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Environmental Security Technology Certification Program

Operational-Scale Demonstration of Propagation Protocols and Comparative Demographic Monitoring for Reintroducing Five Southeastern Endangered and At-Risk Plants

Final Report

Matthew G. Hohmann and Wade A. Wall

February 2018

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14. ABSTRACT
Military installations are subject to extensive encroachment by urban development. The growing burden of managing listed and at-risk plant species falls to installations, which manage much of the remaining suitable habitat. The overall objective of this project was to increase the diversity and success of rare-plant conservation strategies available to managers. We executed an operational-scale demonstration of recently developed protocols for propagating and reintroducing one endangered (*Lysimachia asperulifolia*) and four at-risk plant species (*Amorpha georgiana*, *Astragalus michauxii*, *Lilium pyrophilum*, and *Pyxidanthera brevifolia*) found on multiple southeastern installations. Over three consecutive years we propagated and outplanted 6,075 transplants of different age/size classes of each species to four sites. We monitored survivorship, growth, and reproduction of these outplants and more than 1,500 individuals in natural populations over four years. Using demographic matrix modeling, life-table response experiments, and generalized linear models we compared the vital rates of the different classes and population growth rates (λs) between the natural and reintroduced populations. We also decomposed the influence of vital rates on λs in order to optimize population reintroduction viability and cost. Although many specific performance objectives were not met due to λs <1.0 in both population types, cost thresholds for establishing populations were met.

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Military bases–Endangered plants; Environmental protection; Plant diversity conservation; Plant conservation; Plant reintroduction; Sandhills; Fort Bragg, NC; Population reintroduction; Population growth rate; Vital rates, Comparative demography; Matrix modeling, Life table response experiments; Cost optimization

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ACRONYMS

DoD	Department of Defense
EO	Element Occurrence
ERDC	Engineer Research and Development Center
ESMP	Endangered Species Management Plan
GIS	Geographical Information Systems
GPS	Global Positioning System
INRMP	Integrated Natural Resource Management Plan
LCC	Life-cycle Costs
LTRE	Life Table Response Experiments
NCSU	North Carolina State University
NP	Nature Preserve
PO	Performance Objective
POC	Point of Contact
POV	Privately Owned Vehicle
R&D	Research and Development
SDM	Species Distribution Modeling
SAR	Species at Risk
TES	Threatened and Endangered Species
UNC	University of North Carolina
NCBG	North Carolina Botanical Garden
NGO	Nongovernmental Organization
USFWS	United States Fish and Wildlife Service

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EXECUTIVE SUMMARY

OBJECTIVES OF THE DEMONSTRATION

Military installations in the Southeast are subject to extensive encroachment by urban development (Brown et al. 2005), and increasingly the burden of managing listed and at-risk plant species falls to DoD, which manages much of the remaining suitable habitat (Stein et al. 2008). This stewardship responsibility has an impact on military readiness through restrictions on training area use. While most military installations engage in some form of ecosystem management, listed and at-risk plant populations are still being lost on many military lands (Gray et al. 2003). In cases where population numbers are stable, there is still a need for additional conservation strategies and mitigation options. A potentially valuable strategy in a broader suite of plant-conservation strategies is population reintroduction — the establishment of plant populations in currently unoccupied historical locations using source material from natural populations elsewhere (Maunder 1992). Reintroduction necessarily depends on successful propagation of the target species, but validated propagation methods for most species are not available. However, new propagation and reintroduction protocols have been demonstrated in proof-of-principle experiments for one federally endangered (*Lysimachia asperulifolia*) and four at-risk plant species (*Amorpha georgiana*, *Astragalus michauxii, Lilium pyrophilum*, and *Pyxidanthera brevifolia*) that occur across multiple DoD installations in the Southeast. These protocols could be implemented in the field by DoD users and others upon controlled validation and operational-scale demonstration. Specifically, these protocols need to be (1) verified in production-scale propagation, (2) implemented at multiple operational scale reintroduction sites, (3) evaluated over a longer time period to satisfy regulatory requirements for approval, and (4) optimized for success and cost-effectiveness. Additionally, there is a need to demonstrate the use of comparative demographic analyses for monitoring the success of population reintroductions, as no other method can generate comparably informative metrics about population viability in a similar timeframe.

The overall objective of this project was to increase the diversity and success of rare-plant conservation strategies available to managers by conducting an operational-scale demonstration of the recently developed propagation protocols for reintroducing the target endangered and at-risk plant species. The primary performance objectives were to (1) demonstrate successful reintroduction of four populations per target species at an operational scale, (2) optimize the cost of establishing self-sustaining populations using data collected from natural and reintroduced populations, and (3) secure user acceptance. Based on nine rigorous qualitative and quantitative performance objectives designed to specifically evaluate these three primary objectives separately for each species, successful operational scale reintroduction was left unmet for all species, while cost optimization and user acceptance objectives were met for four of the species.

TECHNOLOGY DESCRIPTION

Rare plant reintroduction efforts have successfully utilized a variety of techniques (e.g., direct seeding, or transplants propagated from seeds and cuttings) to establish new populations. Technologies both necessary and sufficient for reintroduction of the target species exist in the form of newly established propagation protocols, test-plot field trials, and state-of-the-science, population reintroduction, best practice guidelines (Weeks 2004; Marchin et al. 2009; Wall et al. 2010; Kunz et al. 2014). By combining these technologies with comparative demographic modeling methods (Caswell 1989, 1996, 2001; Colas et al. 2008), we assessed the success of our multiple performance objectives with functional and informative metrics. These involved evaluation of the separate contributions of survivorship, growth, and reproduction by transplants of different age/size class to the population growth rate, and the cost per capita for establishment of different outplanted classes. Our conceptual test design was comprised of three phases: propagation, reintroduction, and comparative demographic analysis, which correspond with the reintroduction technologies.

DEMONSTRATION RESULTS

The project team propagated and outplanted 6,075 transplants for four of the five target species over three successive years. These outplants and more than 1,500 individuals within natural populations were demographically monitored over four years. Propagation challenges and exceptionally high transplant mortality ultimately forced abandonment of *P. brevifolia* as a demonstration species. Averaged across the four reintroduced populations of each species, approximately 22.1%, 13.4%, 24.0% and 24.2% of outplants became established for *A. georgiana*, *A. michauxii*, *L. pyrophilum*, and *L. asperulifolia*, respectively. All four species exhibited limited reproduction within one or more reintroduced population.

One qualitative and seven to nine shared quantitative performance objectives were evaluated for each of the species. The qualitative performance objective assessed end-user acceptance of the technologies, and was evaluated with a post-demonstration questionnaire. A mean score ≥4.0 on a five-point Likert scale indicated general acceptance. The first quantitative performance objective assessed successful establishment of four viable populations of each target species. It was evaluated based on whether the population growth rates (λ) of reintroduced populations was greater than natural populations, with λ greater than 1.0 four years post-reintroduction. None of the reintroduced populations of the four species exhibited a λ greater than 1.0 four years post-reintroduction. The second quantitative performance objective assessed equivalent or better *in situ* recruitment in reintroduced versus natural populations of each species. It was evaluated based on observed recruitment rates in the two population types four years post-reintroduction for each species. Limited reproduction precluded recruitment in reintroduced populations, causing this objective to be unmet for all species. The third quantitative performance objective assessed equivalent or better survivorship and transition probabilities for all size classes of each species in reintroduced versus natural populations. It was evaluated four years post-reintroduction based on growth and survival of individuals tracked within the two population types. Neither survival nor transition probabilities representing growth (as opposed to stasis or retrogression) were greater for any size classes of any species. The fourth quantitative performance objective assessed a maximum cost threshold for growing and outplanting individuals of different size classes to achieve λs greater than 1.0 for each species. It was evaluated using propagation and outplanting costs for three age/size cohorts and elasticities. Propagation and outplanting costs for the most cost-efficient size class was lower than the $175 threshold for all species. The fifth quantitative performance objective assessed improved cost-effectiveness of watering *A. georgiana* and *A. michauxii* outplants, or competition reduction for *L. pyrophilum*. It was evaluated based on whether the cost per surviving individual was lower with the treatment, than without it. The vegetation-removal treatment applied to *L. pyrophilum* was found to be cost-effective, but supplemental watering of *A. georgiana* and *A. michauxii* outplants was not. The sixth quantitative performance objective sought to optimize the cost of establishing self-sustaining populations. It was evaluated as a maximum cost threshold ($10,000) for establishing populations having λs greater than 1.0 four years post-reintroduction for each species. None of the reintroduced populations of the four species exhibited a λ greater than 1.0 four years post-reintroduction. However, we alternately showed that a population of 100 individuals can be established five years post-outplanting for under $10,000 for *A. georgiana* and *A. michauxii*. The seventh quantitative performance objective sought to demonstrate the efficacy and cost-effectiveness of establishing populations via seed addition versus outplants for *A. georgiana* and *L. pyrophilum*. It was evaluated based on whether the cost per recruit from seed exceeded the cost per recruit via outplanting, and whether the seed needed to establish viable populations via direct seeding within an equivalent timeframe did not exceed 10% of average seed availability from natural populations. Neither species recruited within seed-addition plots during the demonstration, suggesting that transplants provide a more efficient and cost-effective approach for reintroducing populations. The last quantitative performance objective assessed the cost-effectiveness of watering *A. georgiana* seed-addition plots. It was evaluated based on whether the cost per recruited individual in irrigated seed-addition plots was less than or equal to the cost per recruited individuation in non-irrigated seed-addition plots. No recruitment occurred in either irrigated or non-irrigated *A. georgiana* seed-addition plots.

IMPLEMENTATION ISSUES

Use of reintroduction as a strategy for rare-plant conservation and mitigation has exhibited varying success (Guerrant and Kaye 2007). Although the highly ambitious performance objectives established for this demonstration were largely unmet, the observed mean percent survival of individuals of *A. georgiana*, *L. pyrophilum*, and *L. asperulifolia* was comparable (~20%) to that reported in a recent review of reintroduction success for 249 species (Godefroid et al. 2011). The survival rates of the other two species (*A. michauxii* and *P. brevifolia*) were reduced by small outplant size during one or more years. Because size is generally correlated with survival, we explored the efficacy and cost-efficiency of three age/size classes in our test design. This task allowed us to identify, for each species, outplant size classes that provided the greatest contributions to population growth rates, but it also undoubtedly negatively impacted the vital rates and growth rate of reintroduced populations. Moreover, natural populations exhibited low recruitment and only two species (*A. georgiana* and *L. pyrophilum*) had λs greater than 1.0. Consequently, it may have been overly optimistic to expect positive population growth rates within reintroduced populations within such a short timeframe. Additional monitoring will be needed to determine the ultimate fate of the populations reintroduced during this demonstration. We remain optimistic about the expanded conservation strategies and new opportunities to share conservation responsibility with partner agencies and organizations that are made possible by the propagation and reintroduction protocols. Scaling up may cause propagule procurement limitations. Use of the demonstrated technologies should not be a substitute for active conservation of the remaining natural populations of the five species, especially given the modest success of the demonstrated reintroduction efforts compared to the effort invested.

1. INTRODUCTION

1.1 BACKGROUND

The southeastern United States is known both for its large number of military installations and high plant diversity. The Southeast also has a large number of rare plant species (Estill and Cruzan 2001; Sorrie et al. 2006). Twenty-five federally threatened and endangered (TES) plant species occur on southeastern DoD installations in U.S. Fish and Wildlife Service (USFWS) Region 4. An additional 81 DoD-designated plant species at risk (SAR) are also found on these same installations (NatureServe 2011, 2014), representing a disproportionate percentage (~25%) of all plant SAR known to occur on DoD installations. Finally, an additional 30 plant species known to occur on DoD installations in the Southeast have recently been petitioned for federal listing under the Endangered Species Act (Center for Biological Diversity 2010), representing 17% of all SAR in the region.

As in other regions of the United States, many circumstances threaten the long-term viability of rare plant populations in the Southeast, including habitat loss due to urbanization and development, intensive agricultural and forestry practices, fire suppression, emerging pathogens and invasive species. The situation is compounded in the Southeast, which has experienced high levels of development and population growth in the last 50 years (Brown et al. 2005). Among other reasons, this exurbanization is attributable to the fact that most land in the Southeast is privately owned. This factor differs from other regions of the country with extensive DoD lands, where most property is publicly owned. Consequently, military installations in the Southeast are subject to extensive urban encroachment, and increasingly the burden of managing listed and at-risk plant species is left to DoD (Stein et al. 2008). This stewardship burden has an impact on military readiness through restrictions on training area use. While most installations engage in some form of ecosystem management, listed and at-risk plant populations are still being lost on many military lands (Gray et al. 2003; Wall et al. 2013). Even in cases where population numbers are stable, there is still a need for additional conservation strategies and mitigation options.

Population reintroduction—the establishment of plant populations in currently unoccupied historical locations using source material from natural populations—is a potentially valuable strategy in a broader suite of plant conservation strategies (Maunder 1992). Reintroduction is necessarily dependent upon successful propagation of the target species, but a lack of propagation information for most species limits its use. Fortunately for one federally endangered (*Lysimachia asperulifolia* Poiret) and four at-risk plant species (*Amorpha georgiana* Wilbur, *Astragalus michauxii* [Kuntze] F.J. Hermann, *Lilium pyrophilum* M.W. Skinner and B. Sorrie, and *Pyxidanthera brevifolia* B.W. Wells) that occur across multiple DoD installations (Figure 1),

Figure 1: Flowers of *Amorpha georgiana* (upper left), *Astragalus michauxii* (upper right), *Lilium pyrophilum* (middle left), *Lysimachia asperulifolia* (middle right) and *Pyxidanthera brevifolia* (bottom).

newly available propagation protocols beyond proof-of-principle are available for population reintroductions (Weeks 2004; Marchin et al. 2009; Wall et al. 2010; Kunz et al. 2014), but need to be successfully demonstrated. Specifically, these protocols need to be scaled up to multiple, operational-scale reintroduction sites and evaluated over an adequate time period to satisfy regulatory requirements for approval. These new propagation protocols are a product of significant prior Army investment in listed and at-risk plants in the southeastern United States (e.g., Marchin et al. 2009; Wall et al. 2010), and follow-through demonstration is needed to fully realize and evaluate the payoff of this investment.

