

EXPERIMENTAL REINTRODUCTION OF THE ENDANGERED *ECHINACEA*
LAEVIGATA: COMPARISON OF PLANTING METHODS AND EFFECTS OF LIGHT
INTENSITY ON BIOMASS AND PHOTOSYNTHESIS

by

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ABSTRACT

In an experimental reintroduction of the endangered species *Echinacea laevigata*, we tested several planting methods to determine the optimal method for establishing populations in the wild. High survival rates and comparable growth among plants from all planting methods suggests that reintroduction is a promising conservation strategy for the species and that there are various options for introducing populations.

Plants performed well regardless of age at the time of planting, spacing, and presence or absence of soil amendment. Therefore, reintroduction practitioners may weigh logistical costs and benefits when choosing reintroduction methods for *E. laevigata*. Based on theoretical predictions and our findings, we suggest that an ideal method for establishing populations is to plant adult plants (older than one year) in the spring, without soil amendment. This recommendation is preliminary and contingent on the future survival and reproductive success of reintroduced individuals.

In order to more effectively and efficiently manage *E. laevigata* populations, it is important to understand the role of the light environment in the species' decline. While it

is generally agreed that the species decline is in part due to the lack of fire-maintained, early successional habitat, the extent to which light limits population persistence has not been quantified. We compare the effects of high, medium and low developmental light levels on photosynthetic performance as described by light curves, and on biomass allocation. We found no significant difference in photosynthetic response among plants grown at different light levels. However, plants grown under low light (18 percent of full sun) had significantly lower root and flowering stem dry weight, and number of flower heads than plants grown in full sun or moderate shade (43 percent of full sun). Therefore, in order to optimize biomass and flower production, important factors in population persistence, light levels should be maintained above 43 percent of full sun for *E. laevigata* populations.

INDEX WORDS: *Echinacea laevigata*, Plant reintroduction, Endangered plant species, Environmental management, Light adaptation

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iv
CHAPTER	
1 INTRODUCTION.....	1
2 LITERATURE REVIEW.....	3
3 EXPERIMENTAL COMPARISON OF REINTRODUCTION METHODS FOR THE ENDANGERED <i>ECHINACEA</i> <i>LAEVIGATA</i>	26
4 RESPONSES OF PHOTOSYNTHESIS AND BIOMASS PRODUCITON TO DEVELOPMENTAL LIGHT LEVEL IN <i>ECHINACEA LAEVIGATA</i> , A FEDERALLY LISTED ENDANGERED SPECIES.....	41
5 CONCLUSION.....	57
APPENDIX A.....	59

CHAPTER 1

INTRODUCTION

Echinacea laevigata (Boyton & Beadle) Blake, Asteraceae, or smooth coneflower, is a federally endangered perennial native to the piedmont of the southeastern United States (Cronquist 1980). While historically documented from Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Georgia, Alabama, and Arkansas, it is currently known to exist in southern Virginia, North Carolina, South Carolina and northeast Georgia (U.S. Fish and Wildlife Service 1995). Of the 57 known historical populations, 21 (36 percent) remained at the time of its federal listing in 1992 (U.S. Fish and Wildlife Service 1995). The majority of known populations exist on roadsides and utility rights-of-way, while the few undisturbed populations are declining in number and size as succession shades out the understory (Emanuel 1996; Gaddy 1991). In Georgia, there are only four known smooth coneflower populations (U.S. Fish and Wildlife Service 1995).

Habitat destruction is a primary cause of endangerment for many species and the smooth coneflower is no exception. Additionally, it is thought smooth coneflower requires disturbance and that suppression of fire is a significant cause for its decline (Gaddy 1991). The vulnerable location of many populations in human-use areas (roadsides, right-of-ways, fields) adds to the uncertainty of their survival. Populations located on private land have little legal protection under the Endangered Species Act.

Protection, monitoring and management are vital to the preservation of the remaining smooth coneflower populations. The recovery plan for *E. laevigata* calls for wild-reintroduction and safeguarding throughout the range of the species (U.S. Fish and Wildlife Service 1995).

The objective of this study was to learn how to more effectively reintroduce and manage *E. laevigata* populations. We present an experimental reintroduction of *E. laevigata* that tests the effectiveness of several planting methods for establishing populations in the wild. Secondly, in order to better manage the light environment for *E. laevigata* populations, we assess the effect of developmental light intensity on photosynthesis, biomass allocation and flower production.

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CHAPTER 2

LITERATURE REVIEW

I. SMOOTH CONEFLOWER

Introduction

Echinacea laevigata (Boyton & Beadle) Blake, Asteraceae, is a federally and state listed endangered species native to the piedmont of the southeastern United States. While there is little published on the species, the nine-member genus *Echinacea* is well studied. *Echinacea* is an exclusively North American genus, which ranges from the midwest to the eastern United States. The most widespread species, *E. angustifolia*, extends from southern Texas north into Canada (McGregor 1968). Along with *E. laevigata*, *E. tennesseensis*, a central Tennessee cedar glade endemic, is also endangered. Interest in *Echinacea* stems from its long history as a medicinal herb, and more recently from its popularity as a garden ornamental.

Echinacea laevigata, or smooth coneflower, is historically documented from Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Georgia, Alabama, and Arkansas, although accounts of Maryland and Alabama occurrences may not be from authentic *E. laevigata* populations (Gaddy 1991). It is currently known to exist in southern Virginia, North Carolina, South Carolina and Georgia (U.S. Fish and Wildlife Service 1995). Of the 57 known historical populations, 21 (36 percent) remained at the time of its federal listing in 1992 (U.S. Fish and Wildlife Service 1995). The causes for most of the extirpations are unknown. Only 1 of the remaining populations is increasing in size, 7 are stable, and 13 are declining (Gaddy 1991). Most populations are very small

with half containing less than 100 plants (U.S. Fish and Wildlife Service 1995). The largest and likely healthiest populations are in North Carolina with 3 out of 6 sites containing over 1000 individuals (Gaddy 1991). The majority of known populations exist on roadsides and utility rights-of-way, while the few undisturbed populations are declining in number and size as succession shades out the understory (Emanuel 1996; Gaddy 1991). In Georgia, there are 4 known smooth coneflower populations found only in Stephens County (U.S. Fish and Wildlife Service 1995) and Habersham County, where a population was recently discovered (Cindy Wentworth, U.S. Forest Service, personal communication).

Biology

A rhizomatous perennial, which flowers in May through July, smooth coneflower is found in meadows, fields, roadsides and woodlands (Cronquist 1980; Radford et al. 1968). Its primary mode of reproduction is sexual (Gaddy 1991). However, vegetative reproduction from rhizomes has also been observed making it difficult to distinguish genetically distinct individuals (Apsit & Dixon 2001). All species of *Echinacea* were found to be self-sterile when flower heads were bagged (McGregor 1968). More recently, *E. purpurea* was found to be primarily outcrossing, but partially self-compatible when hand pollinated with pollen from the same capitulum (Leuszler et al. 1996). *E. laevigata* apparently has no known specialized pollinators; homopterans, heteropterans, lepidopterans, coleopterans, orthopterans and hymenopterans have been observed visiting the flowers (Emanuel 1996). Its seed is an achene with no apparent specialized dispersal mechanism. Reproductive success appears inadequate for maintaining population size in the wild (Gaddy 1991). Bare soil, rich in magnesium and/or calcium, is thought to be a

requirement for germination and growth (Gaddy 1991). The species' germination requirements have not been studied. The majority of seedlings appear to be clustered in the vicinity of adult plants. Seed dispersal by animals is likely, but has not been documented (Gaddy 1991).

Populations of *Echinacea laevigata* sampled from North Carolina, South Carolina and Virginia were found to have moderate levels of genetic diversity, comparable to that of the widespread congener *E. angustifolia* DC. Significant population structure was documented with each population containing about 90 percent of the total genetic variation. Further, there was a positive correlation of genetic and geographical distances, suggesting the populations follow an isolation-by-distance model. Because of the unequal partitioning of genetic variation among populations, each population contributes to the overall genetic variation for the species. This partitioning also has implications for the collection of material for *ex situ* conservation and reintroduction, as populations may be locally adapted (Apsit & Dixon 2001).

