family Syrphidae accounted for 45.5% of all insect visits to gentian flowers. Insect visitation on *G. autumnalis* flowers was negatively correlated with the density of

heterospecific flowers. Staminate phase flowers produced more nectar and experienced greater insect visitation than pistillate flowers. Average floral seed set was greatest in managed sites and increased significantly following disturbance events. An analysis of the relative importance of gentian floral constancy, gentian floral density, and total site visitation of *Bombus*, Syrphidae, and all other insect visitors on average fruit seed set across all sites showed that these variables explained 64.27% of the model. Floral constancy explained 49.04% of the cumulative r^2 , while gentian floral density accounted for 14.72%, *Bombus* total site visitation 18.83%, Syrphidae total site visitation 6.2%, and all other insect total site visitation 11.21%. Managed sites that experienced an increase in the number of flowering plants received lower average floral visitation rates when compared to pre-management levels, yet averaged significantly greater fruit seed set. The increased average fruit seed set following an increase in the number of potential mates may suggest a mate finding allee effect for female fitness in *Gentiana autumnalis* populations.

Conservation Implications

These data on several populations of *Gentiana autumnalis* in New Jersey represent the first reported life history information collected for this species in the state. We provide baseline data on the demography of select populations as well and identify areas of focus for future conservation management planning. This study highlights the need to incorporate a holistic approach when planning for the management of this complex perennial.

Most sites included in this study had few gentian plants, and were primarily composed of non-reproductive individuals. Transition matrices and associated sensitivity analyses identified the transition of vegetative plants to flowering individuals as the key stage influencing population growth rate across sites. A focus on increasing the number of seedlings and flowering plants, and enhancing the transition of vegetative individuals to reproductive individuals, will have positive impacts on the viability of these populations

Management practices should focus on maintaining an open canopy and the reduction of woody shrub competition through prescription burning when feasible. Sites that had a greater percentage of open canopy and limited woody shrub competitions had greater gentian densities, percentage of flowering individuals and seed set. Introducing a management regime to known *G. autumnalis* sites that have been left unmanaged has the potential to benefit populations within the same year. Repeated disturbance in such sites is advisable in order to remove woody plant species. While mechanical disturbance is an effective measure in maintaining early-successional sites, every effort should be made to use prescription burns as a management practice. The nutrients added to the soil after burning may improve the ability to flower in *G. autumnalis*, which often appears to lack the resources needed to flower in successive years. In late-successional sites, it may be beneficial to first remove the established woody plant community before burning in order to limit the resprouting potential of these species.

Management regimes must also be planned around the flowering phenology and growing season of this species. If management is not timed correctly, the disturbance

event may negatively affect the target population. One study site (561) was mowed annually during flowering, effectively preventing seed set in a population exhibiting prolific flowering. Conservation groups had already urged county officials to postpone mowing until October, but a lack of reliable data and understanding of the flowering phenology of *G. autumnalis* led to negative impacts on seed set and potential seedling establishment. Similarly, disturbance during the growing season prior to flowering (April through August) may negatively impact individual plants and limit flowering.

Planning should also take into account both positive and negative interactions with other organisms. We now know that *G. autumnalis* supports high levels of mycorrhizal colonization on its roots, and is visited by insects from four different orders. Supporting the abundance and diversity of these mutualists by managing for a diverse plant community may positively influence the demography of *G. autumnalis*. Lastly, understanding levels of herbivory and grainivory in *G. autumnalis* populations over time will be important in understanding biotic threats to this species. Changes over time in the abundance of these herbivores may influence gentian site productivity.

Future Research Directions

This study provides baseline data on the life history and demography of select populations of *Gentiana autumnalis*. While conducting research on the site conditions, demography, and reproductive system of this species, additional questions arose that were outside of the scope of this study. Answering these questions will improve our understanding of the life history of this species, what makes it rare, and how to better manage for its conservation.