Historically, the success of rare-plant reintroductions has been low. Recent advances in restoration science have highlighted the complexity of factors—such as genetics, site conditions, and growth stage of individuals outplanted—that are relevant for successfully establishing new populations (Godefroid et al. 2011). Higher success rates are more likely to be achieved through comprehensive reintroduction strategies that attempt to address multiple factors important for positive population growth rates. Unfortunately, comprehensive strategies are rarely adopted; reintroduction efforts are usually piecemeal because of external constraints such as limited information, duration, and funding support. Although failures are often not published in peer-review journals or included in technical reports, anecdotal evidence shows that many failures result from not properly accounting for factors that influence reintroduction success. In many reintroduction efforts the objectives are poorly defined or unmeasurable, the metrics for success are vague or hard to quantify, and cost evaluations are limited in terms of money and time. Those shortcomings are unfortunate because resource limitations mean that managers, biologists, and other conservation stakeholders need better evidence to determine which methods and technologies will maximize the return on investment. End users need to know not only that populations of species can be established, but also at affordable costs.

Federal listing of the four SAR targeted by this proposal would have significant negative impacts on training activities at the six installations where they occur: Camp Lejeune, Camp Mackall, Fort Bragg, Fort Gordon, Fort Jackson, and Military Ocean Terminal Sunny Point. On Fort Bragg and Camp Mackall alone, the five species occur at 420 different sites that total 602 acres. The scattered distribution of these populations fragments the installation landscape, reducing training potential (Figure 2), which is a primary concern of military trainers. Operational-scale demonstration of propagation and reintroduction technologies for the aforementioned rare plants are expected to provide multiple benefits, including improved reintroduction success, lower reintroduction costs, reduced restrictions on training land use, expanded conservation strategies, new opportunities to share conservation responsibility with partner agencies and organizations, and reduced likelihood that SAR become federally listed.

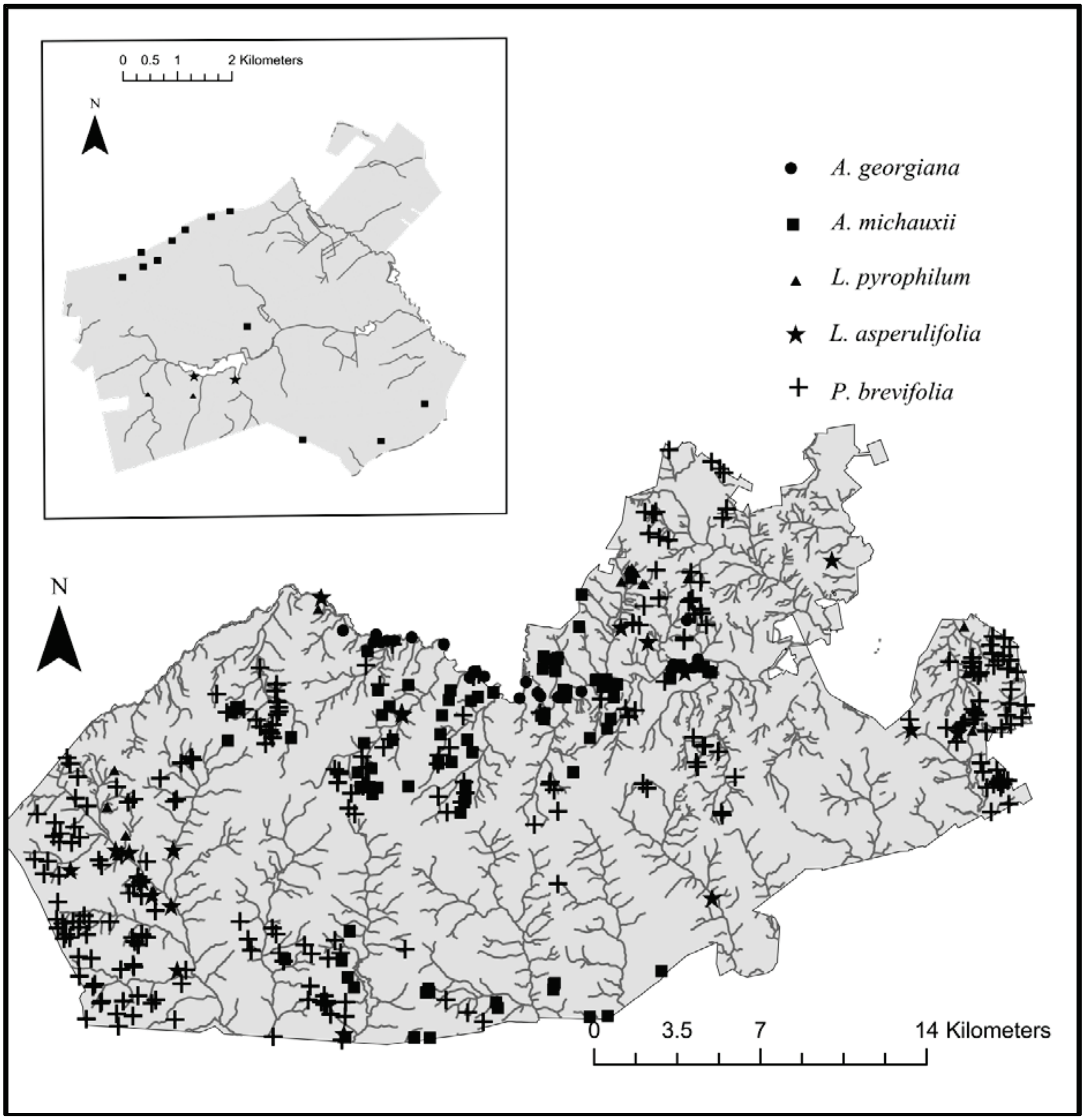

Figure 2: Distribution the five demonstration species across Fort Bragg and Camp Mackall (insert).

1.2 OBJECTIVE OF THE DEMONSTRATION

The overall objective of this project was to perform an operational scale demonstration of recently developed propagation protocols for reintroducing one endangered and four at-risk plant species that occur across multiple military installations in the Southeast. The project's general performance objectives were to (1) demonstrate that four populations per target species can be successfully reintroduced at an operational scale, (2) optimize cost for establishment of self-sustaining populations using data collected from natural and reintroduced populations, and (3) secure user acceptance. The overarching objectives were to increase the diversity and success of rare-plant conservation strategies available to end users.

1.3 REGULATORY DRIVERS

All federal land management agencies are required to comply with federal environmental laws and regulations. This demonstration specifically addressed the compliance challenges posed by the Endangered Species Act (ESA) of 1973. Protection of threatened or endangered plants under the ESA varies depending on whether the species is located on federal or private property. The no-take provisions under the act, which prohibit landowners from causing harm to listed species, apply only to animals. Listed plants occurring on federal lands are protected from removal, possession, import/export, interstate or foreign commerce, and malicious damage. In contrast, listed plant species are offered few protections on private lands because the ESA is invoked only when a federal action is involved. Although it is lawful for a State to have more restrictive laws and regulations governing the taking, possession, and transporting of plants, such laws are uncommon. None of the States within the ranges of the five target species have more restrictive regulations than the ESA concerning rare plants, and in South Carolina rare plants are not offered any protections (Table 1) (see Georgia Wildflower Preservation Act of 1973 [OCGA 12-6-170], North Carolina Plant Protection and Conservation Act [02 NCAC 48F .0301-.0413], South Carolina Nongame and Endangered Species Conservation Act [South Carolina Code Ann. 50-15-10], Virginia Endangered Plant and Insect Species Act [10 Code of Virginia 3.1 1020-1030]). Consequently, successful conservation of rare plants depends on federal and State land management agencies, as well as willing private landowners.

This demonstration also directly addressed the proactive and integrated approach to sustainable land management demanded by recently issued DoD Instruction 4715.3, "Natural Resources Conservation Program." This instruction states DoD components and installations should (1) ensure that sensitive natural resources, such as biologically or geographically significant ecosystems or species, are monitored and managed for their protection and long-term sustainability, (2) ensure no net loss to the training and testing capability and capacity of the installation and enhance those capabilities to the maximum extent practicable, (3) participate in off-installation conservation banks and recovery credit systems for federally listed TES if they are cost-effective and contribute to species recovery, (4) establish policies and procedures for the management of SAR, prioritizing proactive management that has the greatest potential to prevent the listing of SAR, and (5) consider entering into cooperative agreements with States, local governments, non-governmental organizations and individuals to provide for the maintenance and improvement of natural resources or conservation research on, or off DoD installations.

Table 1: State protection status of five target species in the states where they occur. "None" indicates that the species occurs in the state but is not protected, and "NA" indicates that the species does not occur in the state.

Species	State Protection Status			
	Georgia	**North Carolina**	**South Carolina**	**Virginia**
Amorpha georgiana	Endangered	Endangered	none	NA
Astragalus michauxii	Threatened	Special Concern, Vulnerable	none	NA
Lilium pyrophilum	NA	Endangered	none	none
Lysimachia asperulifolia	NA	Endangered	none	NA
Pyxidanthera brevifolia	NA	none	none	NA

2. TECHNOLOGY/METHODOLOGY DESCRIPTION

This chapter describes the demonstrated technologies, including their advantages and limitations.

2.1 TECHNOLOGY/METHODOLOGY OVERVIEW

Reintroduction is not only a common recovery strategy for federally listed plant species, but also a potential means for curtailing the need to list at-risk plant species in the face of continued population declines. Technologies both necessary and sufficient for reintroduction of one endangered and four at-risk plant species found on multiple DoD installations in the Southeast have recently been developed. These technologies exist as newly established propagation protocols (Weeks 2004; Marchin et al. 2009; Wall et al. 2010; Kunz et al. 2014), test-plot field trials, and state-of-the-science population reintroduction best-practice guidelines (Maschinski et al. 2012). These technologies combined with comparative demographic modeling methods are the focus of this demonstration and validation project. A generic overall flow diagram of the technology is shown in Figure 3.

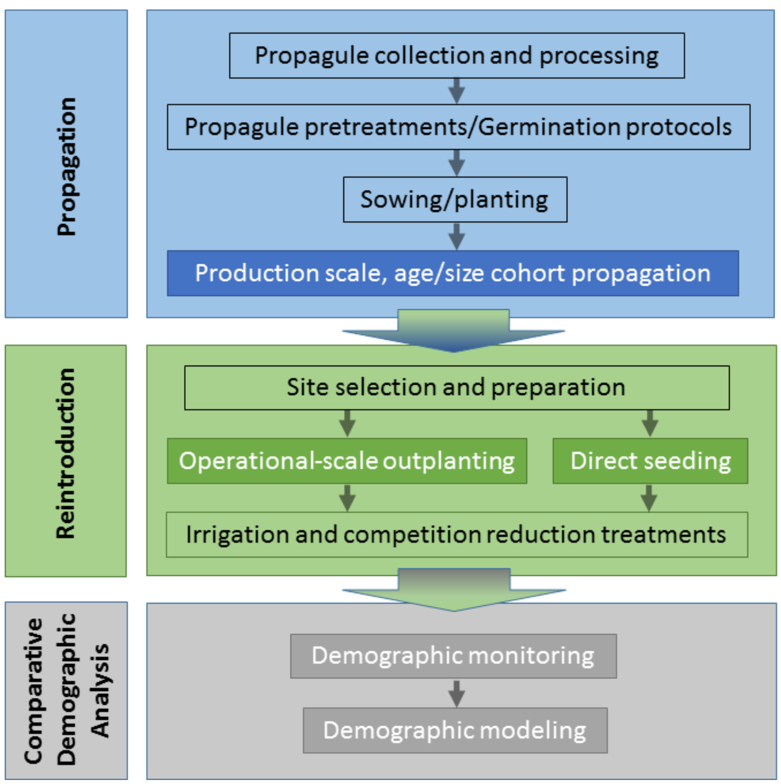

Figure 3: Flow diagram showing sequential steps of the methodology and the technology components evaluated (contrasting bubbles).

Technology development (e.g., germination, propagation, outplanting trials, reintroduction best practice guidelines) was completed by the project team members and others prior to initiation of this ESTCP demonstration/validation project. We successfully germinated and propagated *A. georgiana* under greenhouse conditions (Marchin et al. 2009). Twenty *A. georgiana* individuals that remained at the end of the germination and growth experiment were outplanted at a previously occupied site in a pilot study, with 75% survivorship two years post-reintroduction and half of the surviving plants successfully producing seeds (W. Hoffmann unpub. data). The North Carolina Botanical Garden (NCBG) and University of North Carolina at Chapel Hill (UNC) had collected and successfully germinated *L. pyrophilum* seeds from three existing populations using established protocols for hypogeal seed (Baskin and Baskin 1998). In addition, 575 one- and two-year-old plants propagated from two source populations were available for reintroductions prior to the start of this demonstration. Direct seeding of *L. pyrophilum* into over 13 seed-addition plots using two separate methods yielded successful recruitment and survivorship under field conditions for three years (M. Kunz and W. Wall unpub. data). *Lysimachia asperulifolia* had been successfully propagated from vegetative material with demonstrated survivorship of outplanted individuals for more than 6 years (Kunz et al. 2014). We had also successfully germinated *P. brevifolia* seeds from multiple populations and across multiple years (Wall et al. 2010). Finally, successful germination and field establishment of *A. michauxii* had been demonstrated previously (Weeks 2004), with survivorship of outplanted individuals for more than 6 years. No capability to reintroduce these species existed prior to our development of these propagation protocols.