Habitat

Echinacea laevigata is associated with calcium and magnesium rich soils associated with underlying mafic rock: amphibolite, dolomite, or limestone in Virginia; gabbro in Virginia and North Carolina; and diabase dikes in North Carolina and South Carolina and marble in Georgia and South Carolina (Gaddy 1991; U.S. Fish and Wildlife Service 1995). In Georgia and South Carolina, all natural populations are associated with soils of the Poor Mountain-Chauga Belt (Gaddy 1991). Smooth coneflower community types are characterized as xeric hardpan forest, diabase glades, or dolomite woodlands in the case of Virginia populations (Schafale & Weakley 1990). Openings may be

maintained by shallow soil and harsh edaphic conditions associated with aspect and/or poor moisture retention of the soil (U.S. Fish and Wildlife Service 1995). Populations are generally found in open woods, or human maintained clearings such as roadsides and utility rights-of-way. Historically, they were likely found in prairie habitats and post oak-blackjack oak savannas, which were maintained by lightning fires and burning performed by native Americans (Barden 1997; Komarek 1974; U.S. Fish and Wildlife Service 1995). Plant species associated with *E. laevigata* populations vary with location. Species lists are given in the recovery plan (U.S. Fish and Wildlife Service 1995) and in the status report by Gaddy (1991). In a thorough site survey conducted in 1990 all sites were found to have less than 30 percent cover (Gaddy 1991).

Threats

Habitat fragmentation reduces the flow of genes between populations reducing the effective population size of disjunct populations (Wright 1943). Small population size has been shown to increase the likelihood of population decline and extinction (Ellstrand & Elam 1993; Newman & Pilson 1997). Because most *E. laevigata* populations are very small (Murdock 1992), they are at risk of genetic decline from inbreeding depression.

Habitat destruction is a primary cause of species endangerment and the smooth coneflower is no exception. The vulnerable location of many populations in human-use areas (roadsides, right-of-ways, fields) leaves their future uncertain. Populations located on private land have little legal protection under the Endangered Species Act.

The Forest Service began intensive fire suppression activities in the early 20th century. The interruption of this natural process has greatly decreased the proportion of early successional habitat in southeastern forests (Komarek 1974). It is thought that fire

suppression is a significant cause for the decline of the disturbance-requiring smooth coneflower (Gaddy 1991).

Wild harvest of many *Echinacea* species for their medicinal value long ago reached unsustainable levels and continues to increase. In the late 19th century concern over collection of *E. angustifolia* and *E. pallida* began to arise as single orders for the herb ranged from 200 to 40,000 pounds (Foster 1991). In 1989, it was estimated that two million *E. purpurea* roots were harvested that year (Kindscher 1989). Demand and concern continue for many species throughout their ranges, encouraging cultivation of several species. While the wild collection of whole *E. laevigata* plants has not been observed, the threat is real. Other unusual, rare or endemic *Echinacea* species have been negatively impacted due to wild collection, including the endangered *E. tennesseensis* (Foster 1991). Public interest in all species of *Echinacea* for their ornamental value also threatens the plant's future (Foster 1991; Sheldon et al. 1997). On several occasions, flowers or seed have been removed from entire roadside populations of *E. laevigata* (C. Wentworth, U.S. Forest Service, personal communication).

Management and Recovery

Protection, monitoring and management are vital to the preservation of the remaining smooth coneflower populations. In Virginia, The Nature Conservancy and the Virginia Department of Conservation and Recreation (Division of Natural Heritage) developed a landowner contract program to encourage the voluntary protection of privately owned populations. The Forest Service in North Carolina works with the North Carolina Department of Transportation to ensure safe roadside mowing regimes. In Georgia, most populations are on U.S. Forest Service land where plants are monitored

and protected from destructive mowing and road grading activities. *Ex situ* conservation at the North Carolina Botanical Garden involves maintaining a collection of live plants and seed storage. Seeds are also stored at the U.S. Department of Agriculture's national seed storage lab in Colorado. These *ex situ* collections are merely a safety net should wild populations become extirpated (Komarek 1974; U.S. Fish and Wildlife Service 1995).

Smooth coneflower management requires clearing of competing vegetation by various means. Several season-specific clearing methods, including burning, hand-clearing and mowing, have been evaluated by land managers. At a Virginia site owned by The Nature Conservancy, mowing in 1987 yielded a near 100 percent increase in stems after four years. The North Carolina Department of Agriculture's Plant Conservation Program began a prescribed-burning program in 1994 for the largest known population, which seems to have benefited the population. Similarly, prescribed burning was initiated for Georgia populations on U.S. Forest Service land and results are being monitored. Mechanical removal of woody species on a four year cycle was chosen by the U.S. Army Corps of Engineers after evaluation of management alternatives to prescribed burning (Komarek 1974; U.S. Fish and Wildlife Service 1995).

In a 1996 thesis, Carlen Emanuel identified habitat characteristics of Oconee Co., South Carolina, *Echinacea laevigata* colonies and compared the success of six combinations of silvicultural management techniques for the species. Treatments were chosen to reduce shading and competition, disturb the substrate, and promote reproduction. The treatments were as follows:

- I. removal of understory trees,
- II. removal of understory trees and winter prescribed burning,

- III. removal of understory trees, clipping other competing vegetation and leaf blowing forest floor,
- IV. removal of all trees,
- V. removal of all trees and winter prescribed burn,
- VI. removal of all trees, clipping other competing vegetation and leaf blowing forest floor and
- VII. no manipulation.

It was determined that none of the treatments had a negative impact on smooth coneflowers. Leaf blowing of the forest floor was more beneficial than prescribed burning or canopy removal for regeneration. Removal of all trees plus leaf blowing resulted in the greatest increase in blooming and leaf production (Emanuel 1996).

Treatment effects on seedling recruitment could not be determined in the research period.

The U.S. Fish and Wildlife Service Recovery Plan (1995) provides a comprehensive outline of requirements for the recovery of *Echinacea laevigata*. The plan calls for protective management, monitoring, and survey of suitable habitat for undiscovered populations and potential reintroduction sites for the establishment of populations within the historic range. It recommends research be conducted on the species' biology and ecosystem management tools.

Protection of viable populations using tools such as management agreements, acquisition, registry, and cooperative agreements are called for. Public education, and law enforcement should be implemented to prevent further destruction of extant populations. Cultivated sources of plant material should be maintained. The plan also calls for yearly assessment of recovery efforts and redirection thereof when necessary.

II. PLANT REINTRODUCTION AS A CONSERVATION TOOL

Overview

Introduction, reintroduction and supplementation or augmentation of plant and animal populations are conservation strategies aimed at creating new or enhancing existing populations. Supplementation is “most natural” of the three; it involves adding individuals to an existing population to increase its numbers or in some cases to introduce genetic diversity to the population. Reintroduction refers to the introduction of a population to an area where it is presumed to have once occurred. The “least natural” of the three strategies is the introduction of a population to a location where it is unlikely to have occurred historically. Introducing populations is a somewhat controversial practice. In general it is agreed that it should only be used in cases where sufficient historical habitat no longer exists, or in cases where climate change has or is projected to displace the range for the species. The application of these techniques vary in scope, with the simplest involving one species and the most complex involving the recreation of an entire community. They may be used for restoration of a habitat, to increase biodiversity, and in management and recovery of threatened and endangered species. Here, I will discuss reintroductions, but the same points generally apply to introductions and supplementations as well.

The value of reintroduction as a conservation tool must be carefully weighed. It has been argued that it is impossible to recreate nature and opponents worry that it may be used in environmental mitigation, ultimately to the detriment of the species and communities it seeks to protect. Reintroduction is no substitute for preservation of existing populations, but in many cases it is a last hope for species or communities that

have declined to the point where preservation alone cannot ensure persistence (Falk & Olwell 1992; Janzen 1988). It is also vital in restoring disturbed areas to a more natural state. In determining the appropriateness of reintroduction or restoration for a particular species or location the following should be considered: Does the action benefit the species, the site, the existing population (in the case of supplementation), and the future evolutionary potential of the species (Morse 1996)?

Plant reintroductions have become increasingly popular in the past decade. Recovery plans for over half of the cases of threatened and endangered plant species recommend reintroduction. While becoming a common practice, there are few carefully designed, experimental reintroductions published. As damaged ecosystems and species loss are sure to rise in the coming decade, so too will efforts to restore and protect these natural resources. Experimental reintroductions that document failures and successes are vital in insuring the integrity of this promising conservation tool.

Done correctly, even the simplest case of reintroducing a single species is enormously complex. It requires a multidisciplinary approach, significant funding and a long-term strategy to ensure its goals are met. Therefore, reintroductions should be thoroughly researched, planned, and experimental in nature (Kutner & Morse 1996). Such experiments have the potential to answer important ecological questions if designed properly (Kutner & Morse 1996). Detailed discussions and guidelines on the various aspects of plant reintroductions are provided by Falk et al (1996), Guerrant and Pavlik (1998) and The Center for Plant Conservation (1991). Some of the more critical considerations relevant to initiating a plant reintroduction project are outlined here.

Site selection

Selecting reintroduction sites is one of the most crucial steps in reintroducing plant species. The numerous considerations important in selecting a reintroduction site include physical, biological, logistical, and historical factors. Further, the cause of rarity influences the appropriateness of reintroduction sites (Fiedler & Laven 1996).