- 1) What is the lifespan of individual *G. autumnalis* plants? The three year duration of this study was not long enough to identify the longevity of these plants. Long term monitoring will be needed to answer this question.
- 2) How long can *G. autumnalis* remain dormant? This study identified the activation of dormant plants after disturbance, but it is not currently known how long these plants can remain in that state. A long term, manipulative study will be needed to address this question.
- 3) How does *G. autumnalis* respond to repeated prescribed burn disturbance? This study found that *G. autumnalis* responds positively within the same year of a prescribed burn, but reverts back to pre-disturbance levels of flowering and seed set a year removed from disturbance. A study designed to prescribe burn populations of *G. autumnalis* with varying fire intervals will be needed to determine if the increased disturbance frequency maintains post-disturbance levels of flowering and seed set.
- 4) How variable are soil nutrients in *G. autumnalis* sites? Temporal variation, and variation caused by management practices may influence the concentrations of available nutrients in the soil. A study analyzing the concentration of soil nutrients throughout the year and pre- and post-disturbance will be needed to address this question.
- 5) What level of mycorrhizal fungi diversity is present in roots of *G. autumnalis* as well as in the macroplot in which they are sampled? Fungal diversity can influence the benefits of mutualism accrued to the host plant, and the fungal diversity in the surrounding site may be indicative of what species are

available for inoculation. A study using molecular techniques to assess the soil for fungal diversity will be needed to address this question.

- 6) Are the reported levels of moth infestation typical of *G. autumnalis* sites?

Infestation levels over a long sampling period will better elucidate how these moths are impacting *G. autumnalis* populations and if these moths undergo cyclic variation in their abundance. A long term study identifying floral and fruit damage from *Endothenia hebesana* and *Chloroclamys choloroleukaria* will be needed to answer this question.

- 7) What is the most effective pollinator of *Gentiana autumnalis*? This study identified insect visitors and the rate in which they visited *G. autumnalis* flowers, but understanding the efficiency and effectiveness of specific insects will provide a better understanding as to how insect visitation may influence seed set. A study measuring pollen removal and receipt by individual insects, and observing the visitation and seed set of individual flowers will be needed to answer this question.

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Appendix A

Global and state element ranks as defined by NatureServe.

Global Element Ranks

G1 Critically imperiled globally because of extreme rarity (5 or fewer occurrences or very few remaining individuals or acres) or because of some factor(s) making it especially vulnerable to extinction.

G2 Imperiled globally because of rarity (6 to 20 occurrences or few remaining individuals or acres) or because of some factor(s) making it very vulnerable to extinction throughout its range.

G3 Either very rare and local throughout its range or found locally (even abundantly at some of its locations) in a restricted range (e.g., a single western state, a physiographic region in the East) or because of other factors making it vulnerable to extinction throughout its range; with the number of occurrences in the range of 21 to 100.

G4 Apparently secure globally; although it may be quite rare in parts of its range, especially at the periphery.

G5 Demonstrably secure globally; although it may be quite rare in parts of its range, especially at the periphery.

State Element Ranks

S1 Critically imperiled in New Jersey because of extreme rarity (5 or fewer occurrences or very few remaining individuals or acres). Elements so ranked are often restricted to very specialized conditions or habitats and/or restricted to an extremely small geographical area of the state. Also included are elements which were formerly more abundant, but because of habitat destruction or some other critical factor of its biology, they have been demonstrably reduced in abundance. In essence, these are elements for which, even with intensive searching, sizable additional occurrences are unlikely to be discovered.

S2 Imperiled in New Jersey because of rarity (6 to 20 occurrences). Historically many of these elements may have been more frequent but are now known from very few extant occurrences, primarily because of habitat destruction. Diligent searching may yield additional occurrences.

S3 Rare in state with 21 to 50 occurrences. Includes elements which are widely distributed in the state but with small populations/acreage or elements with restricted distribution, but locally abundant. Not yet imperiled in state but may soon be if current trends continue. Searching often yields additional occurrences.

S4 Apparently secure in state, with many occurrences.

S5 Demonstrably secure in state and essentially ineradicable under present conditions.