State-of-the-science population reintroduction best practices, recently consolidated into a set of guidelines by the Center for Plant Conservation, were available for use as a framework in conducting our demonstration (Maschinski and Haskins 2012). Adopting these guidelines was intended to help ensure success of our operational-scale demonstration, but the guidelines can also promote acceptance by regulatory agencies involved in determining the appropriateness of reintroduction as a suitable conservation strategy for the five subject species. These guidelines, which expand upon previous work (Falk et al. 1996; Vallee et al. 2004), summarize critically important steps necessary for any successful reintroduction effort, including (1) assessing the appropriateness of reintroduction as a conservation strategy for target species, (2) designing the reintroduction in light of necessary legal, management, funding, biological, horticultural, and site condition considerations, (3) implementing the reintroduction, (4) providing site and plant maintenance, and (5) designing monitoring plans to assess reintroduction success. These guidelines also incorporate new insights gained by meta-analyses of numerous plant reintroduction efforts, particularly in relation to the importance of applying demographic approaches to monitor population viability. Demographic analysis of (re)introduced populations has largely been overlooked by conservation scientists, regulators, and rare-plant managers. Although these statistical modeling methods have been developed previously, the methods have rarely been utilized to analyze data collected from reintroduced populations and, to our knowledge, had not been used to help determine success in demonstration/validation projects. It is this new emphasis on the application of demographic analyses (Colas et al. 2008) that particularly warranted validation in our proposed operational-scale demonstration. This approach to monitoring reintroductions offers highly informative, defensible, and temporally expedient metrics by which to evaluate success.

There are numerous potential constraints on the use of reintroduction as a conservation strategy for rare plants. Beyond the basic capability to successfully propagate species from seed and/or vegetative stock, in many cases it is essential to understand species population genetic structure, demographic vital rates (e.g., survivorship, growth, seed production, and seedling establishment), habitat preferences and physiological tolerances. In 2011, ERDC, NCSU and Fort Bragg completed an Army direct-funded, four-year research effort examining various aspects of the population genetics, demography, phylogeography, physiology, and habitat requirements of multiple southeastern plant SAR. For three of the five species proposed in this demonstration (*A. michauxii, L. pyrophilum, P. brevifolia*), we had preliminary demographic data available (Wall et al. 2012). Demographic monitoring of natural *A. georgiana* populations on Fort Bragg was also initiated in 2011 as part of a separate four-year, Army direct-funded research effort focused on elucidating rare plant physiological, demographic and community-scaled response to varying fire regimes. Additionally, stem-count data for all known populations of all five species were available on Fort Bragg and Camp Mackall from two surveys, one conducted from 1991–1993 and the other in 1999. In addition, a third survey was conducted in 2005 to record presence/absence of populations.

Population genetic data for the five species were also available to assist in selecting sites and identifying suitable source populations. The population genetic structure of *A. georgiana* (Straub and Doyle 2009), *A. michauxii* (Wall et al. 2014), *L. pyrophilum* (Douglas et al. 2011), and *P. brevifolia* (Godt and Hamrick 1995; Wall et al. 2010) were known in detail. A preliminary population genetic study of *L. asperulifolia* had been performed, with the data suggesting low levels of genetic variation within populations and significant differentiation between populations (Edwards 2007). A separate study of *L. asperulifolia* found that low seed set is due to pollinator limitation, varying levels of fertility, and S allele incompatibility within populations, which can lead to individuals within a population being unable to successfully reproduce (Franklin et al. 2006). This information was used to guide the selection of reintroduction sites and populations for vegetative source material in a manner that will limit detrimental effects of inbreeding.

Applications of the demonstrated propagation and reintroduction technologies are expected to grow where operational-scale demonstration shows acceptable efficacy and cost efficiency. DoD TES managers will be able to greatly expand the types of conservation actions they implement and facilitate additional conservation partnerships. For example, successful operational-scale demonstration of population reintroduction will increase the likelihood that TES managers, regulators and decision-makers will adopt proactive (as opposed to reactive) conservation strategies. Population reintroductions can be used to enhance metapopulation connectivity and gene flow between natural populations that would otherwise be separated and vulnerable to stochastic genetic and demographic processes. They also provide opportunities to adopt mitigation actions, thereby precluding gradual losses of populations that can push species toward federal listing. Additionally, non-DoD entities will be able to expand their participation in conservation of the target species by reintroducing populations to their landholdings. This would increase the number of documented viable populations for the respective species, which could contribute greatly to TES recovery (i.e., delisting or downlisting in the case of *L. asperulifolia*), or significantly reduce the likelihood that SAR will become federally listed.

The results of this demonstration were also intended to provide a basis for designing, costing, and validating reintroduction efforts for other threatened, endangered, and at-risk plant species that share similar life history traits with the five targeted species. When summarized as reintroduction guidelines, this information may have broad applicability throughout DoD. For example, the most obvious and immediate applicability is to three SAR from the Southeast that are congeneric to the target species. These congeneric species are *Amorpha confusa* (Wilbur) S.C.K. Strab, Sorrie, and Weakley, *Lilium iridollae* Henry, and *Lysimachia fraseri* Duby. *Amorpha confusa*, which was recently split from its sister species *A. georgiana* (Straub et al. 2009), is found on Military Ocean Terminal Sunny Point. *Lilium iridollae* has been recently proposed for federal listing under the ESA and occurs on Eglin Air Force Base, Harold Outlying Landing Field and Saufley Field. *Lysimachia fraseri* occurs on Fort McClellan. Additionally, there are fourteen SAR within the genus *Astragalus* that occur on numerous DoD installations across the country.

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY/METHODOLOGY

The availability of proven alternative propagation methods for the five target species is limited. The NCBG has explored bud-scale propagation of *L. pyrophilum*, which has been used for other North American lilies (Heus 2003) but has had only limited success. Plants produced by this method had poor survivorship in greenhouse trials (M. Kunz and J. Randall, unpub. data) and the method requires physical disturbance of mature rhizomes in natural populations. In a pilot study ERDC-CERL and Fort Bragg were able to divide individual *P. brevifolia* clumps into separate physiological units and transplant into new locations, showing survival and reproduction of individuals for as long as six years. However, the number of individuals that can be propagated by this method is substantially fewer than what can be produced from seed. Splitting and transplanting also requires physical disturbance of mature plants from natural populations.

The main advantage of the technologies featured in this demonstration (see Figure 3 and section 2.1) is that they are cost-effective compared to micro-propagation techniques such as tissue culture, which has been used with other species. Tissue culture also has the potential for reducing genetic diversity in reintroduced populations if selection of source genotypes is limited. Limitations of our technique are (1) reliance on seed sourced from natural populations and (2) moderately long propagation times (greater than 3 years) for one species (*L. pyrophilum*). Major cost considerations are labor required for propagation and facility costs. Reductions in the time spent propagating individuals, can potentially reduce the cost of establishing new populations.

Once propagation and reintroduction technologies become available for rare species, and population reintroductions are attempted, managers want to know whether these reintroductions are successful. Unfortunately, success is often poorly defined or evaluated in ways that are either uninformative, prone to failure, or produce overly optimistic results. Presently, methods tend to rely solely on censuses of surviving individuals, which are then used to estimate short-term survival rates or population trends in the rare case where monitoring is conducted for a sufficiently long duration (10 years or more). In contrast, comparative demography can separate contributions of survivorship, growth, and reproduction by stage class to the population growth rate, and cost per capita for establishment of species outplanted at different stages. These metrics are information-rich and scientifically defensible. They represent vast improvements over the *status quo* approach for documenting population viability, which is based on simple regression analysis and requires at least ten years of count data to achieve a trend estimate with sufficient power.

3. PERFORMANCE OBJECTIVES

The performance objectives (POs) developed for this demonstration were specifically designed to evaluate success based on end-user needs, with emphasis given to robust assessment of economic feasibility and population viability. Performance objectives related to propagation and reintroduction technologies are summarized collectively in Table 2. The first PO represents a qualitative assessment of demonstration success, while all other POs focus on quantitative assessment. Demographic studies benefit from collection of data over multiple time steps. Consequently, the performance time for all POs spans multiple years. Detailed descriptions of the performance objectives follow.

PO1: Gain user acceptance

PO1 was a qualitative performance objective focused on characterizing end-user acceptance of the new technologies. This was a critical component of the overall demonstration, since without end-user acceptance the new technologies are unlikely to affect real-world change. The metric we used to evaluate whether PO1 was met was the willingness of end users to utilize the new technologies. This metric was estimated in two different ways—through observation of end-user acceptance and the results of a graded survey with results on a Likert scale of 1–5. The data required to evaluate the metric were Integrated Natural Resource Management Plan (INRMPs) and rare-plant management plans, non-DoD management plans for relevant areas adjacent to installations, and results from graded surveys of end-users acceptance. Surveys were developed during the course of the project. Success criteria were based on (a) acceptance of the new technologies and/or incorporation of the new technologies into both DoD and relevant non-DoD management plans, and (b) attaining an average score ≥4.0 for questions on the graded survey. Since documenting integration of the technologies into the management plans of multiple organizations is subject to many uncontrollable external factors, the graded survey was added to ensure a means of assessing user acceptance.

Table 2: Performance objectives used to evaluate propagation and population reintroduction technologies (continued to next two pages).

Performance objective	Metric	Data requirements	Success criteria	Results
Qualitative Performance Objectives				
1. User acceptance	Willingness of users to adopt demonstrated technologies	a. Installation INRMPs and rare plant management plans, non-DoD management plans, b. Results of a graded survey of user acceptance	a. Outright adoption and/or incorporation of technologies into installation INRMPs and rare plant management plans, as well as non-DoD management plans, b. average score for questions on graded survey distributed to potential users ≥ 4.0	Technology adoption *A. georgiana:* Yes *A. michauxii*: Yes *L. pyrophilum*: No *L. asperulifolia*: Yes *P. brevifolia*: No Survey results *A. georgiana:* Yes *A. michauxii*: Yes *L. pyrophilum*: Yes *L. asperulifolia*: Yes *P. brevifolia*: No
Quantitative Performance Objectives				
2. Demonstrate establishment of four self-sustaining, viable populations for all species.	Population growth rate(s)	Vital rates of reintroduced and natural populations	Individual population growth rates of reintroduced populations ≥ natural populations with population growth rates > 1 four years post-reintroduction	*A. georgiana:* No *A. michauxii*: No *L. pyrophilum*: No *L. asperulifolia*: No *P. brevifolia*: No
3. Demonstrate equivalent or better *in situ* seedling recruitment in reintroduced versus natural populations of *A. georgiana*, *A. michauxii*, *L. pyrophilum*, and *P. brevifolia.*	*In situ* seedling recruitment rate	Seed production and *in situ* seedling establishment for both natural and reintroduced populations	Seedling recruitment rates in each reintroduced population ≥ recruitment rates in natural populations four years post-reintroduction	*A. georgiana:* No *A. michauxii*: No *L. pyrophilum*: No *P. brevifolia*: No

Performance objective	Metric	Data requirements	Success criteria	Results
4. Demonstrate equivalent or better survivorship and transition probabilities in reintroduced versus natural populations for all size classes of all species.	Survivorship and transition probabilities for all size classes in natural and reintroduced populations	Survivorship and transition data for individuals across multiple years and populations	Survivorship and transition probabilities for all size classes in each reintroduced population ≥ natural populations four years post-reintroduction	Survivorship *A. georgiana:* No *A. michauxii*: No *L. pyrophilum*: No *L. asperulifolia*: No *P. brevifolia*: No Transition probabilities *A. georgiana:* No *A. michauxii*: No *L. pyrophilum*: No *L. asperulifolia*: No *P. brevifolia*: No
5. Meet maximum cost threshold for growing and outplanting individuals of different size classes for all species to achieve positive population growth rate.	Cost per individual necessary to achieve a positive population growth rate	a. Production and outplanting cost per unit time per individual, b. Distinct contributions of survivorship, growth, and reproduction by size class to the population growth rate	Production and outplanting cost per individual for most cost-efficient size class does not exceed $175.00	*A. georgiana*: Yes ($11.99) *A. michauxii*: Yes ($18.32) *L. pyrophilum*: Yes ($32.82) *L. asperulifolia*: Yes ($38.55) *P. brevifolia*: No
6. Demonstrate improved cost-effectiveness of watering (*A. georgiana*, *A. michauxii*) or site maintenance (*L. pyrophilum*) on transplant survival.	Cost per surviving individual	Fixed and variable costs of providing water and site maintenance	Cost per surviving individual of watered or site-maintained transplants ≤ non-watered and/or site maintained transplants	Watered *A. georgiana:* No *A. michauxii*: No Site Maintained *L. pyrophilum*: Yes
7. Optimize cost for establishment of self-sustaining populations of all species.	Cost per capita for population establishment	Fixed and variable costs of propagating and reintroducing different size classes	Cost of establishing populations with growth rates ≥ 1 does not exceed $10,000	*A. georgiana:* No *A. michauxii*: No *L. pyrophilum*: No *L. asperulifolia*: No