The physical characteristics of potential reintroduction sites are the most measurable criteria for determining its appropriateness. Physical features important for the success of the species can include shading, hydrology, soil (texture, type, pH, etc.), slope, and aspect. Biological considerations include the presence of pollinators, dispersal agents, and mycorrhizal associates; plant community structure and successional stage; presence of competitors and herbivores; and the potential for genetic contamination via hybridization, outbreeding depression or other means. Logistical considerations such as land ownership; degree of protection; long-term site protection; ease of access for researchers, monitors, and managers are critical in ensuring long-term success of the reintroduced population (Fiedler & Laven 1996).

Depending on the species and the cause(s) for the decline, reintroduction sites may or may not be best located within the species native range. If a reason for the species' decline is tied to its range, for example in the range of significant projected climate change, it would not be advantageous to establish populations within this range. Because rare species have limited ranges and distributions, specialized habitat requirements and limited dispersal ability, they are at high risk to climate change (Kutner & Morse 1996). In such cases, the historical range may at least provide baseline information on the ecological and habitat requirements (Kutner & Morse 1996). Moving

a species beyond its range raises the challenge of choosing new sites that ensure pollinators, dispersers, and other requirements (White 1996) and is controversial because of the potential for misuse in mitigation (Fiedler & Laven 1996).

Creating populations

The goal of reintroduction should be to create a founding population that over time will develop into a self-sustaining population with the demographic complexities, genetic diversity, and potential for natural selection typical of a natural population. A carefully designed population, with attention to composition, placement and aftercare, has the potential to meet reintroduction goals. Critical to the success of the population is high survival and rapid growth of the founding population (Guerrant 1996).

Small populations experience loss of genetic diversity (Ellstrand & Elam 1993). The negative effect of genetic bottleneck inherent in creating populations with small effective population size may be greatly reduced by rapid growth of the founding population (Nei et al. 1975). Therefore it is important to design reintroduced populations with high rates of survival and growth. By modeling the effects of plant size and developmental stage of reintroduced individuals on population extinction risk and population growth, Guerrant (1996) found a significant reduction in extinction risk by introducing individuals from the next-to-smallest size class relative to introducing the smallest size class or seeds. It was further found that using larger individuals has no increasing benefit on survival. However, maximizing population growth rates requires the use of large size class individuals. Therefore, the models suggest that the use of large class size individuals for creating the founding population provides the best chances for population persistence and growth. Simulations for plants with various life histories gave

similar results. The extent to which the predictions of the models, developed from wild populations, apply to reintroduced populations, must be tested empirically (Guerrant 1996).

While introducing larger size class individuals best reduces extinction risk and increases growth rate in the introduced population, the benefit must be weighed against feasibility in terms of available plant material, and financial, human, and equipment resources. Options for introducing individuals range from simply scattering seeds to various methods of transplanting whole plants or plant parts. Each has associated costs and benefits and must be considered on a case-by-case basis (Guerrant 1996).

The source of plant material is a critical consideration in any reintroduction as it determines the genetic makeup of the reintroduced population. It is a complex topic and generalizations cannot be made without further theoretical and empirical work. While difficult to ascertain, adaptation of genotypes to local conditions should be considered (Guerrant 1996). Introduction of ecotypes from outside a population may cause outbreeding depression to the detriment of the native population (Falk & Olwell 1992). Material for reintroduction should be collected from 10 to 50 individuals to maximize the sample's representation of the total genetic variation in the population (Center for Plant Conservation 1991). Environmental gradients within the habitat, self-incompatibility, or restricted gene flow can be indicative of high diversity among individuals in a population. In such cases, the upper end of the recommended range should be sampled (Guerrant & Pavlik 1998).

While the preservation of genetic diversity is an important consideration in all plant reintroductions, some cases may warrant additional measures to minimize

deterioration of existing genetic diversity. Such cases include species that have low genetic diversity and species that have declined to an extremely low number of individuals. Several practices may be employed to maintain genetic diversity. Equalizing founder representation (introducing equal number of genetically distinct individuals) has been shown to reduce the likelihood of inbreeding and results in higher genetic diversity than choosing founders randomly. Further, selective breeding of the founder population may also increase genetic diversity. Equalizing family size, including the number and sex of offspring per parent in the founding population, has been shown to double effective population size, reduce inbreeding, and prevent deterioration of genetic diversity. However this method presents a tradeoff between maximizing genetic diversity and impeding natural selection. Another method for reducing the loss of genetic diversity is to allow immigration or introduce immigrants into the founding population. Even a small number of immigrants can significantly reduce deterioration of genetic diversity caused by inbreeding. The potential benefits of these methods are not without cost. The subtleties and complexities inherent in managing for genetic diversity are discussed by Guerrant (1996).

Choosing sites and designing populations mark only the beginning of a reintroduction strategy. The success or failure of a reintroduction can only accurately be judged in the long term. Pavlik (1996) provides a framework for defining, and measuring success in reintroduction projects. Here the goals of reintroduction are described in the short term as the establishment of a population that is able to carry on the basic life-history processes (establishment, reproduction, and dispersal) with a low probability of extinction and in the long term, as being as capable as its natural equivalent of integrating

into the ecosystem and being capable of evolution and/or migration in response to environmental changes. Success is defined as meeting objectives that fulfill goals of abundance, extent, resilience, and persistence (Pavlik 1996). Methods for measuring these parameters are detailed by Sutter (1996). Monitoring will eventually consume the greatest amount of time and commitment in reintroduction projects, requiring years or decades. Often neglected, it is a most critical element in the execution of meaningful reintroductions (Pavlik 1996; Sutter 1996).

III. ADAPTATION TO LIGHT INTENSITY

Sunlight intensity is a key environmental factor that determines plant species distributions in communities (Bazzaz & Carlson 1982). Species vary in their ability to adapt to various levels of light (Boardman 1977). Their ability to adapt to given light conditions is reflected in their designation as “shade” or “sun” species. Species that can only persist in high light environments are designated obligate sun species while those that cannot acclimate to high light intensity are obligate shade species. Otherwise, a species able to acclimate to a given light environment may develop into shade or sun forms (Boardman 1977). Light adaptability, the ability to persist under changing light levels, should be distinguished from plasticity. Plasticity is the ability to grow well under high and low developmental light levels, provided the level is held constant (Lüttge 1997).

A plant’s genetic ability to adapt to the light environment is the result of a combination of complex anatomical and physiological factors (Björkman & Holmgren 1963; Boardman 1977). As outlined in Table 2.1, species adapted to sun or shade

extremes have characteristic differences in their photosynthetic light response, biochemistry, chlorophyll, anatomy, chlorophyll a/b ratios, leaf morphology, canopy size and orientation, resource allocation, and reproductive effort (Boardman 1977; Givnish 1988).

Photosynthetic response curves of plants grown under high versus low developmental light levels are useful in identifying a species' light adaptability (Baskauf & Eickmeier 1994; Björkman & Holmgren 1963; Givnish 1988). Light response curves generated by plotting CO₂ fixation against a range of photosynthetically active radiation (PAR) intensities describe photosynthetic properties of leaves (Lüttge 1997; Zeiger & Taiz 1998). Because light response curves for sun and shade plants often have characteristic cardinal points, they are valuable in understanding a plant's light adaptability (Björkman & Holmgren 1963). Dark respiration rate, the rate of CO₂ efflux in the dark due to respiration, is characteristically lower in shade plants (Björkman 1981; McClendon & McMillen 1982). Light compensation point, the light intensity where the amount of CO₂ given off by respiration rate equals the amount of CO₂ uptake by photosynthesis is also lower in shade plants. As light intensity increases, there is a proportional increase in photosynthetic rate to the point of light saturation, where the photosynthetic rate is no longer light limited. Quantum yield, the slope of the line below light levels approaching saturation, is a measure of photosynthetic efficiency in terms of the number of molecules of CO₂ per mole of photons absorbed (McCree 1981). Generally, it is higher in shade plants. Sun plants compensate for their lower efficiency by having higher light saturation points and maximum photosynthetic rates (Lüttge 1997; Zeiger & Taiz 1998). However, because a sun species may generate a light response

curve similar to that of a shade species when grown in shaded conditions, characterization of species into obligate sun or shade plants cannot be made solely on light curve properties. Further, such leaf level factors cannot always account for dynamics at the whole plant level (Givnish 1988).