Appendix B (continued)

Species	Run	FPP	LC	Sight	Sight2	NPR	Calico	Atsion	Stock	561
Sub-shrubs										
<i>Rubus hispidus</i>	X	X								
<i>Vaccinium angustifolium</i>	X	X	X	X	X					
<i>Vaccinium macrocarpon</i>		X	X			X		X		
<i>Vaccinium pallidum</i>	X			X	X			X		
Vines										
<i>Smilax glauca</i>	X							X	X	X
Forbs/Herbs/Graminoids										
<i>Amphicarpum amphocarpum</i>	X	X	X	X	X			X	X	X
<i>Andropogon virginicus</i>	X	X	X	X	X		X	X	X	
<i>Arenaria caroliniana</i>	X									
<i>Aristida dichotoma</i>	X						X	X		
<i>Aristida longispica</i>	X									
<i>Aristida purpurascens</i>	X									
<i>Bartonia virginica</i>	X									
<i>Calamovilfa brevipilis</i>	X									
<i>Carex pensylvanica</i>	X	X					X			
<i>Carex tosa</i>	X									
<i>Cirsium arvense</i>										X
<i>Cladium mariscoides</i>	X									
<i>Cyperus dentatus</i>	X									
<i>Cyperus retrorsus</i>	X									
<i>Dichanthelium leucothrix</i>	X	X				X			X	
<i>Dichanthelium sabulorum</i>	X									
<i>Digitaria ischaemum</i>	X	X	X					X		X
<i>Digitaria sanguinalis</i>	X							X		
<i>Diodia teres</i>	X	X	X			X	X			
<i>Drosera rotundifolia</i>									X	
<i>Euphorbia ipecacuanhae</i>	X									
<i>Eurybia compacta</i>	X	X								
<i>Eurybia spectabilis</i>	X									
<i>Euthamia tenuifolia</i>	X	X								
<i>Gamochaeta purpurea</i>	X									
<i>Helianthemum canadense</i>	X									
<i>Hypericum canadense</i>	X									
<i>Hypericum gentianoides</i>	X								X	
<i>Ionactis linariifolius</i>	X	X								

Appendix B (continued)

[illegible]

Curriculum Vita: Ryan R. Rebozo

Education:

Ph.D. Environmental Science, March 2015: Drexel University, Philadelphia, Pennsylvania. *Dissertation:* The Influence of Land Management on the Demography of the Pine Barren gentian (*Gentiana autumnalis*) in New Jersey.

B.S. Ecology and Natural Resources, Minor in Entomology and Economic Zoology, May 2010: Rutgers University, New Brunswick, New Jersey.

Research Experience:

2010-2014, Research Assistant, Drexel University: *Gentiana autumnalis* demography, rare plant survey, *Pituophis melanoleucus* spatial ecology, and *Rhynchospora knieskernii* monitoring.

2008-2010, Research Assistant, Rutgers University: Small mammal predator avoidance, small mammal spatial analysis.

Teaching Experience:

2014, Adjunct Faculty, Philadelphia University: Current topics in biology: Botany

2012-2014, Teaching Assistant, Drexel University: GIS and Environmental Modeling, Biology: Physiology and Ecology, Field Botany of the New Jersey Pine Barrens, Introduction to Field Methods in Earth Science, and Entomology

Publications in Preparation:

1. Rebozo, R., Struwe, L., and Bien, W.(in prep) *Gentiana autumnalis* (Pine Barren gentian) and *Endothenia hebesana* (Verbena Bud Moth): A rare plant species threatened by a common moth.
2. Rebozo, R., Struwe, L., and Bien, W.(in prep) The effects of land management practices on the demography of the rare Pine Barren gentian (*Gentiana autumnalis*) in New Jersey.
3. Rebozo, R., Livshultz, T., and Struwe, L. (in prep) Insect visitation and seed set of the Pine Barren gentian (*Gentiana autumnalis*) under varying disturbance regimes.
4. Rebozo, R., Dighton, J., and Bien, W. (in prep) Arbuscular mycorrhizal fungal colonization and its role in the demography of the rare Pine Barren gentian (*Gentiana autumnalis*).

Awards:

1. Bayard Long Award for Botanical Research, Philadelphia Botanical Club
2. Louis Stokes Alliance for Minority Participation Bridge to the Doctorate Fellowship
3. Robert Hanna Soil and Water Conservation Award