Performance objective	Metric	Data requirements	Success criteria	Results
				P. brevifolia: No
8. Demonstrate efficacy and cost-effectiveness of establishment of individuals via seed-addition plots versus transplanted individuals for *A. georgiana*, *L. pyrophilum*.	a. Cost per recruited individual in seed-addition plots, b. Seed needed to establish viable populations is not limited by availability	Cost per recruit within seed-addition plots, transition probabilities, transplant cost, and seed production data	a. Cost per individual recruited in seed-addition plots does not exceed cost per individual transplant, b. Seed needed to establish viable populations via direct seeding within an equivalent timeframe for establishing transplants does not exceed 10% of average seed availability from natural populations	Cost *A. georgiana:* No *L. pyrophilum*: No Seed needed *A. georgiana:* No *L. pyrophilum*: No
9. Demonstrate cost effectiveness of watering *A. georgiana* seed-addition plots.	Cost per recruited individual	Cost per recruit within seed-addition plots, fixed and variable costs of providing supplemental irrigation	Cost per recruited individual in irrigated seed-addition plots ≤ cost per recruited individual in non-irrigated seed-addition plots	*A. georgiana:* No No individuals recruited into either irrigated or non-irrigated seed-addition plots

PO2: Establishment of four self-sustaining viable populations

PO2 addressed the establishment of four self-sustaining populations of the five target species. While end-user acceptance of the new technologies is critical, PO2 demonstrated that the developed technologies can be scaled up to an operational population reintroduction scale. This was of significant importance to the project and was, along with PO7 (cost optimization), one of the two most important POs that determined user acceptance. The metric that we used to calculate the success of PO2 was the population growth rate for individual reintroduced populations of the five target species. The data required to calculate the population growth rates for the five target species included survivorship, growth, and seed production for each of the size classes, as well as germination rates and seedling survivorship for each of the target species. Detailed information regarding how these data were collected is presented in section 5.5 (Sampling Protocol). Our measure of success was whether the population growth rate of reintroduced populations was greater than or equal to natural populations with growth rates greater than 1. The performance time for this objective was four years post-reintroduction. For PO2 through PO9, the endpoint criterion is met when we have collected data across multiple time steps in order to incorporate temporal variation into our demonstration. Our study design provided four, three and two time steps for the individuals outplanted in 2012, 2013 and 2014, respectively.

PO3: Equivalent or better *in situ* seedling recruitment in reintroduced vs. natural populations

PO3 was common to all five target species and established a goal of demonstrating equivalent or higher rates of *in situ* seedling recruitment (and asexual stem recruitment in the case of *L. asperulifolia*) in reintroduced populations relative to natural populations. The metric we used to evaluate success was *in situ* recruitment rate. For all species except *L. asperulifolia,* recruitment rate was defined as the number of seedlings established divided by the total seed production in a population. To calculate the metric we developed estimates of seed production and seedling establishment for each reintroduced and natural population. For *L. asperulifolia,* recruitment rate was defined as the number of stems counted or estimated within each reintroduced and natural population at time $t+1$ divided by the number of individuals at time t. The performance objective was considered met if the *in situ* recruitment rates in the reintroduced populations was greater than or equal to *in situ* recruitment in the natural populations. The performance time for this objective was four years post-reintroduction.

PO4: Equivalent or better survivorship and transition probabilities in reintroduced vs. natural populations for all size classes

PO4 established a goal of demonstrating equivalent or better survivorship and transition probabilities for all size classes in reintroduced populations of the five target species relative to natural populations. This PO was relevant for evaluating population reintroduction because it specifically compared demographic vital rates, which not only contribute to overall population growth rate and viability but are also informative for determining effective size structures within reintroduced populations. The metrics for this PO were population-based estimates of survivorship and transition probabilities for all size classes. Survivorship was computed as the number of individuals alive at time *t+1* divided by the number of individuals alive at time *t*. Transition probabilities were calculated by dividing the number of individuals in size class X at time *t+1* by the number of individuals in size class X at time *t*. The performance time for this objective was four years post-reintroduction. The performance objective was considered met if the survivorship and transition probabilities for all size classes of the reintroduced populations were equal to or greater than the natural populations.

PO5: Meet maximum cost threshold for growing and outplanting individuals of different size class to achieve positive population growth rate

PO5 was common to all five target species and established a goal of meeting a maximum cost threshold for growing and outplanting individuals of different size class to achieve positive population growth rates. The metric was the cost in dollars per individual necessary to achieve a positive population growth rate. The metric was calculated by dividing the production and outplanting cost (in dollars) by the survivorship probabilities of the size classes calculated for PO4. For example, if cost was $100 and survivorship was 100%, then cost would be $100.00 per individual. However, if cost was $100.00 but survivorship was only 0.5, then the cost per outplanted individual would be calculated as $200 ($100/0.5). Data requirements for PO5 were cost in dollars for production and outplanting per individual and the survival probabilities of each size class for the five target species. PO5 was met if the most-efficient size class for outplanting of the five target species did not exceed $175.

PO6: Improved cost-effectiveness of site maintenance (competitor reduction) or watering for transplant survival

PO6 focused on demonstrating a reduced cost for the successful establishment of outplanted individuals that receive either site maintenance (competition reduction; *L. pyrophilum*) or supplemental watering (*A. georgiana, A. michauxii*) treatments relative to the cost determined in PO5. This PO was important because it addressed relevant biotic and abiotic factors that may potentially limit population reintroduction success. The metric used to determine success was the cost in dollars per individual. Data requirements were the fixed and variable costs associated establishing individuals that received either treatment. Cost was determined as it was for PO5, with the only difference being that the cost of the treatments (competitor reduction or supplemental watering) were included. The performance objective was met if the cost per surviving individual was lower when provided either supplemental water or competitor-removal treatments relative to the transplants that did not receive any treatments.

PO7: Optimize cost for establishment of self-sustaining populations

PO7 was to optimize the cost of establishing self-sustaining populations of the five target species. This PO was relevant because rare-plant conservation is greatly underfunded, so minimizing costs is important for user acceptance. The metric was the cost per self-sustaining population. A self-sustaining population was defined as a population with a population growth rate greater than or equal to 1. Data requirements were the fixed and variables costs in dollars for the successful establishment of individuals at different size classes. PO7 was considered successfully met if the cost in dollars per self-sustaining population did not exceed $10,000.

PO8: Demonstrate efficacy and cost-effectiveness of establishment of individuals via seed-addition versus transplanted individuals

PO8 demonstrated the efficacy and cost-effectiveness of establishing individuals via seed-addition plots relative to transplanting individuals for *A. georgiana* and *L. pyrophilum*. Reintroducing populations via seed-addition may not be feasible for all rare species, as many have naturally low recruitment rates. Our data collected prior to this project suggested that *A. georgiana* and *L. pyrophilum* recruit from seed quite readily and that survivorship is high enough to warrant further exploration as a means of population establishment. The metrics for determining success for this PO were (a) cost per recruited individual via seed-addition plot and (b) number of seeds needed to establish viable populations does not exceed seed availability. Data requirements were cost in dollars per recruit within seed-addition plots, cost of establishment of individuals by size class for the two target species, transition probabilities of seedlings, and seed production per population. We considered PO8 met if (a) the cost of establishment per individual in seed-addition plots was less than the cost of establishment via transplanting and (b) the seed needed to establish viable populations via direct seeding within an equivalent timeframe for establishing transplants did not exceed 10% of average seed availability from natural populations.

PO9: Cost-effectiveness of watering seed-addition plots

PO9 demonstrated the cost-effectiveness of watering *A. georgiana* seed-addition plots. This PO was important because it addressed a relevant abiotic factor that may potentially limit the utility of seed-addition as a means of population reintroduction. The metric utilized to measure success was the cost in dollars for each established seedling. Data required were the cost per seedling in both irrigated and non-irrigated seed-addition plots. If the cost per established seedling in the irrigated seed-addition plots is less than or equal to the cost per established seedling in the non-irrigated seed-addition plots, we will consider PO9 successful.

4. SITE DESCRIPTION

The following sections highlight our site selection process, site characteristics, location and history, and site-related permits and regulations.

4.1 SITE SELECTION

The operational scale demonstration of the plant population reintroduction technologies took place at Fort Bragg and Weymouth Woods Sandhills Nature Preserve (NP). The five target species occur broadly across Fort Bragg and Camp Mackall, while single element occurrences (EO) of *L. pyrophilum* and *P. brevifolia* are known from Weymouth Woods (shown in Figure 1, page 2). Collectively, Fort Bragg, Camp Mackall and Weymouth Woods Sandhills NP serve as substantial population centers for all five target species, with approximately 67%, 41%, 44%, 27% and 90% of all known, range-wide occurrences of *A. georgiana*, *A. michauxii, L. pyrophilum, L. asperulifolia* and *P. brevifolia*, respectively. Consequently, these sites represented the unique case where sufficient numbers of potential reintroduction sites and natural populations for demographic comparison are in close proximity.

Four population reintroduction sites were selected for all five target species prior to January, 2012 based on a suite of criteria (Figure 4). First, we identified historical sites where the species are no longer present and assessed their suitability based on habitat condition and type of military training, as well as current and projected use and management (Table 3). For the purposes of the demonstration these historical sites met the needs of both the regulatory and end-user community's requirements. We also coordinated with other regional experts to assess the condition of candidate sites, preferentially choosing those with appropriate plant community type(s) (see section 4.3, Site Characteristics), canopy openness, fire regime, and intact hydrological processes among other parameters. When selecting sites we also attempted to ensure that the target species reintroduction was compatible with military training, as well as other current and planned uses. Finally, where available, we took into account the spatial population structure of the specific target species by positioning reintroduction sites in locations to allow for gene flow and metapopulation processes.

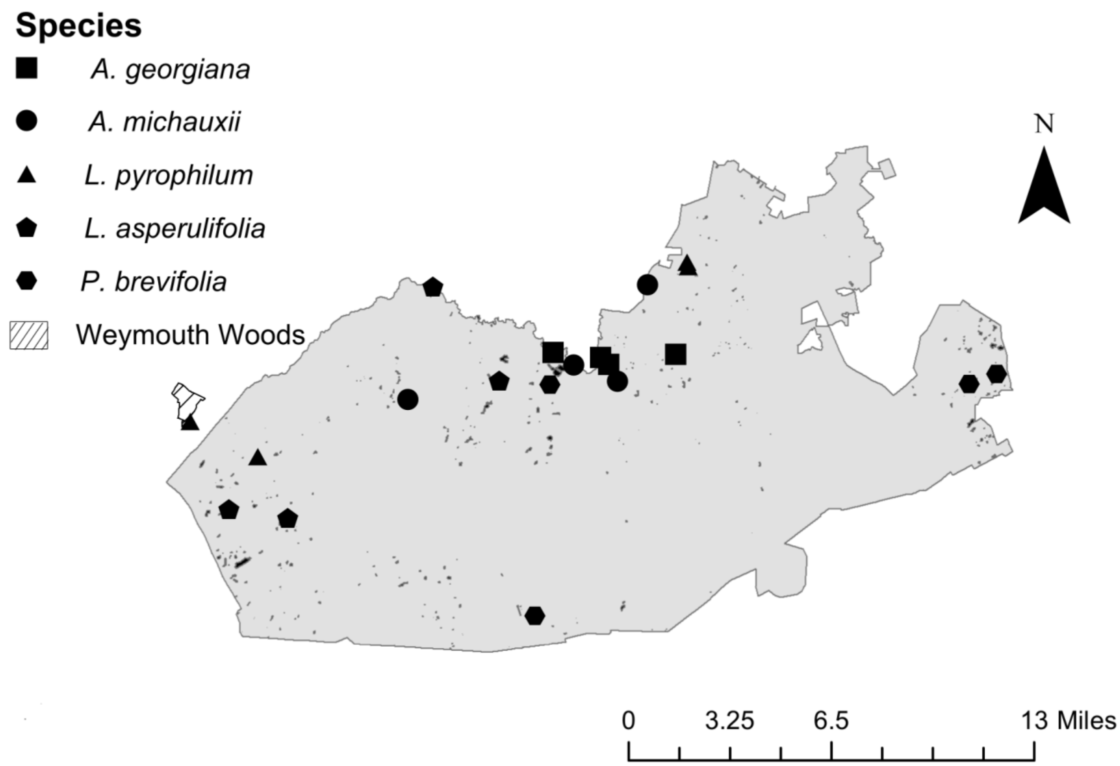

Figure 4: Location of demonstration sites (black polygons) on Fort Bragg (gray) and Weymouth Woods (hatch). Smaller irregular polygons represent distribution of element occurrences for the five target species.

Table 3: Criteria, sub-criteria and associated evaluation used to select population reintroduction sites.

Criteria	Sub-criteria	Evaluation
Habitat condition	Plant community type(s)	Field assessed community type is representative of type(s) known to be inhabited by species
	Canopy openness	Canopy cover ≤ 50%
	Fire regime	Mean fire return interval during the past 20 yrs not ≥ 4 yrs
	Hydrology	Field assessed hydrological integrity of wetland sites is intact
Spatial structure -	none	Site ≤ 1km from one or more extant element occurrence or subpopulation
Compatible land use and management	Military use	Site located ≥ 100 m from bivouacs, staging areas, off-road trails, ranges, drop zones, landing zones, etc.
	Other land use and management	Site located ≥ 50 m from disturbances such as wildlife food plots, pinestraw raking, scheduled timber harvests, etc.