Plant productivity depends not only on the photosynthetic rate on a leaf area basis, but also on the total leaf area intercepting light, and resource allocation to photosynthetic and nonphotosynthetic tissue (Björkman & Holmgren 1963; Givnish 1988). Maximization of leaf area for light capture is critical in low light environments. A plant may increase its leaf area by increasing specific leaf area (SLA), the ratio of leaf area to total leaf dry matter. While nearly universal among shade and sun species, SLA adjustment is highly variable (Blackman et al. 1955). Shade tolerant species often, but not always, increase their SLA in response to shading, while shade intolerant species may not adjust or even decrease their SLA (Björkman & Holmgren 1963). Leaf area may also be maximized by increasing canopy leaf dry weight per unit whole plant dry weight or leaf weight ratio (LWR) (Blackman et al. 1955; Hunt 1978). Increases in leaf area are largely at the expense of root growth, as root weight ratio (RWR) declines correspond to increases in LWR (Evans & Huges 1961; Whitehead 1973). Root growth is generally sacrificed for increases in leaf area in shade adaptation as light capture becomes more important and water and nutrients are generally more abundant in shade (Evans & Huges 1961; Whitehead 1973).

While the ecophysiology of *E. laevigata* has not been studied, that of two related species has. Baskauf and Eickmeier (1994) compared the plasticity of the photosynthetic response of the endangered *E. tennesseensis* and the widespread congener *E. angustifolia*

to high and low developmental water and light regimes. They determined that both species exhibited similar photosynthetic responses to growth light and water regimes and concluded that narrow physiological tolerances did not explain *E. tennesseensis*' rarity (Baskauf & Eickmeier 1994). Both *Echinacea* species, while considered early successional, had photosynthetic rates below the range expected for sun species (Larcher 1980). Other species measured in the same stressful habitat as *E. tennesseensis* had similar low rates of photosynthesis (Baskauf & Eickmeier 1994), which are typical of plants growing in stressful environments (Chapin et al. 1987; Grime 1977; Pearcy et al. 1987).

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Table 2.1 Characteristic differences between plants adapted or acclimated to sunny vs. shady extremes in irradiance level. From Givinish (1987).

Trait	Sun	Shade
<i>Leaf-level</i>		
Photosynthetic light response		
Light saturation rate	High	Low
Compensation irradiance	High	Low
Saturation irradiance	High	Low
Biochemistry		
N, Rubisco, and soluble protein content / mass	High	Slightly lower
Chlorophyll <i>a</i> / chlorophyll <i>b</i> ratio	High	Low
Chlorophyll / soluble protein ratio	Low	High
Anatomy and ultrastructure		
Chloroplast size	Small	Large
Thylakoid/grana ratio	Low	High
Morphology		
Leaf mass / area	High	Low
Leaf thickness	High	Low
Stomatal size	Small	Large
Stomatal density	High	Low
Palisade / spongy mesophyll ratio	High	Low
Mesophyll cell surface / leaf area ratio	High	Low
Leaf orientation	Erect	Horizontal
Iridescence, lens-shaped epidermal cells	None	Rare
Reddish leaf undersides	Very rare	Infrequent
<i>Canopy-level</i>		
Leaf area index	High to low	Low
Phyllotaxis	Spiral	Distichous
Twig orientation	Erect	± Horizontal
Asymmetric leaf bases	Very rare	Infrequent
<i>Plant-level</i>		
Fractional allocation to leaves	Low	High
Fractional allocation to roots	High	Low
Reproductive effort	High	Low

CHAPTER 3

EXPERIMENTAL COMPARISON OF REINTRODUCTION METHODS FOR THE ENDANGERED *ECHINACEA LAEVIGATA*

INTRODUCTION

Echinacea laevigata, (Boyton & Beadle) Blake, Asteraceae, or smooth coneflower, is a federally and state listed endangered species native to the piedmont of the southeastern United States. It was historically documented from Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, and Georgia. It is currently known to exist in southern Virginia, North Carolina, South Carolina and Georgia (U.S. Fish and Wildlife Service 1995). A rhizomatous perennial that flowers in May through July, smooth coneflower is found in meadows, fields, roadsides and woodlands (Cronquist 1980; Radford et al. 1968). Its rarity is thought to be due primarily to development activities and habitat loss from fire suppression (Gaddy 1991). Because there is considerable public interest in all species of *Echinacea* for their medicinal and ornamental value, wild harvesting also threatens the species' future (Foster 1991; Sheldon et al. 1997).

In Georgia, smooth coneflower is found only in Stephens and Habersham Counties. The majority of known populations exist on roadsides and are at risk from road maintenance activities and collecting, while the few undisturbed populations are declining in number and size as succession shades out the understory (Emanuel 1996; Gaddy 1991).

When a species has declined to the point that preservation of extant populations cannot insure its survival, augmentation and reintroduction of new populations become important conservation tools (Falk 1987; Guerrant & Pavlik 1998). The recovery plan for *E. laevigata* calls for wild-reintroduction and safeguarding throughout its range (U.S. Fish and Wildlife Service 1995). Species reintroduction is complex, requiring significant funding, research and a long-term strategy. While hundreds of plant reintroductions have been conducted in the past two decades, few have been successful due to a lack of careful documentation, thoughtful experimental design and theoretical context (Guerrant & Pavlik 1998). In many cases transplantations fail because plants die, fail to reproduce, or simply because they are insufficiently documented and monitored (Pavlik 1996; Sutter 1996). Properly designed experimental reintroductions can answer important ecological questions (Guerrant & Pavlik 1998; Kutner & Morse 1996).

Here we present an experimental reintroduction of *E. laevigata* designed to test the effectiveness of several planting methods for establishing populations in the wild. Establishment of plants in the field may be achieved by a variety of means (Guerrant 1996). These range from the simplest, least costly method of directly seeding individuals into the site to more expensive techniques involving transplantation of plants grown *ex situ* with varying amounts of microsite manipulation and aftercare.

Because there are benefits and risks associated with each planting method, it is impossible to make generalizations about which method is ideal. Instead, methods for establishing founding populations in the field should be considered on a case-by-case basis (Guerrant 1996). By modeling the effect of developmental stage and size of reintroduction material on extinction risk and population growth rate, Guerrant (1996)

predicted that the use of large size class individuals provides the best chances for survival and rapid growth. This is an important consideration, as the negative effect of genetic bottlenecks inherent in creating populations with small effective population size can be greatly reduced by rapid growth of the founding population (Nei et al. 1975). These models were developed from wild populations; the extent to which they apply to reintroduced populations must be tested empirically (Guerrant 1996).

Introduction by direct seeding of plants into the wild may not be ideal in terms of extinction risk and population growth, but it can have other advantages. The use of seeds as propagules is the least costly method in terms of time, labor and finances. There is a risk of introducing disease into the wild associated with transplanting individuals raised *ex situ* or transplanted from other wild locations (Falk & Olwell 1992; Maunder 1992), which can be minimized by reintroducing populations via seed. Direct seeding also allows natural selection to act on the seeds in their natural environment, preventing artificial selection for weak individuals. However, without the benefit of cultural practices that enhance germination, growth, and survival of seedlings, it requires the greatest number of seed. Since collection of large numbers of seeds from endangered species may negatively impact natural populations, direct seeding is a tradeoff to consider carefully (Guerrant 1996).

Among methods for transplanting plants raised *ex situ*, bare-root is presumably the most difficult for establishment, but it reduces the risk of introducing soil pathogens and weeds. While introducing seedlings or adult plants with soil increases the likelihood of individual survival, introduction of pathogens with soil or artificial selection for genes that are deleterious in the wild could weaken wild populations. Adult plants presumably

have an advantage over young seedlings. However, they take considerably more time and resources to produce. These considerations led us to investigate differences in survivorship and size among these reintroduction strategies.

In this experiment, we test the relative effectiveness of various planting methods: transplanting seedlings bare-root versus rooted in potting soil, and transplanting adult plants (1-3 yr. old) in potting soil. While it is logistically easier to cluster seedlings under one protective cage, such crowding may negatively impact seedlings. Therefore, we also tested the effect on survival and growth of clustering seedlings versus planting them individually.

MATERIALS AND METHODS

Plant material for reintroduction of the smooth coneflower was propagated from 500 seeds collected in October, 1999 from 46 individuals of the largest roadside population in the Chattahoochee National Forest, Stephens Co., Georgia. In October 2000, 200 more seeds were collected from 20 individuals from the same population. This population has the largest number of healthy, flowering plants known in Georgia (Gaddy 1991). Collecting from many individuals (10-50) in the largest possible population provides the best chance of maximizing genetic diversity in the reintroduced population (Center for Plant Conservation 1991; Guerrant & Pavlik 1998). In January of each year, seedlings were sown uncovered on sand in sealed petri dishes and stratified at 4° C for six weeks. Upon the development of the first true leaf, the seedlings were planted in 10.16-cm square pots with Fafard 3B[®] growing mix composed of peat moss (45%), processed pine bark, perlite, and vermiculite with a nutrient starter charge. They were maintained in the greenhouse until two weeks prior to transplantation when they were

moved outdoors for acclimation. The seedlings were watered as needed and no fertilizer was applied.