4.2 SITE LOCATION AND HISTORY

Collectively, Fort Bragg and Weymouth Woods Sandhills NP span four counties in south-central North Carolina. The 363 hectare Weymouth Woods Sandhills NP resides entirely within Moore County, while the majority of Fort Bragg's 73,469 hectares reside within Hartnett, Hoke, and Cumberland Counties. These adjacent sites are located in the Sandhills ecoregion of the Atlantic Coastal Plain physiographic province of North Carolina (Griffith et al. 2002; Bailey 1996).

The Sandhills region of North Carolina was first settled by Europeans in the middle to late 1700s. Prior to military use, the area occupied by Fort Bragg and Weymouth Woods Sandhills NP was primarily under sparsely distributed, low-intensity subsistence agricultural and forestry use. Fort Bragg was established as a permanent Army installation in 1922, only five years after the temporary Camp Bragg was created. Over its history the installation has supported diverse training missions, but the 82^{nd} Airborne Division has been the dominant resident component since the early 1940s. Although highly diverse, much of the training is concentrated within seven major drop zones, four impact areas, numerous ranges and airfields. As a consequence, many portions of the installation have not been subjected to repeated high-intensity ground disturbance associated with mechanized infantry and artillery. Under the 2005 Base Realignment and Closure requirements the installation has had an increase in troops and training activities.

Weymouth Woods Sandhills NP was established in 1963, but had previously been a private forest reserve since the early 1900s. Its mission is to preserve the natural features unique to the Sandhills region and to support education and research. It contains some of the only known virgin old-growth longleaf pines in North Carolina.

4.3 SITE CHARACTERISTICS

The Sandhills ecoregion of North Carolina is characterized by rolling hills having moderate to steep side slopes (Wells and Shunk 1931). Fort Bragg and Weymouth Woods Sandhills NP form a large contiguous block of longleaf pine-wiregrass ecosystem and have many ecological and physiographic similarities. The topographic variability characteristic of the region generates substantial moisture gradients that influence plant communities at a scale of several hundred meters. Soils of the highly dissected uplands are well drained sands and loamy sands, supporting fire adapted and xeric vegetation. In low-lying areas the soils are hydric, supporting mesic and less-fire-tolerant vegetation. Sorrie et al. (2006) describe the floristic diversity and 26 natural community types found on Fort Bragg and Weymouth Woods Sandhills NP. The five target species occur in a variety of plant communities with limited overlap between several species (Table 4).

Table 4: Plant communities inhabited by target species on Fort Bragg and Weymouth Woods Sandhills Nature Preserve.

Species	Plant Communities*
A. georgiana	Little River Flatwoods
A. michauxii	Xeric Sandhill Scrub Pine/Scrub Oak Sandhill – Mixed Oak subtype Pine/Scrub Oak Sandhill – Blackjack Oak subtype
L. asperulifolia	Sandhill Streamhead Pocosin Ecotone
L. pyrophilum	Sandhill Streamhead Pocosin Ecotone Small Stream Swamp
P. brevifolia	Xeric Sandhill Scrub Pine/Scrub Oak Sandhill – Clay/Rock Hilltop subtype

* from Sorrie et al. (2006)

Using the Köppen-Geiger system, the climate in North Carolina is classified as Cfa: temperate, without a dry season, and having hot summers (Peel et al. 2007). Normal monthly min/max temperatures on the installation are 33.1/52.7 °F in January and 71.9/90.7 °F in July (Figure 5), with 200-220 mean annual frost free days. Mean annual precipitation in the North Carolina Sandhills is 46.5 inches. Although June, July and August are the wettest months, high temperatures and evapotranspiration rates can produce drought conditions in near-surface soils of sandy upland sites within a few days after heavy precipitation events (Wells and Shunk 1931). In addition to the intra-annual variation in precipitation, longer-term periods of drought and wetness that persist for approximately 30 years have been documented (Stahle et al. 1988). For most species in the Southeast, the demographic consequences of these long-term patterns is poorly known. The North Carolina Sandhills region is also subject to regular tropical storms, but high-intensity storms occur only every few centuries and the region is apparently far enough inland to minimize the damaging effects of wind throw (Batista and Platt 1997; Gilliam et al. 2006; Gilliam and Platt 2006). Although tropical storms are an important natural disturbance affecting mortality of overstory longleaf pines, the demographic response of understory vegetation to this disturbance is poorly known (Menges et al. 2011)

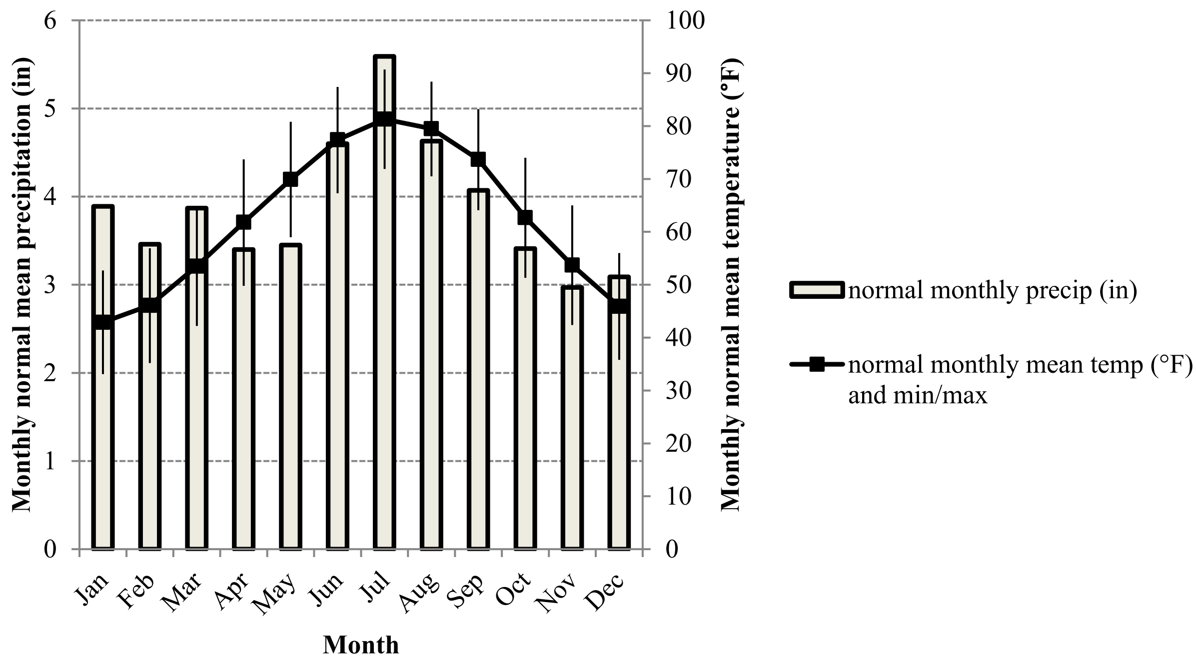

Figure 5: Monthly precipitation (bars) and temperature (mean, minimum and maximum) normals in the North Carolina Sandhills from 1971-2000. Data from the State Climate Office of North Carolina.

5. TEST DESIGN

This chapter provides an outline of the overall test design and the three operational phases of the demonstration.

5.1 CONCEPTUAL TEST DESIGN

Demonstration of our rare plant reintroduction technologies included three operational phases: (1) production-scale propagation, (2) operational-scale reintroduction, and (3) comparative demographic analysis (Figure 6). Although we had no explicit test design of the propagation protocols, we collected relevant data on production success, documenting growth rates, survival and costs of rearing transplants of different size class. This information was used to determine the feasibility and efficiency of production-scale propagation of the target species and was integrated into the demonstration of the reintroduction technology.

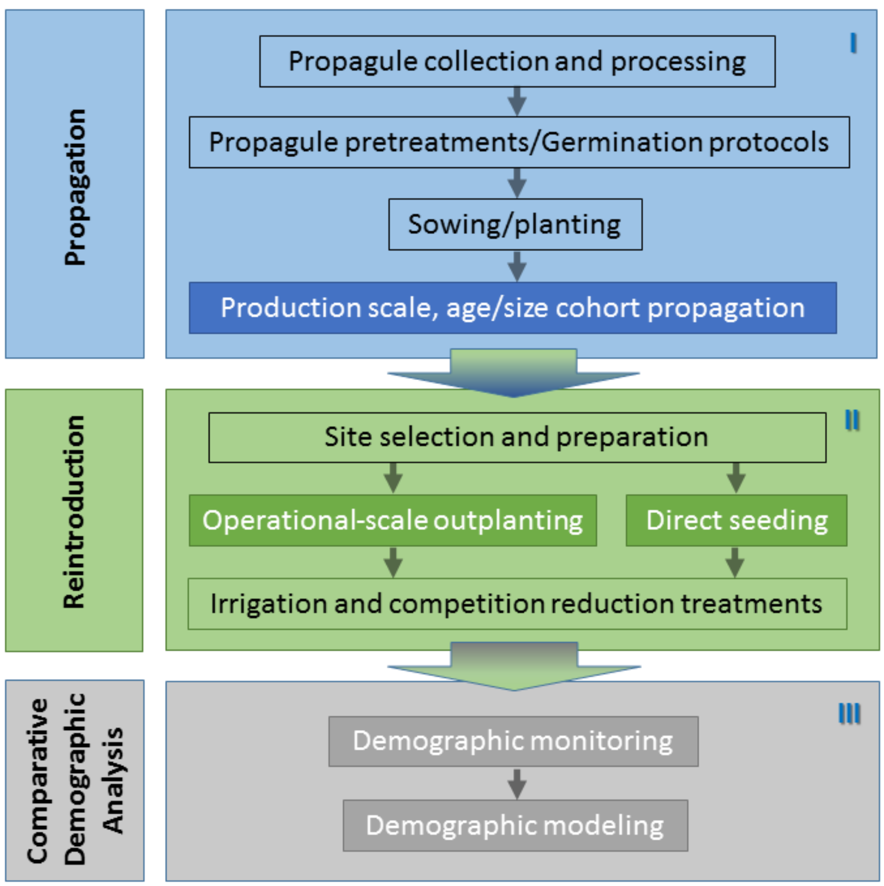

Figure 6: The three operational phases (i.e. propagation, reintroduction, and comparative demographic analysis) of the conceptual test design, showing sequential steps of the methodology, treatments and the technology components evaluated (contrasting bubbles).

Both qualitative and quantitative performance objectives (see Chapter 3, Performance Objectives) were developed to evaluate the operational-scale demonstration of the population reintroduction technologies (phase 2), and thus structured our conceptual test design. The test design addressed the effect of size of propagated individuals at time of outplanting (all species), watering of outplanted individuals (*A. michauxii*, *A. georgiana*), site maintenance (*L. pyrophilum*), and watering of seed-addition plots (*A. georgiana*) on the population growth rate and cost-effectiveness of establishing self-sustaining populations of the target species. The third phase of the test design included collecting and analyzing data on the demographic vital rates (survivorship, growth, and reproduction) of the reintroduced individuals for the duration of the demonstration. Vital rates were assessed as outlined in section 5.5 (Sampling Protocol). Identical types of demographic data collected from natural populations functioned as controls to validate the new technologies. Data collected from natural and reintroduced populations were analyzed using generalized linear mixed effects models and life table response experiments to assess the effectiveness of population reintroduction as a conservation strategy for the five target species. Specific details of data analysis methods are provided in Chapter 6, Performance Assessment.

5.2 BASELINE CHARACTERIZATION AND PREPARATION

Four reintroduction sites for each species were spatially delimited and then characterized using the North Carolina Natural Heritage Program standardized classification of the natural communities of North Carolina (Schafale and Weakley 1990). The approach was similar to wetland delineation in that extant vegetation was utilized to locate suitable habitat. While the utilization of other collected data, such as soil moisture or soil nutrient levels, may have assisted in providing a more accurate delineation of habitat, the financial costs were too high and represented a low return on investment. We collected the following data from each of the reintroduced and natural populations: slope, aspect, soil series, and canopy cover. Slope and aspect were measured in the field using a clinometer and compass, respectively. Soil series was assessed from available GIS layers, and canopy cover was estimated using a spherical densiometer. These data were not included in any of the planned formal analyses, but were used to interpret results from the demonstration.

All sites were prepared for the demonstration with prescribed fires during the 2012 growing season. To reduce the possible short-term negative effects of fire on the vital rates of small individuals, we attempted to reintroduce all populations into recently burned sites that were not scheduled to be burned for three years. Fire prior to reintroduction also reduced competition from currently established vegetation and provided bare mineral soil for seed germination. All reintroduction sites were demarcated to indicate reintroduction work was ongoing and to avoid possible anthropogenic disturbance.

5.3 DESIGN AND LAYOUT OF TECHNOLOGY AND METHODOLOGY COMPONENTS

Technology components were represented in all three operational phases of our conceptual test design (see Figure 3). In this section we describe technology components associated with phase 1 (Propagation). Dates and durations over which technology components were demonstrated are shown in Figure 7. Technology components associated with phase 2 (Reintroduction) are inherently related to section 5.4 (Field Testing), and are described there. Technology components associated with phase 3 (Comparative Demographic Analysis) are inherently related to section 5.5 (Sampling Protocol) and are described there.

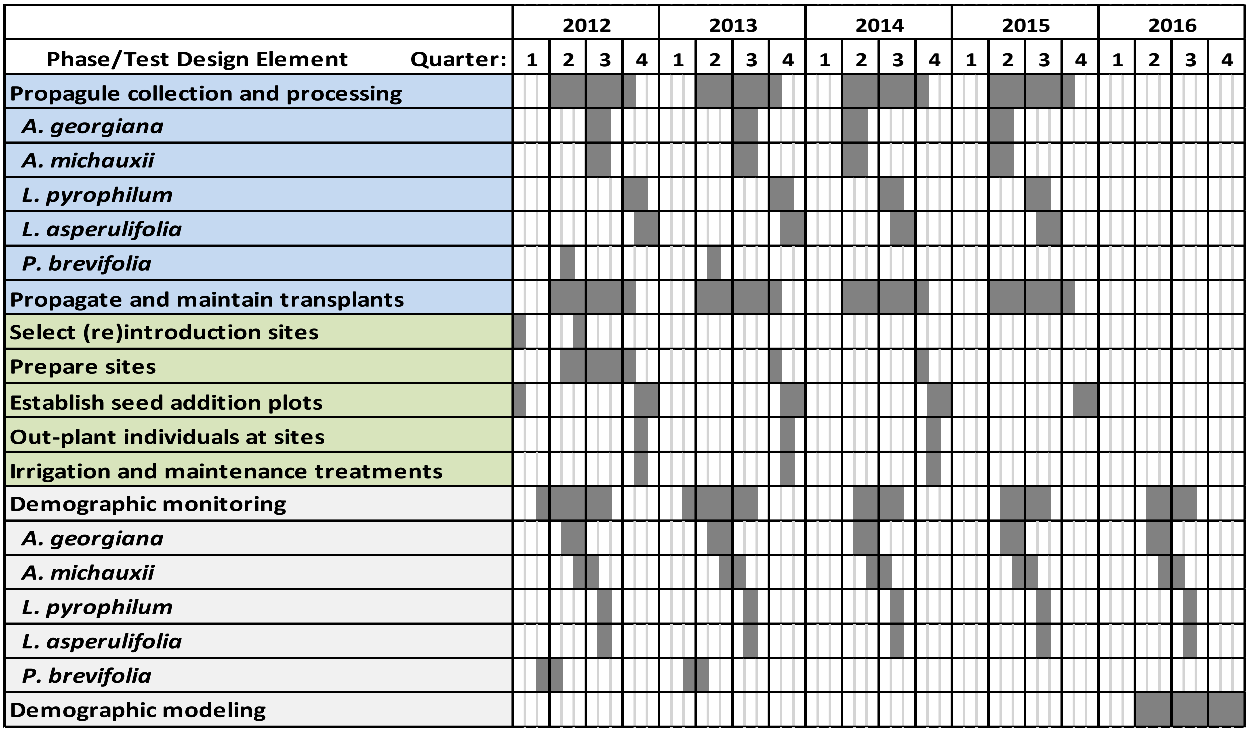

Figure 7: Timelines associated with the demonstration's three operational phases and test design.

As the figure above shows, seeds were collected from *A. georgiana, A. michauxii, L. pyrophilum,* and *P. brevifolia* natural populations over multiple years for use in propagating transplants and comparing the efficacy of direct seeding (see section 5.4, Field Testing). *Amorpha georgiana* was propagated following Marchin et al. (2009). Seeds were mechanically scarified and placed on damp blotter paper within covered Petri dishes. Seeds were kept moist and under light (12 light/12 dark) with temperatures above 20 ˚C. Our previous experience propagating this species suggested germination rates of ~80% should be reached within seven days (Renee Marchin pers. comm.). Upon initiating germination, the radicles of germinated seeds were inoculated with a *Rhizobium* specialized for *Amorpha* spp. (Prairie Moon Nursery, Winona, MN) and placed into 38 cell deep plug trays filled with a media of 2:2:1 sand, peat, and sieved pine bark. Plants were watered every other day and foliar fertilized biweekly at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters Inc., Allentown, PA). After one month of growth, plants received 100 day slow-release fertilizer (13N:13P_2O_5:13K_2O Nutricote Total, Type 100; Arystra Life-Science America, Inc., New York, NY) at approximately 20 g/0.09 m^2. Our test design included rearing transplants of three different age/size cohorts: three, six, and eight months (Figure 8).

Figure 8: ***Amorpha georgiana*** **growing in a shade house.**

Propagation of *A. michauxii* followed recommendations found in Weeks (2004), with minor modifications. Germination rates of 95% were reached by nicking both inner and outer seed coats opposite the hilum with a razor blade. After scarification seeds were treated with *Rhizobium* specific to *Astragalus* spp. (Prairie Moon Nursery, Winona, MN) and sown directly at an approximate depth of 3 mm into 38-cell deep seedling trays filled with media consisting of starter-size Gran-I-Grit (North Carolina Granite Corporation, Mount Airy, NC), Black Gold Seedling Mix (Sun Gro Horticulture, Agawam, MA), vermiculite, filtered pine bark compost in a ratio of 5:2:2:1, and inoculated with native soil. Plug trays were placed in a greenhouse and watered from below as needed to keep the soil moist for two weeks. Germination began within 48 hours and was completed within 7 days.

As *A. michauxii* plants became established, they were watered from above one day after the soil surface began to dry, usually one to two times a week, and foliar fertilized biweekly at 50 ppm ($20N:20P_2O_5:20K_2O$ with micronutrients; Jack's Classic, JR Peters, Inc., Allentown, PA). After one month of growth, plants received 100 day slow-release fertilizer ($13N:13P_2O_5:13K_2O$ Nutricote Total, Type 100; Arystra Life-Science America Inc., New York, NY) at approximately 20 g/0.09 m^2. Our test design included rearing transplants of three different age/size cohorts: three, six, and eight months (Figure 9).

Figure 9: *Astragalus michauxii* cohorts ready to be outplanted.

Propagation of *L. pyrophilum* followed recommendations found in Baskin and Baskin (1998). Seeds collected from *L. pyrophilum* populations were placed in a jar with damp, milled sphagnum (Figure 10). Seeds and sphagnum were sprayed with a low concentration of hydrogen peroxide to reduce microbial/fungal growth. Jars were sealed and kept at 21.1 ˚C for approximately 120 days. At the end of 120 days jars were kept at 4.4 ˚C for 60–90 days. Seeds may form a bulblet during this time.


Figure 10: *Lilium pyrophilum* capsule and seeds (left); seeds germinating in sphagnum (right).

Stratified seeds and bulblets were then sown into #606 deep inserts (TO Plastics, Clearwater MN) containing a soil mix of 1:1 sand and peat and placed into standard 11x21.5 in. flat trays. Trays were placed in sub-irrigation and maintained wet at all times. Plants produce a single leaf the first growing season and in subsequent seasons may continue to produce single leaves (Figure 11) or possibly a short stem with one to many whorls of leaves. Regardless of leaf type, the bulb will continue to grow. Plants were foliar fertilized monthly during the growing season at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters Inc., Allentown, PA). Plants should be of suitable size for outplanting (bulbs approximately 1 cm diameter) after several years. Our test design included rearing bulbs of three different age cohorts: one, three, and four years.

Figure 11: *Lilium pyrophilum* seedlings.

Because germination from seeds is relatively rare for *L. asperulifolia*, propagation is somewhat different than for the other target species. Rhizomes were collected from natural populations on Fort Bragg during the dormant season, as this is recognized to increase survivorship (Kunz et al. 2014). The color of rhizomes harvested in the fall varies from light pink to tan. White to pink rhizomes represent new growth and oftentimes will be associated with a new bud or hibernacle; tan rhizomes are a year old, senescent, and no longer viable (Figure 12). Harvested rhizomes were divided into segments containing at least three nodes, placed in a 1:1 peat moss and sand, and kept at 4.4 ˚C for 90 days, followed by 30 days under mist at 15.6 ˚C/ 4.4 ˚C. At this point, the soil temperature was raised to 21.1 ˚C to maximize length of growing season; rhizomes were grown under these conditions for approximately 90 days to allow for increased rhizome length. Plants were watered daily and foliar fertilized biweekly during the growing season at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters Inc., Allentown, PA). After stems senesced in the fall, the rhizomes were excavated and divided in preparation for reintroduction or another round of propagation. The test design included outplanting rhizomes having 1–12 nodes and varying in length (range = 1.9-23.0 cm, x = 8.8 cm, sd = 3.2 cm).

Figure 12: ***Lysimachia asperulifolia*** **rhizomes in a flat prepared for propagation. Pink viable rhizomes are on the left and tan senescent rhizomes are on the right.**

Pyxidanthera brevifolia seeds that had been allowed to after-ripen (period of time needed to allow for physiological changes that must precede germination in fully developed seeds) for a minimum of two months were placed on Petri dishes with damp blotter paper under light conditions and 14–18 ˚C temperatures; these conditions have yielded 78% germination in *P. brevifolia* seeds (Wall et al. 2010). Seedlings were then placed in 225 mL pots in a 1:2 mixture of peat moss and sand that was inoculated with 20 g of soil from the relocation site(s). Pots were watered every three days with a nutrient solution and flushed weekly with deionized water. As the plants became established, they were watered from above one day after the soil surface began to dry, usually one to two times a week, and foliar fertilized biweekly at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters, Inc., Allentown, PA). Although the described propagation protocols were previously successful at small scale production, we were unsuccessful in implementing them at production scales despite multiple attempts. Seedling survivorship and growth rates were disappointingly low, and 100% mortality post-outplanting forced us to cancel our demonstration for this species.

5.4 FIELD TESTING

Dates and duration of field testing, which paralleled our second operational phase, Reintroduction, were presented in Figure 7 (page 24). In addition to testing direct seeding and operational-scale outplanting technologies, we also evaluated several different treatments that were thought to likely improve outplant survival and growth.

We tested the efficacy and cost-efficiency of direct seeding as a technology relative to propagating and reintroducing transplants (see Chapter 3, Performance Objectives). Fifteen seed-addition plots were established in reintroduced populations each fall over three years to assess the efficacy of direct seeding of all species (Figure 13). No pretreatments were applied to seeds; they were simply scattered within plots without any attempt to promote burial. An irrigation treatment was also applied to a subset of *A. geogiana* seed plots. Seed-addition plots were also established in natural populations of all species except *L. asperulifolia*, which did not produce seeds during any years of the demonstration. These seed plots were used to inform our comparative demographic analyses (operational phase 3).

Figure 13: Setting up an *A. georgiana* seed-addition plot (right) and an irrigation setup within a seed-addition plot (left).

Propagated individuals were transplanted into the four reintroduction sites for each of the species over the course of three years, except *P. brevifolia*, which was abandoned as a target species after propagation and reintroduction efforts failed. Site maps for all reintroduction sites are presented in appendix B. Outplanting took place at the same time each year (late fall) to avoid possible confounding effects of time of outplanting (Figure 14). Plants of all species were planted in transects or in a grid pattern to assist future monitoring. Spacing plants at greater than 1 m facilitated researcher movement between plants during monitoring and provided room for any recruitment of second generation seedlings. For *A. georgiana* and *A. michauxii*, holes were dug to match the dimensions of the plug trays. After placing plants into the holes with the root crown at the soil surface, the soil was gently packed in and watered. In the case of *L. pyrophilum,* small (<2.5 cm diameter) bulbs were planted closer to the surface (~3 cm) than large (>3 cm) bulbs (~6 cm) and all were oriented with the scale pointing upward. *Lysimachia asperulifolia* rhizomes were outplanted by simply piercing a slot in the soil with a shovel or trowel, and then positioning rhizomes at a depth of approximately 2–5 cm. After positioning rhizomes, the gap at the surface was pinched together. Individual planting sites were then watered in to facilitate contact between the rhizomes and soil. The different age/size classes were planted at random within transects/grids to minimize the influence of any spatial autocorrelation in environmental conditions across reintroduction sites. The number of transplants of each species outplanted into sites is summarized in section 5.6 (Sampling Results).

Figure 14: Fall outplanting of *A. michauxii* (left) and a *L. pyrophilum* bulb (right).

We implemented several different treatments to assess the effects of supplemental watering and site maintenance on survivorship and growth, and to perform a cost-benefit analysis of different treatments. Two hundred fifty *A. georgiana* and 181 of *A. michauxii* were outplanted and divided into watered (treatment) and not watered (control) using a factorial design in order to permit a cost-benefit analysis of supplemental irrigation. Irrigation was provided using a gravity-fed drip system (sprinklerwarehouse.com) on a timer (Rain Bird, Azusa, CA) (Figure 15). The watering treatment was applied only during the first growing season after outplanting.

Additionally, we tested the efficacy and cost-effectiveness of reducing above-ground competition for *L. pyrophilum*. Site maintenance (treatment) or not maintained (control) were applied to 82 bulbs in a factorial design. The maintenance treatment included removal of above-ground biomass in the immediate proximity of transplants at outplanting using hand tools, in order to reduce competition and increase light availability.

Figure 15: Setting up gravity-fed irrigation system at an *A. georgiana* reintroduction site.

5.5 SAMPLING PROTOCOL

The sampling protocol was synonymous with phase three (Comparative Demographic Analysis) of the conceptual test design shown previously in Figure 3 (page 6), specifically the demographic monitoring technology component. All reintroduced individuals were uniquely marked with aluminum tags secured to the ground next to them (Figure 16) and georeferenced using a Trimble GeoXT (Trimble Navigation Limited, Sunnyvale, CA). Individuals from natural populations were similarly tagged and georeferenced. We collected data on vital rates from both reintroduced and natural populations of all species, except *P. brevifolia*, for the duration of the project (Table 5). Individuals were monitored over multiple years to assess survival, growth, and reproduction, which was necessary for ascertaining the contribution of these individuals to population growth, to account for potential dormancy (in the case of *L. pyrophilum*), and to better encompass inter-annual weather variability into demographic modeling. As described above, seedling recruitment was quantified annually within established seed-addition plots. Neither seed nor bulb predation were explicitly distinguished from other sources of mortality Additionally, we established 1 m^2 plots around both natural and reintroduced reproductive individuals to monitor *in situ* seedling recruitment not associated with seed-addition plots.