There are two experimental sites; the “lower site” is approximately 100 meters from the parent population and the “upper site” is approximately 2 kilometers away. The sites were chosen based on their proximity to the parent population and seclusion from human activities, their close resemblance to natural coneflower habitat, appropriateness for land management, and ease of access. The lower site’s southeast (142 degree) aspect and 43 percent slope and the upper site’s south-southwest (214 degree) aspect and 25 percent slope fall within the ranges of the species natural occurrence in Georgia (James Sullivan, Toccoa, GA, personal communication). The lower site, which is 100 meters uphill from the seed source population was previously thinned by a bark beetle infestation which left it relatively open. The upper and lower sites’ soil pH, 6.1 and 5.7 respectively, are within the range of natural populations. Plant communities at the two sites are somewhat different. The lower site has a more diverse species composition with *Pinus echinata* and numerous hardwoods comprising the overstory. The upper site is dominated by *Pinus echinata* and *Quercus marilandica*. All plant species present in each experimental plot were recorded (Appendix A).

In April 2000, all woody vegetation less than 15 cm diameter at breast height (dbh) was cleared manually from a 15 x 15 meter area at each site in accordance with Emanuel’s (1996) finding that smooth cone-flowers responded most vigorously to hand clearing of trees and brush as opposed to other clearing methods. After clearing, the two sites had similar amounts of shading with two or three medium size trees (20-30cm dbh) remaining. There was little understory prior to clearing.

Reintroduction Treatments

Plants were planted out in late April of 2000 and 2001. Following a split-plot randomized block design, treatments were randomly assigned to three 6 x 5 meter blocks each year at each site with two replicates per treatment per block (Figure 3.1). The treatment units were 1 meter square. Plants were planted according to the following treatments: single adult, clumped seedlings (3 plants in a triangle, each plant 10 cm apart), or spaced seedlings (3 plants in a triangle, each plant 40 cm apart). In 2001, bare-root spaced seedlings (3 plants in a triangle, each plant 40 cm apart) were introduced as an additional treatment.

From the 1999 and 2000 seed sources, greenhouse-raised plants were transplanted to the experiment plots in April 2000 and 2001, respectively. Plants were transplanted from their pots without disturbing roots. Fafard 3B[®] potting soil used for cultivation was left intact with the roots. In the case of bare-root transplants, the soil was rinsed from the roots immediately before planting and disposed of. Holes were dug with bulb planters to insure that all seedlings were planted in uniformly sized holes. Adults were planted into holes that approximated as much as possible the size and shape of their pots. Soil surrounding the hole was not loosened. Because adult plants were limited in number, the spacing variable was only tested among seedlings. All plants were covered with a 65 cm diameter, cone-shaped cage constructed of 6.35 mm hardware cloth to protect against deer and rodent damage. The site was visited weekly and all plants were watered to soil saturation as needed when one or more plants at the site became wilted. Flowering stems were removed from all plants during their first season.

Competing vegetation and woody regeneration were removed or cut back monthly throughout the growing season. Plant survivorship and leaf width and length was recorded for all leaves on each plant in September of 2000 and 2001. The number of flowering stems per individual was recorded in September 2001 for the cohort reintroduced in 2000. Protective cages were removed from individuals one year after transplantation.

A linear relationship between leaf width and leaf area was derived by least squares regression from a sample of 30 randomly selected leaves. Leaves were hand traced in the field. Scanned images of the leaf tracings were analyzed with NIH Image software (National Institute of Health, Washington, DC). Treatment effects on estimated total leaf area per plant were compared with Tukey-Kramer HSD. Treatment effects on percent survival were analyzed with ANOVA (JMP 4.0, SAS Institute Inc., Cary, NC).

RESULTS

Survivorship was high for all transplantation methods in both years. In 2000, 92 percent of the adult transplants, 94 percent of the clustered seedlings, and 92 percent of individually planted seedlings survived their first growing season through late September 2000. By the end of the second growing season, September 2001, the number of survivors from the April 2000 outplanting fell to 75, 94 percent, and 75 percent respectively. The majority of these plant losses were due to consumption of the rhizomes by voles during the winter months. The plants required water just once weekly during a severe drought year. While the plants did not appear to increase much in size, they wilted infrequently. The adult plants lost a considerable number of leaves in the first two months, but appeared stable by September, when leaf measurements were taken. The plants that were not eaten over the winter performed well in their second season; many

flowered and no wilting was observed. However, immediately following removal of the protective cages in April 2001, over half the flower stems were destroyed by deer browsing. In 2001, 100 percent of the adult transplants, 97 percent of the clustered seedlings, 92 percent of individually planted seedlings, and 95 percent of bareroot planted seedlings survived the first growing season. There was no significant difference in survival rates among any of the planting treatments in either year (Tables 3.1 and 3.2).

The linear model $\hat{Y} = 0.67 + 1.16x$, where \hat{Y} represents the estimated leaf area and x represents the square root of the leaf width ($r^2=0.95$), was used to estimate leaf areas based on leaf width. There was no significant difference in size as measured by leaf area among seedling spacing treatments in the first season of each year. However, by the end of the second growing season, there was significantly greater total leaf area in clustered seedlings than individually planted seedlings in the 2000 outplant group (Table 3.1). There was a 4-fold decrease in total leaf area in the second growing season of adult plants planted in 2000. These plants had an average of 3.6 flowering stems per plant, which was significantly higher than for either group of seedlings (Table 3.1). Large reductions in leaf area from September 2000 to September 2001 were also observed in both seedling groups from the year 2000 cohort (Table 3.1). There was no significant difference in growth as measured by leaf area among any of the seedling transplantation methods in the first season of plants outplanted in 2001 (Table 3.2). As expected, adult plants had greater leaf area than any of the seedling groups in both years (Tables 3.1 and 3.2).

DISCUSSION

High survival rates, regardless of the method of transplantation, make the reintroduction of *E. laevigata* a promising recovery strategy. The large reduction in leaf area from September 2000 to September 2001 in all treatment groups planted in Spring of

2000 is most likely the result of drought stress and herbivory. The only significant difference in leaf area among comparable treatment groups was found in the year 2000 cohort at the end of the second growing season, when leaf area among the clumped seedling group was significantly higher than among the spaced seedling group. One would expect competition for water among clumped individuals to decrease growth. The most likely explanation for this unexpected result is that clumped individuals were spaced so closely that they shared planting reservoirs and consequently they had a larger volume of loosened soil in which to grow. Therefore, it may be advisable to loosen a larger area of soil in the root zone when planting. Further, it appears that the logistically easier method of planting several individuals close together under one protective cage has no negative impact on growth.

After six months in the field, seedlings planted bareroot had 95 percent survival rates and leaf areas comparable to seedlings planted with potting soil, making this a viable method of transplanting seedlings. This method minimizes the introduction of disease or weeds into the site, and is the most cost and labor effective without compromising survivorship or growth in the founding population. Planting seedlings first generation from the wild reduces the risk of introducing artificially selected traits associated with raising plants *ex situ*. Plant material was not available to test the effectiveness of planting adult plants bare-root.

Another consideration in selecting propagules for creating a founding population in plant reintroduction is the relative rate at which the plants begin to reproduce and their relative reproductive output. The loss of genetic diversity through the bottlenecks inherent in small founding populations is minimized by rapid population growth

(Guerrant 1996). After two seasons, individuals introduced as seedlings had significantly fewer flowering stems per plant. Reproductive output, measured by flowering capacity and seed set must be monitored over the course of several generations to determine the potential effect of transplant method on population growth rate.

Herbivory appears to be the biggest threat to survival and reproduction in the reintroduced populations. While there is no record of herbivory on *E. laevigata* in the wild, destruction of plants by deer and voles has been observed in garden settings (personal observation; Ken Cleveland, Clarksville, GA). Planting seedlings bareroot may reduce the attractiveness of such plants to herbivores, which could be attracted to the relative lushness of plants grown in amended soil. Burrowing animals, such as voles, may also be attracted to the loose nature of the artificial soil. No further herbivory was evident following the initial episode that immediately followed cage removal in 2001.

Our preliminary findings indicate that *E. laevigata* has great potential for reintroduction success. Each transplantation method tested resulted in high initial survival rates. Monitoring will be required for several more seasons in order to determine the relative reproductive capacity of the treatment groups over time. Until further information from monitoring is available, it is recommended that *E. laevigata* reintroduction efforts use adult plants to provide the best chance of persistence and rapid population growth. Should the seedling transplants survive and begin to reproduce at rates comparable to the adults, it would be advisable to introduce seedlings bare-root in order to minimize the risk of introducing pathogens to the reintroduction site.

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Table 3.1 Means (\pm SE) of total leaf area (cm^2), percent survival and number of flowering stems per plant of *Echinacea laevigata* reintroduced in 2000 under three treatments: *C*, 3 seedlings clustered 10 cm apart with Fafard 3B[®]; *S*, seedlings planted individually with Fafard 3B[®]; and *A*, adults planted with Fafard 3B[®]. Different letters within columns represent significant differences (Tukey-Kramer HSD, $p < .05$).

2000 cohort treatment	Mean leaf area (cm^2) per plant		Percent survival		Flowering stems per plant
	Sept. 2000	Sept. 2001	Sept. 2000	Sept. 2001	Sept. 2001
<i>C</i>	188.7 \pm 19.2b	91.3 \pm 16.6b	.94a	.94a	0.4 \pm 0.2b
<i>S</i>	173.7 \pm 26.7b	42.6 \pm 18.9c	.92a	.75a	0.1 \pm 0.2b
<i>A</i>	811.7 \pm 154.6a	187.2 \pm 122.0a	.92a	.75a	3.6 \pm 0.4a

Table 3.2 Means (\pm SE) of total leaf area (cm^2) per plant, and percent survival of *Echinacea laevigata* reintroduced in 2001 under four treatments: *C*, 3 seedlings clustered 10 cm apart with Fafard 3B[®]; *S*, seedlings planted individually with Fafard 3B[®]; *B*, seedlings planted individually with bare-roots; and *A*, adults planted with Fafard 3B[®]. Different letters within columns represent significant differences (Tukey-Kramer HSD, $p < .05$).

2001 cohort treatments	Total leaf area (cm^2)	Percent survival
Date	September 2001	September 2001
<i>C</i>	151.3 \pm 20.9b	.97a
<i>S</i>	154.3 \pm 20.2b	.92a
<i>B</i>	169.6 \pm 25.6b	.95a
<i>A</i>	553.6 \pm 20.4a	100a

CHAPTER 4

RESPONSES OF PHOTOSYNTHESIS AND BIOMASS PRODUCTION TO DEVELOPMENTAL LIGHT LEVEL IN *ECHINACEA*, ASTERACEAE, A FEDERALLY LISTED ENDANGERED SPECIES

INTRODUCTION

Echinacea laevigata, listed as federally endangered in 1992, is native to the southeastern foothills of Appalachia. Its historical range is from southeast Pennsylvania to northeast Georgia (Cronquist 1980). It is one of nine *Echinacea* species, all perennials in the Asteraceae family (McGregor 1968; Radford et al. 1968). Of the 57 known historical populations, 21 (36 percent) remained at the time of its federal listing in 1992 (Murdock 1992). The causes for most of the extirpations are unknown. Only 1 of the remaining populations is increasing in size, 7 are stable, and 13 are declining (Gaddy 1991). The cause for the species' decline across its entire range is somewhat mysterious. Many aspects of its natural history, population genetics and ecophysiology are poorly understood. Explanations for *E. laevigata*'s decline include wild harvest, habitat destruction, and fire suppression (Foster 1991; Gaddy 1991; U.S. Fish and Wildlife Service 1995).

Echinacea laevigata community types are characterized as xeric hardpan forest, diabase glades, or dolomite woodlands (Schafale & Weakley 1990). *Echinacea laevigata* is an early to mid-successional species with populations generally found in open woods, or human-maintained clearings such as roadsides and utility rights-of-way (U.S. Fish and Wildlife Service 1995). Openings may be maintained by shallow soil and harsh edaphic conditions associated with aspect and/or poor moisture retention of the soil. Historically,

they were likely found in prairie habitats and post oak-blackjack oak savannas (U.S. Fish and Wildlife Service 1995) that were maintained by lightening fires and burning performed by Native Americans (Barden 1997; Komarek 1974). Field observations suggest that the species requires high light environments to persist (Gaddy 1991; U.S. Fish and Wildlife Service 1995). However, the role of light limitation in the decline of *E. laevigata* populations is unclear.

The species recovery plan calls for habitat management to allow existing populations to persist and increase in size. It further calls for reintroduction of new populations, also requiring habitat management. Light intensity is a critical variable in a plant's environment. Understanding light requirements is necessary in creating and maintaining habitat for certain species. Photosynthesis experiments that use photosynthetic response curves and comparative biomass allocation to quantify light requirements are common in horticultural and ecological sciences (Marler et al. 1994; Rothstein & Zak 2001; Walters & Field 1987). They have also been used in investigations into the causes of plant rarity (Baskauf & Eickmeier 1994; Smith et al. 1993). However, such experiments have yet to be utilized in the context of plant reintroduction. Here we apply these methods to determine the light requirements for the persistence of *E. laevigata* in order to effectively manage existing populations and create new ones.

In order to assess *E. laevigata*'s ability to adapt to shade, we tested the following hypotheses: 1) *E. laevigata* will generate light curve characteristics typical of a sun adapted species (high dark respiration, light compensation point, maximum photosynthetic rate, and saturating light intensity; low quantum efficiency at low light

levels; and no photoinhibition at high light levels, with the inability to adjust these parameters when grown in the shade); and 2) shaded growth conditions will significantly reduce biomass and flowering.

MATERIALS AND METHODS

Plants for both experiments were grown from seed collected from a relatively large (over 300 individuals) roadside population in Stephens County, Georgia. For the photosynthesis experiment, 30 one-year-old plants were placed in dark refrigeration for 10 weeks at 5°C to stimulate dormancy and simultaneous emergence of individuals. Plants used for the biomass allocation experiment were 30 greenhouse-raised, one-year-old plants over-wintered together outdoors in full sun. These plants were dormant from November 2000 until the application of light treatments. In mid April, plants for both experiments were potted from 10.16 cm-square pots to 3.8 liter round pots with Fafard 3B[®] growing mix composed of peat moss (45%), processed pine bark, perlite, and vermiculite with a nutrient starter charge. All plants were moved to the outdoor propagation area at the State Botanical Garden of Georgia, Athens, GA, and equally divided (segregated design) under high (HL), medium (ML), and low (LL) light levels: 100 percent, 43 percent, 18 percent, of ambient light, respectively. Black polypropylene shade fabric covering rectangular frames provided shade. Each treatment area was approximately four square meters. On a clear August day, at 11:30 a.m., average PPF (photosynthetic photon flux), was calculated for each light treatment block with a LI-250 light meter and LI-190SA quantum sensor (LI-COR, Lincoln, NE) by averaging measurements at leaf height from the four corners and middle of the block. PPF for the HL, ML, and LL treatments were: $1881.2 \pm 11.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, $812.4 \pm 13.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $344.8 \pm 13.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the time of measurement. Temperature among the

treatments varied up to 10°C. Plants were watered to saturation every other day. Pots were rotated daily to the next position in each treatment area to minimize the effect of pot location on the plant's development. No fertilizer was applied during the study period.

For the photosynthesis measurements, one plant from each of the treatments was measured per day. Light response curves plotting CO₂ assimilation versus PPF were generated for six plants chosen randomly from each light treatment. To generate the light curves, CO₂ assimilation was measured at 10 consecutively decreasing light intensities: 2000, 1300, 900, 650, 400, 250, 150, 80, 20, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Photosynthetic rates were measured on the youngest fully expanded leaf. Net CO₂ assimilation was measured using a portable photosynthesis system with a quarter-liter leaf chamber (model 6250: LI-COR). An adjustable frequency QB6200 lamp (Quantum Devices, Inc., Barneveld, WI) mounted directly onto the leaf chamber provided light. The lamp was calibrated using a LI-250 light meter and LI-190SA quantum sensor (LI-COR, Lincoln, NE). Plants were watered to saturation and brought into the lab the evening before measurements were taken. Before measurements began, each plant was allowed to reach a steady gas exchange rate for one hour. To insure gas exchange rate equilibrium during measurements, plants were held at constant PPF for 15 minutes between changing light intensities. The CO₂ concentration in the chamber was maintained within the range of 300-500 ppm and the relative humidity between 40 and 85 percent. The chamber temperature was maintained at 28 ± 2 °C. After gas exchange measurements were taken, the area of the portion of the leaf enclosed in the chamber was measured with a LI-3000 area meter (LI-COR, Lincoln, NE) to calculate CO₂ assimilation on a leaf area basis.