Figure 16: ***Amorpha georgiana*** **transplant (foreground) marked with a pin flag and aluminum tree tag to allow annual demographic monitoring.**

Table 5: Vital rate data collected in natural and reintroduced populations.

Vital rate	Data collected	Number of samples	
		reintroduced populations	**natural populations**
Reproduction	Number of fruits per individual All species	All	All
	Number of seeds per raceme *A. georgiana*	≥ 10% of fruits	≥ 2.5% of fruits
	Number of seeds per fruit *A. michauxii* *L. pyrophilum* *L. asperulifolia* *P. brevifolia*	≥ 10% of fruits	≥ 2.5% of fruits
	Number of stems *L. asperulifolia*	All	All
	Number of seedlings (*in situ* and seed-addition plots) *A. georgiana* *A. michauxii* *L. pyrophilum* *P. brevifolia*	All seedlings	All seedlings
Survivorship	Presence/absence of marked individuals and seedling recruits *A. georgiana* *A. michauxii* *L. pyrophilum* *L. asperulifolia* *P. brevifolia*	All transplanted individuals and seedling recruits (*in situ* and seed-addition plots)	All marked individuals and seedling recruits (*in situ* and seed-addition plots)
Growth	Height (cm) *A. georgiana* *A. michauxii* *L. pyrophilum* *L. asperulifolia*	All transplanted individuals	All marked individuals
	Width (cm) *A. georgiana*		
	# leaves per leaf whorl *L. pyrophilum*	10% of marked individuals	10% of marked individuals
	Major axis (cm) *P. brevifolia*	All transplanted individuals	All marked individuals
	Minor axis (cm) *P. brevifolia*	All transplanted individuals	All marked individuals

The method used to quantify growth and seed production was species-specific and designed to match different growth forms. For the semi-woody, erect shrub *A. georgiana* we measured height and width of individuals. To quantify seed production, we counted the number of racemes and, for a subset of racemes, the number of seeds per raceme. For the herbaceous legume *A. michauxii*, growth was quantified by measuring the height, and seed production was estimated by counting the number of capsules produced per individual and then obtaining an average number of seeds per capsule. Growth of the herbaceous *L. pyrophilum* was estimated by measuring the height of individual stems and counting the number of leaf whorls per stem. Seed production was estimated by counting the number of capsules per individual and, for a subset of capsules, counting the number of seeds. Growth of herbaceous, rhizomatous *L. asperulifolia* was quantified by measuring the height, number of leaf whorls and number of flowers for each stem identified. *In situ* asexual reproduction in *L. asperulifolia* was documented by comparing annual counts of the number of stems in both reintroduced and natural populations. Finally, growth and reproduction in the prostrate, evergreen subshrub *P. brevifolia* was estimated by measuring the inter-annual change in area occupied by marked individuals and counting the number of capsules and seeds produced for a subset of individuals in different size classes (Figure 17).

Figure 17: *Pyxidanthera brevifolia* flowering (left), mature capsules (middle), and capsule and seeds (right).

Data on these vital rates allowed us to create transition matrices. Transition matrices are essentially N*N matrices where N is equal to the number of size classes for the species and the matrix elements are the transition probabilities both between size classes and stasis. The columns represent size at time *t* and the rows represent size at time *t+1*. Transition matrices allow for the projection of population growth rates through time through the use of matrix algebra. More in-depth explanation of the process is contained in Chapter 6 (Performance Assessment). For example, suppose we have a two size-class matrix (small and large) and that 10 individuals are in the small size class at time *t* and 20 individuals are in the large size class (Figure 18), with the columns representing time *t* and the rows representing *t+1*. Further suppose that at time *t+1*, five of the individuals in the small size class have died (mortality is 50%), two have moved to the large size class (20%), and three have remained in the small size class (30%). Let us suppose that three out of the initial 20 large individuals are now in the small size class (15%) and 16 out of 20 large individuals are in the same large size class (80%), with one individual dead at time step $t + 1$. From the estimated transition matrix, the population growth rate is the dominant right eigenvalue (λ_i) of the right eigenvector (w_i) that satisfies the following formula: $Aw_i = \lambda_i w_i$ (Caswell 2001). In other words, eigenvectors are non-zero vectors that, when multiplied by the square matrix A, are equal to the multiplication of the same eigenvector to a scalar, also known as the dominant right eigenvalue. Population growth rates greater than 1 suggest that given the current environment the population will increase over time. Conversely, population growth rates less than 1 suggest that population size will decrease through time.

$t + 1$ \ t	small	large
small	0.3	0.15
large	0.2	0.8

Figure 18: Example matrix for demographic modeling.

5.5.1 Quality Assurance Sampling

Because the demographic data were collected using standard manual tools (i.e., calipers, clicker counters, and tape measures), few quality-assurance samples were needed. The largest potential source of error was observational on the part of the data collector. However, previous experience suggested that incorporating duplicate samples via repeat counts or multiple observers does not represent an efficient use of time for most of the data we collected. Instead, collecting data on more individuals represented a better use of limited time and led to more precise estimates of variables. In addition, binning the various demographic data (e.g., into size classes) further reduced any potential impact of observational error.

5.5.2 Sample Documentation

All site and demographic data collected in the field were recorded in bound, waterproof field notebooks (J. L. Darling Corp., Tacoma, WA). All data were electronically archived on a weekly basis and stored in two locations to prevent data loss. Data collected by NCBG were forwarded to ERDC-CERL after being archived locally. Field logbooks permanently resided with either NCBG or at ERDC-CERL, but both organizations had complete data records. All GIS data layers recorded with a GPS unit by either ERDC-CERL or NCBG, such as coordinates of reintroduced individuals, were archived.

5.6 SAMPLING RESULTS

5.6.1 *Amorpha georgiana*

We outplanted 3,065 *A. georgiana* individuals across four sites and three years. We collected data on survivorship, growth, and reproduction for these individuals as well as 412 individuals within natural populations. Combined across four years of data collection, our demographic dataset for *A. georgiana* includes 15,232 observations. A Kruskal-Wallis rank sum test confirmed the size distribution of outplanted individuals within age cohorts was not consistent across years ($p < 0.001$; Figure 19). Consequently, the relevance of some cohort-based analyses are somewhat limited. As expected, propagation period had a significant effect on stem height, with significant differences in stem height existing between all cohorts within each year (Table 6).

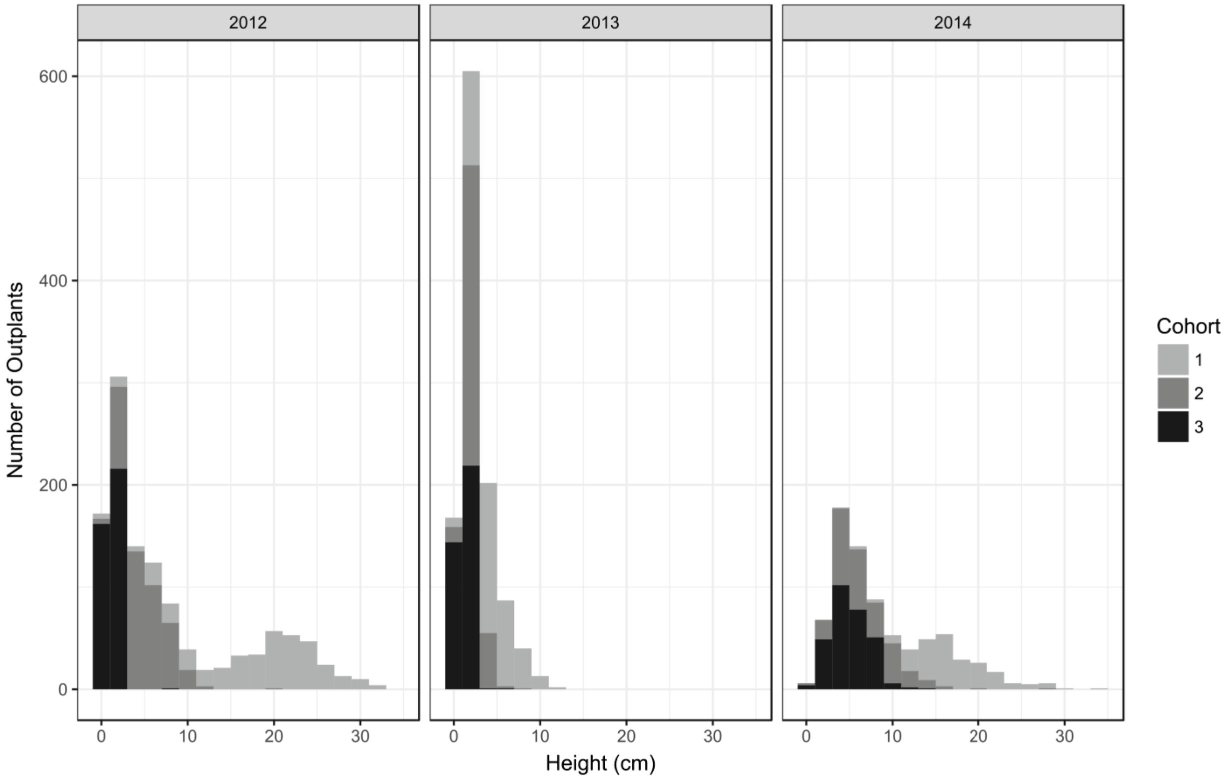

Figure 19: Outplant numbers for *Amorpha georgiana* by cohort and height (cm).

Table 6: *Amorpha georgiana* height (cm) at outplanting by cohort and year.

Year	Cohort	Mean	Standard deviation
2012	1	17.90	7.18
2012	2	5.12	2.43
2012	3	1.13	0.60
2013	1	4.54	2.19
2013	2	2.33	0.84
2013	3	1.17	0.54
2014	1	17.12	4.64
2014	2	7.17	3.57
2014	3	5.45	2.22

For outplanted *A. georgiana* individuals, first-year survivorship was significantly affected by size at outplanting ($p < 0.001$; Figure 20), with individuals greater than 20 cm in height at outplanting having a 90% probability of survivorship. Growth of outplanted individuals, defined as $(size\ at\ time\ t_1\ - size\ at\ t_0)/size\ at\ \ t_0$, was negatively correlated with initial height. Unsurprisingly, smaller individuals tended to exhibit greater relative growth compared to larger individuals ($p < 0.001$; Figure 21).

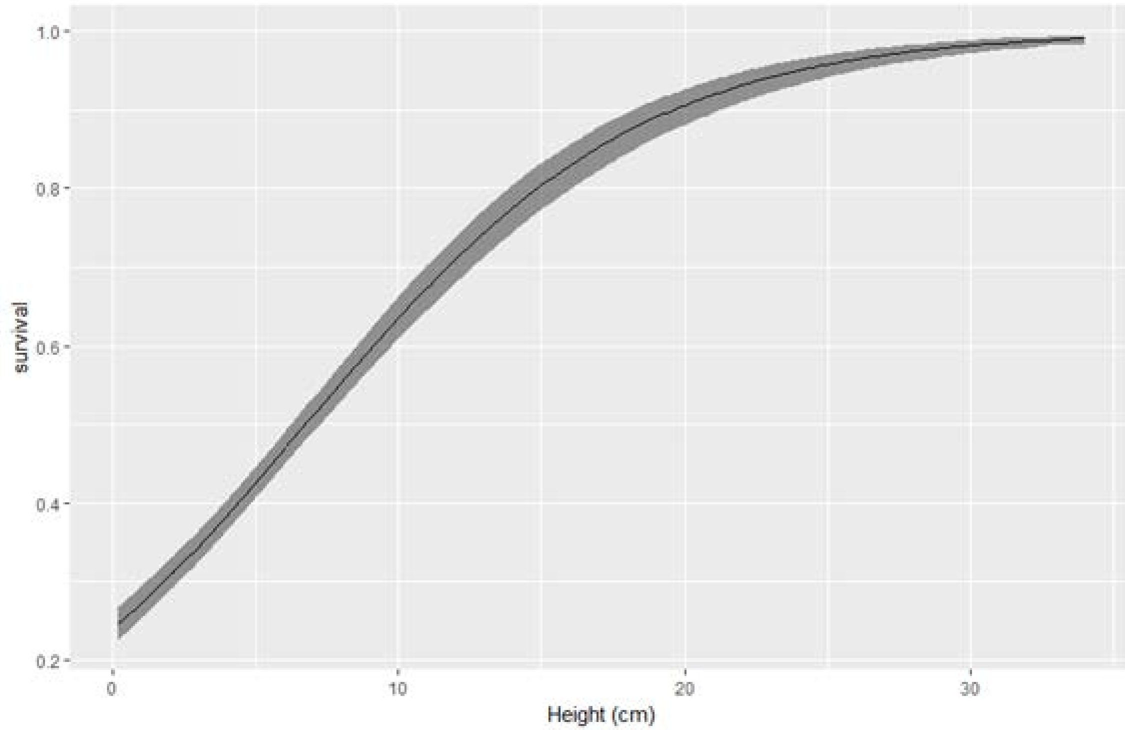

Figure 20: Survival as a function of outplant height (cm). Line represents best fit and dark gray represents 95% confidence intervals for *Amorpha georgiana*. $B_0 = -1.1541$, $B_1 = 0.1707$