For biomass measurements, plants were harvested on September 6 and 7, 2001. Number of flowering stems and flower heads were recorded. *Echinacea laevigata*'s growth habit includes thick fleshy roots, basal rosettes, and erect flowering stems with flowers borne in heads (Radford et al. 1968). For the purpose of our biomass measurements, we divided plants into four parts: roots, leaves (all leaves in the basal rosette), flowering stems (including stem, stem leaves, and peduncle), and flower heads (including the involucre, ray and disk flowers). Measures of relative growth and resource allocation included: total, root, leaf, flower head and flowering stem biomass; total leaf area; specific leaf area; and the ratio of biomass in flowering stems, roots, and leaves (Blackman et al. 1955; Hunt 1978). Leaf areas for every leaf from each plant were calculated from scanned leaf images with NIH Image software (National Institute of Health, Washington, DC). Roots, leaves, flowering stems and flower heads were dried at 80°C for 48 hours and then weighed. Specific leaf area (SLA), a measure of leaf thickness, was calculated as:

$$\text{SLA} = \text{total leaf area} / \text{total leaf weight}$$

for each plant. Resource allocation was calculated as the ratio of total biomass allotted to leaves, flowering stems and roots: leaf weight ratio (LWR), stem weight ratio (SWR), and root weight ratio (RWR). Leaf area ratio (LAR) was calculated as:

$$\text{LAR} = \text{total leaf area} / \text{total dry weight}$$

as a measure of leafiness (Blackman et al. 1955; Hunt 1978).

Light curve data were fit by least squares regression to a nonlinear curve (Sigma Plot 6.0, SSPS Science, Chicago IL) (Potvin et al. 1990). Net CO₂ at saturation (A_{sat}) and light saturation point (LSP) for each light treatment were derived from this model. Dark

respiration (DR), and maximum net CO₂ assimilation (A_{\max}) were taken directly from the data. Quantum yield (Φ) was derived by fitting net CO₂ assimilation versus the three PPF values 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and below with linear regression (Bazzaz & Carlson 1982). From the linear equation, light compensation point (LCP) was calculated by setting net CO₂ assimilation to zero.

Means for these parameters were analyzed with linear regression at the $p \leq .05$ level of significance (JMP 4.0, SAS Institute Inc., Cary, NC). In the case of LWR, where the unequal variance assumption was violated, means were compared with Welch analysis of variance. Data are presented as means \pm standard error.

RESULTS

At the end of the growth period, plants from all treatments appeared healthy. There was no visible yellowing or dieback. Plants from shaded treatments were deeper green in color. Several individuals damaged by insect herbivory were removed from the study.

Dry weight and leaf area

There were significantly fewer flowers and lower total biomass in LL plants compared to ML and HL treatment groups ($p \leq .05$). Root biomass was significantly lower for the LL group ($p \leq .05$). Flowering stem biomass in the LL group was significantly lower than the ML group, but not significantly different from the HL group. The leaf biomass did not differ significantly among any of the treatment groups. Biomass in the ML and HL groups was not significantly different (Figure 4.1). There was no significant difference in the mean leaf area ($p = .47$), LWR ($p = .16$), SWR ($p = .54$), RWR ($p = .98$), total leaf area ($p = .55$), or LAR ($p = .08$) among the light treatments.

Mean SLA was significantly different for each treatment group with the LL group having the highest and the HL group having the lowest SLA value (Table 4.1)

Photosynthetic response to light

For the nonlinear equation, coefficient of determination (r^2) values ranged from .91 to .99 with 14 of the 18 curves having r^2 values greater than .95. For the linear fit, r^2 values were between .78 and .83. Presumably, these low r^2 values could be improved by taking more photosynthesis measurements in the PPF ranges below the LSP. There was no significant difference among any of the three treatment groups detected for the light curve parameters A_{\max} , LSP, DR, LCP and Φ (Table 4.2, Figure 4.2). There was no decline in CO_2 at high PPF that would indicate photoinhibition observed in any of the treatment groups.

DISCUSSION

There was no detectable difference in any of the light curve parameters for plants grown under full sun, moderate or deep shade. We therefore accept our hypothesis that *E. laevigata* will exhibit light curve characteristics typical of a sun adapted species. It appears that light levels during growth do not alter the photosynthetic capacity of *E. laevigata*; quantum yield, maximum rate of photosynthesis, light saturation point, light compensation point and dark respiration rate were not significantly different among the light treatments (Table 4.2). No photosynthetic adaptation to shade was detectable. While not significant, there was a noticeable trend of lower maximum photosynthetic rates among the high light treatment group. This is a possible result of heat stress, as temperature among the light treatment groups was not controlled. When grown in the shade, shade plants generally exhibit lower respiration due to lower reduced construction

and maintenance costs, and thus have lower light compensation points and higher quantum efficiency at low light levels (Björkman & Holmgren 1963; Marler et al. 1994).

Characterization of species as sun or shade plants cannot be made based solely on light curve properties because they do not account for dynamics at the whole plant level. Plant productivity depends not only on the photosynthetic rate on a leaf area basis, but also on the total leaf area intercepting light, and resource allocation to photosynthetic and nonphotosynthetic tissue (Björkman & Holmgren 1963; Givnish 1988). The results of this study indicate that *E. laevigata* performs equally well in full sun or moderate (43 percent) shade. However it begins to decline in deeper shade (18 percent), having significantly reduced number of flower heads, and root and flowering stem dry weight (Table 4.1, Figure 4.1). We therefore accept our hypothesis that shaded growth conditions will significantly reduce biomass and reproductive capacity. Leaf thickness, measured by SLA, decreased with increasing shade. However, this adaptation to shade was insufficient to maintain productivity in deep shade. Deep shade plants had at least half the number of inflorescences as plants grown in moderate or full sun. Further, they had between 51 and 58 percent less total biomass. This reduction in biomass was the result of both significantly smaller roots and flowering stems; both of these weights approximately doubled in plants grown in full sun or moderate shade.

The inability of *E. laevigata* to adjust photosynthetically to low light levels, coupled with its reduction in biomass and flowering capacity, suggest that it cannot adapt to extreme shade conditions. The degree of shade one would expect to occur in deep shade in eastern U.S. forests may be as low as five percent of full sun (Horn 1971). In moderate shade, *E. laevigata* performed as well as in full sun. This is to be expected of

early successional species, which typically have relatively high plasticity to environmental gradients (Bazzaz & Carlson 1982).

Baskauf and Eickmeier (1994) compared the plasticity of the photosynthetic response of the endangered *Echinacea tennesseensis* and the widespread congener *E. angustifolia* to high and low developmental water and light regimes. They determined that both species exhibited photosynthetic responses to growth light and water regimes that were similar to one another and concluded that narrow physiological tolerances did not explain the rarity of *E. tennesseensis*. Photosynthetic rates for *E. laevigata* are similar to those of *E. angustifolia* and *E. tennesseensis* (Baskauf & Eickmeier 1994), which are quite low relative to herbaceous heliophytes that typically have maximum photosynthetic rates between 13 and 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Larcher 1980).

Our findings that *E. laevigata* cannot adapt to extreme shade are consistent with the belief that one explanation for the species decline is its requirement for a moderate to high light environment (Emanuel 1996; Gaddy 1991; U.S. Fish and Wildlife Service 1995). The species' ability to reproduce sexually is greatly diminished in deep shade where it produces fewer flower heads. While vegetative reproduction by root division has been observed in *E. laevigata* (Apsit & Dixon 2001), it does not appear to be a significant mode of reproduction in nature. Until recently, the species was thought to only reproduce by seed (U.S. Fish and Wildlife Service 1995). *Echinacea laevigata* produces numerous viable seed although it is functionally self-sterile (McGregor 1968). Because it appears that the species relies heavily on sexual reproduction, one would expect a reduction in the number of flowers to have a significant impact on population size. While it is not known how light levels affect germination of *E. laevigata*, it appears that

moderate shading has little impact on the species' ability to grow and flower. Therefore, management activities that maintain light levels greater than 42 percent shade should be sufficient for population persistence provided other factors are not limiting.

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Table 4.1 Mean (\pm SE) values for biomass parameters of *Echinacea laevigata* grown under 100, 43, and 18 percent ambient light for 5 months. Means with different letters within rows (*) are significantly different (Tukey-Kramer HSD, $p < .05$).

	Light regime (% of ambient light)		
	100	43	18
No. flower heads per genet*	7.0 \pm 1.0a	5.9 \pm 0.9a	3.0 \pm 0.7b
Total dry wt (g)*	43.3 \pm 4.7a	49.4 \pm 4.3a	25.2 \pm 3.4b
Total leaf dry wt (g)	4.1 \pm 1.1a	2.0 \pm 1.1a	2.9 \pm 0.8a
Flower head dry wt (g)	2.2 \pm 0.7a	2.9 \pm 1.8a	3.2 \pm 0.5a
Flowering stem dry wt (g)*	15.8 \pm 2.6a,b	21.2 \pm 2.4a	9.4 \pm 2.0b
Root dry wt (g)*	23.4 \pm 3.5a	26.2 \pm 3.3a	13.7 \pm 2.6b
Total leaf area (cm ²)	338.3 \pm 116.1a	224.8 \pm 107.4a	374.4 \pm 85.7a
Leaf area/leaf (cm ²)	16.7 \pm 7.4a	29.0 \pm 6.8a	25.6 \pm 5.4a
Leaf area ratio	7.8 \pm 6.4a	4.7 \pm 5.9a	20.4 \pm 4.7a
Specific leaf area*	84.6 \pm 10.8a	116.4 \pm 10.0b	146.5 \pm 8.0c
Leaf weight ratio	0.09 \pm .04a	0.04 \pm .04a	0.14 \pm .03a
Stem weight ratio	0.39 \pm .07a	0.43 \pm .07a	0.34 \pm .05a
Root weight ratio	0.52 \pm .05a	0.53 \pm .05a	0.52 \pm .04a

Table 4.2 Mean (\pm SE) values for light curve parameters of *Echinacea laevigata* grown under 100, 43, and 18 percent ambient light for 5 months.

Light regime (% ambient)	A_{\max}	LSP	DR	Φ	LCP
	$(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})$			$(\mu\text{mol photons m}^{-2} \text{ s}^{-1})$	
100	5.67 ± 1.22	657.40 ± 43.56	$-0.86 \pm .26$	$0.029 \pm .004$	15.37 ± 5.21
43	7.57 ± 1.07	776.67 ± 46.42	$-0.61 \pm .10$	$0.027 \pm .003$	22.45 ± 2.66
18	7.05 ± 1.22	753.69 ± 91.28	$-0.50 \pm .07$	$0.029 \pm .003$	17.47 ± 2.25
p-value	0.30	0.23	0.13	0.85	0.68

Abbreviations: Maximum net leaf CO₂ assimilation (A_{\max}), light saturation point (LSP), dark respiration (DR), quantum yield (Φ), and light compensation point (LCP).

Figure 4.1 Mean values for biomass in *Echinacea laevigata* grown under 100, 43, and 18 percent ambient light for 5 months. Error bars represent standard error for the means.

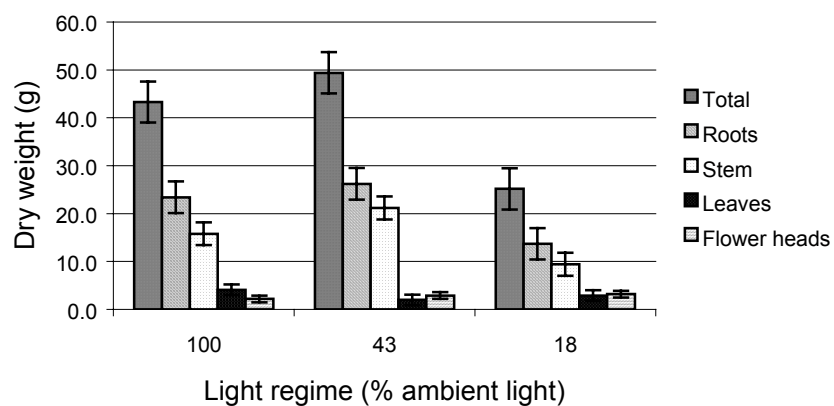
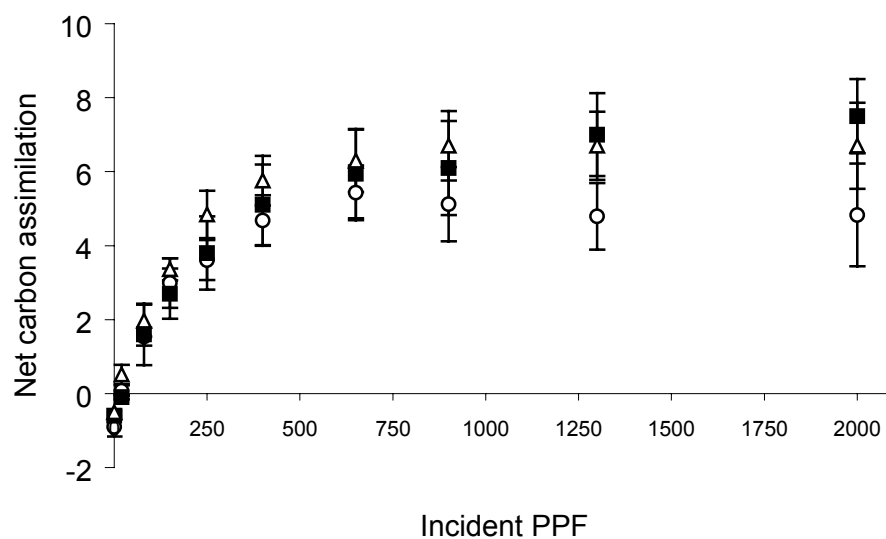


Figure 4.2 Photosynthetic light response curves plotting CO₂ assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) versus PPF ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) for *Echinacea laevigata* grown under 100 (○), 43 (■), and 18 (Δ) percent ambient light for 5 months. Data points represent means. Error bars represent standard error for the means.



CHAPTER 5

CONCLUSION

The U.S. Fish and Wildlife Services' recovery plan for *Echinacea laevigata* requires that at least 15 geographically distinct, self-sustaining populations be protected in each of two counties in Virginia, North Carolina, and South Carolina, and one county in Georgia and that these populations be stable or increasing for ten years before delisting can be considered. Currently, there are seven populations in Virginia, six in North Carolina, eight in South Carolina, and three in Georgia. Therefore, reintroduction must play an integral role in recovery.

The results of experimental reintroduction of *E. laevigata* suggest that the successful establishment of new populations is possible. Transplanted individuals had high survival rates regardless of plant age, spacing or use of supplementary soil. The high survival rates occurred with minimal watering (weekly to monthly) even in the extreme drought years during which the experiments took place. Before final recommendations of planting method may be made, monitoring of flowering and seed set must continue for five to ten years. Such monitoring will allow comparison of flowering and seed production among the treatment groups, an important consideration for creating populations. Our preliminary findings suggest that all planting methods tested are viable options for establishing individuals. Until further information is obtained we recommend that reintroduced populations be established using adult plants. Introduction of adult plants theoretically lowers the extinction risk and increases the growth rate of the new

population. In this experiment, adult plants developed four times more flowering stems after two seasons. Should the seedling transplants survive and begin to flower at rates comparable to the adults within the monitoring period, they could be recommended for establishing populations. Where seedlings are used to create populations, bare-root transplanting is recommended. This is based on the observation that bare-root individuals survived and grew as well as plants planted in potting soil which increases the risk of introducing soil pathogens.

Our light experiments demonstrate that *E. laevigata* grown in moderate (43 percent) sun to high sun perform equally well in terms of flowering, and biomass parameters. Deeper shade (18 percent) decreased biomass production and flowering. Plants grown in 100, 43, and 18 percent of ambient sunlight produced light curves with no significant differences. Therefore, there was no photosynthetic adaptation to shade observed. Based on these findings we recommend that management practices be aimed at maintaining light levels at *E. laevigata* sites above 43 percent of full sun. This is based on the assumption that our results would hold over successive seasons and that other factors are not limiting.

APPENDIX A EXPERIMENTAL PLOTS SPECIES LIST

LOWER TEST PLOT

GRAMINOIDS

Andropogon gerardii
Danthonia sericea
Dichanthelium sp.
Scleria oligantha

WOODY PLANTS AND VINES

Acer rubrum
Carya sp.
Cornus florida
Crataegus sp.
Fraxinus americana
Nyssa sylvatica
Oxydendrum arboreum
Pinus echinata
Prunus serotina
Quercus alba
Quercus marilandica
Quercus rubra
Smilax bona-nox
Vitis rotundifolia

HERBS

Aristolochia serpentaria
Cacalia atriplicifolia
Euphorbia corollata
Galium pilosum var. *puncticulatum*
Houstonia purpurea
Hypericum hypericoides
Liatris microcephalus
Parthenocissus quinquefolia
Passiflora lutea
Rosa carolina
Salvia urticifolia
Schrankia microphylla
Silphium compositum
Spigelia marilandica
Tetragonthea helianthoides

UPPER TEST PLOT

GRAMINOIDS

Andropogon gerardii
Danthonia sericea
Danthonia sp.
Schizachyrium scoparium
Scleria oligantha

WOODY PLANTS AND VINES

Chionanthus virginicus
Fraxinus americana
Gaylussacia dumosa var. *dumosa*
Oxydendron arboreum
Pinus echinata
Quercus marilandica
Quercus prinus
Vitis rotundifolia

HERBS

Asclepias viridiflora
Coreopsis major
Eryngium yuccifolium
Eupatorium album var. *album*
Euphorbia corollata
Helianthus microcephalus
Hypoxis hirsuta
Lespedeza sp.
Parthenium integrifolium
Pityopsis graminifolia
Stylosanthes biflora
Tephrosia virginiana
Tragia urticifolia