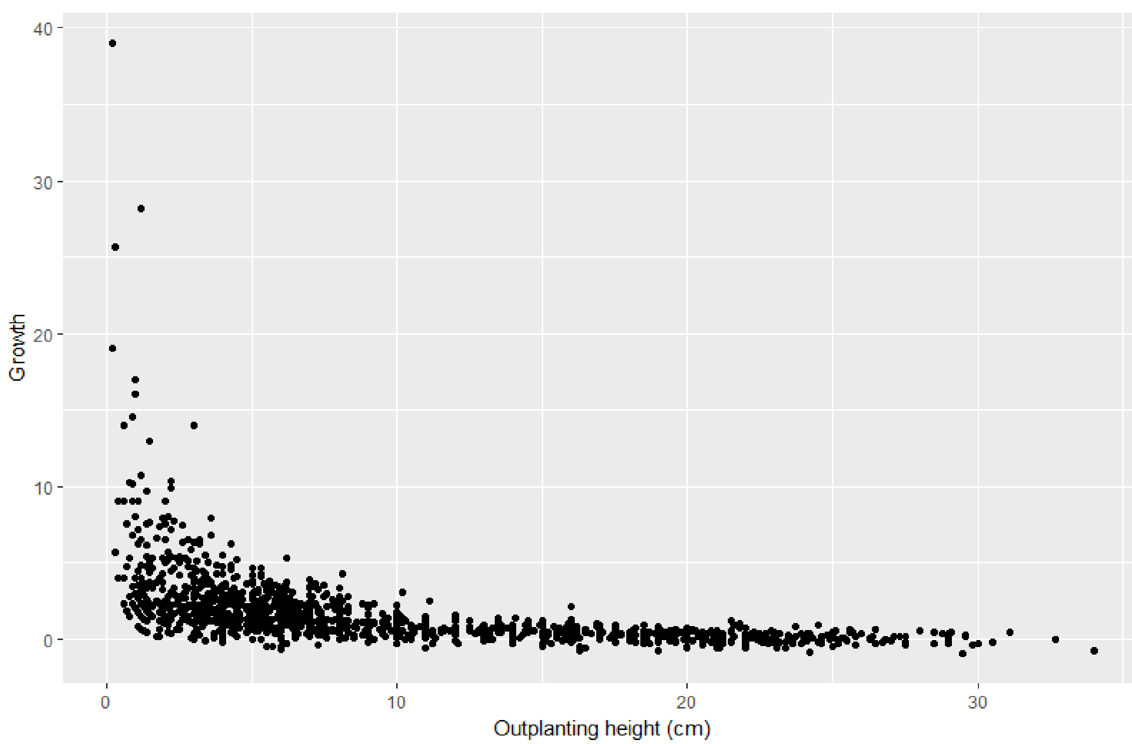

Figure 21: Relationship between outplant size and growth (one year post outplanting) for *Amorpha georgiana.* Model fit to data was nonlinear least squares. $B_0 = 1.897$, $B_1 = -0.2543$

Overall population growth rate was 0.80 within reintroduced populations as compared to the natural population growth rate of 1.06. Bootstrapping identified differences in the transition probabilities between natural and reintroduced populations ($p < 0.05$; Figure 22). Only two transitions were significantly greater in reintroduced populations than in natural populations: stasis of the small-medium size class and retrogression of the medium class. Survivorship of all size classes was higher in natural than in reintroduced populations ($p < 0.05$; Figure 22). The transition matrix elasticities for the natural and reintroduced populations suggest that the natural populations are most sensitive to changes in survivorship of the medium and large size classes, while the reintroduced populations are most sensitive to changes in the survivorship of the small-medium and medium size classes (Figure 23). The life table response experiments comparing the natural and reintroduced transition matrices suggest that the lower population growth rate of the reintroduced populations was mainly driven by the survivorship of the medium and large size classes (Figure 24). As the reintroduced populations become more established, the number and survivorship of individuals within the medium and large size classes should increase. As of 2016, the number of established outplants in the four reintroduction sites was 59 (AMGE10E), 252 (AMGE12A), 189 (AMGE15B) and 177 (AMGE17C).

Natural populations

	seedling	**small**	**small-med**	**medium**	**large**
seedling	0.00	0.01	0.03	0.66	2.22
small	0.19	0.47	0.13	0.01	0.00
small-med	0.06	0.26	0.50	0.10	0.00
medium	0.01	0.01	0.24	0.70	0.18
large	0.00	0.00	0.01	0.18	0.80
Survivorship	0.26	0.74	0.88	0.98	0.99

Reintroduced populations

	seedling	**small**	**small-med**	**medium**	**large**
seedling	0.00	0.00	0.00	0.00	0.10
small	0.19	0.23	0.07	0.01	0.00
small-med	0.06	0.30	0.55*	0.19*	0.22
medium	0.01	0.01	0.17	0.61	0.33
large	0.00	0.00	0.00	0.04	0.33
Survivorship	0.26	0.54	0.79	0.84	0.89

Figure 22: Transition matrices for size classes within natural (top) and reintroduced (bottom) *Amorpha georgiana* populations. Elements in the reintroduced populations matrix identified with an asterisk are significantly higher ($p < 0.05$) than the same elements in the natural populations matrix. Survivorship (proportion) of each size class is shown at the bottom in gray shading.

Natural populations

	seedling	small	small-med	medium	large
seedling	0.00	0.00	0.00	0.02	0.04
small	0.04	0.04	0.01	0.00	0.00
small-med	0.02	0.04	0.07	0.02	0.00
medium	0.00	0.00	0.07	0.23	0.04
large	0.00	0.00	0.01	0.08	0.28

Reintroduced populations

	seedling	small	small-med	medium	large
seedling	0.00	0.00	0.00	0.00	0.00
small	0.00	0.01	0.02	0.00	0.00
small-med	0.00	0.02	0.28	0.10	0.01
medium	0.00	0.00	0.11	0.39	0.02
large	0.00	0.00	0.00	0.03	0.02

Figure 23: Elasticity matrices for natural (top) and reintroduced (bottom) *Amorpha georgiana* populations.

	seedling	small	small-med	medium	large
seedling	0.00	0.00	0.00	-0.03	-0.04
small	0.00	-0.02	-0.01	0.00	0.00
small-med	0.00	0.00	0.01	0.03	0.02
medium	0.00	0.00	-0.03	-0.04	0.02
large	0.00	0.00	-0.01	-0.08	-0.09

Figure 24: Results of life table response experiments comparing transition matrices of natural and reintroduced populations of *Amorpha georgiana*.

Only three outplanted individuals flowered over the course of our demonstration: two in 2015 and one in 2016. We anticipated higher numbers of reproductive individuals during 2016, but unscheduled fires within two reintroduction sites reduced aboveground biomass, limiting the individuals' ability to attain reproductive size. Although observations of flowering within several years post-outplanting is a positive indication of the populations' viability, we have no observations of *in situ* recruitment within reintroduced populations due to a general lack of reproduction. Therefore, reproduction shown in the transition matrix for reintroduced populations was estimated based on data from natural populations.

Supplemental irrigation was provided to 250 individuals within two of our reintroduction sites (AMGE10B and AMGE15B), but had negligible impact on reintroduction success. Survivorship of irrigated plants was marginally greater than non-irrigated plants (79% vs. 70%, respectively; $p > 0.05$). However, at a cost of roughly $20 per individual (see Chapter 7, Cost Assessment), we cannot recommend the use of supplemental irrigation within *A. georgiana* reintroduction sites. (See Chapter 6, Performance Assessment, PO6.)

No *A. georgiana* seedlings recruited into any of our seed-addition plots during any year. Consequently, there is no efficacy or cost-effectiveness in attempting to establish individuals via seed addition versus transplanting individuals.

5.6.2 *Astragalus michauxii*

We outplanted 1,914 *A. michauxii* individuals across four sites and three years. We collected data on survivorship, growth, and reproduction for these individuals as well as 760 individuals within natural populations. Combined across four years of data collection, our demographic dataset includes 7,690 observations. Outplanted individuals were significantly smaller in 2012 and 2013, compared to 2014 ($p < 0.001$), which limited the relevance of some cohort-based analyses (Figure 25). As expected, propagation period had a significant effect on stem height, with significant differences in stem height existing between all cohorts (Table 7).

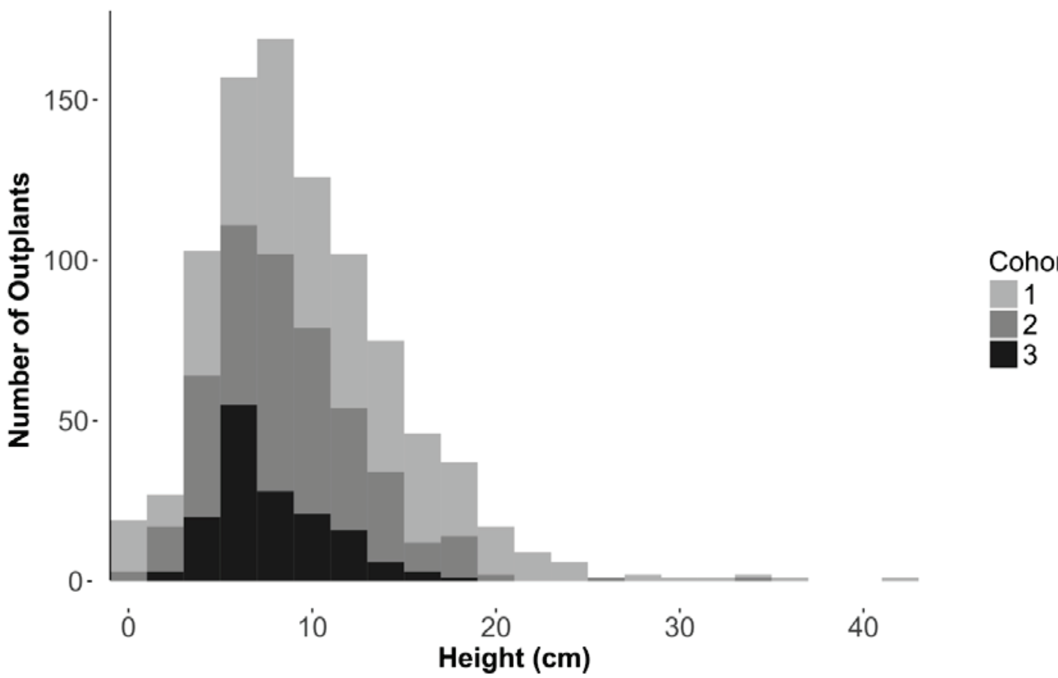

Figure 25: Outplant numbers for *Astragalus michauxii* in 2014 by cohort and height (cm).

Table 7: *Astragalus michauxii* height (cm) by cohort at outplanting.

Year	Cohort	Mean	Standard deviation
2014	1	11.10	6.19
2014	2	9.09	4.35
2014	3	7.93	3.08

For outplanted *A. michauxii* individuals, first-year survivorship was significantly affected by size at outplanting ($p < 0.001$; Figure 26), with individuals greater than 20 cm in height at outplanting having a 92% probability of survivorship.

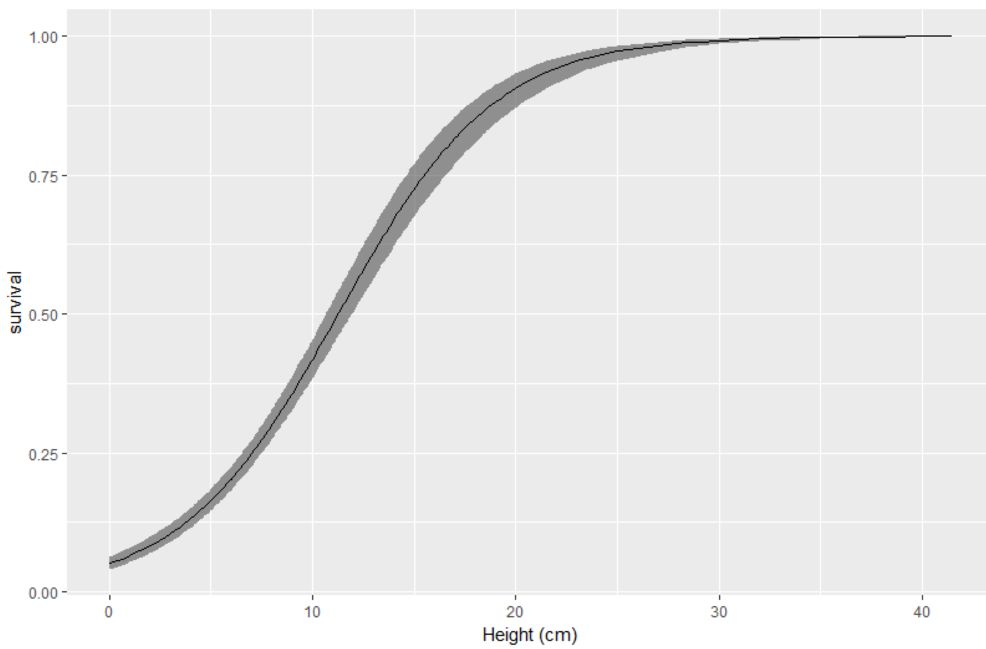

Figure 26: Survival as a function of outplant height (cm). Line represents best fit and dark gray represents 95% confidence intervals for *Astragalus michauxii*. $B_0 = -2.923$, $B_1 = 0.259$

Growth of outplanted individuals was calculated as for *A. georgiana*, and was negatively correlated with initial height ($p < 0.001$; see Figure 27). Unsurprisingly, smaller individuals tended to exhibit greater relative growth compared to larger individuals.

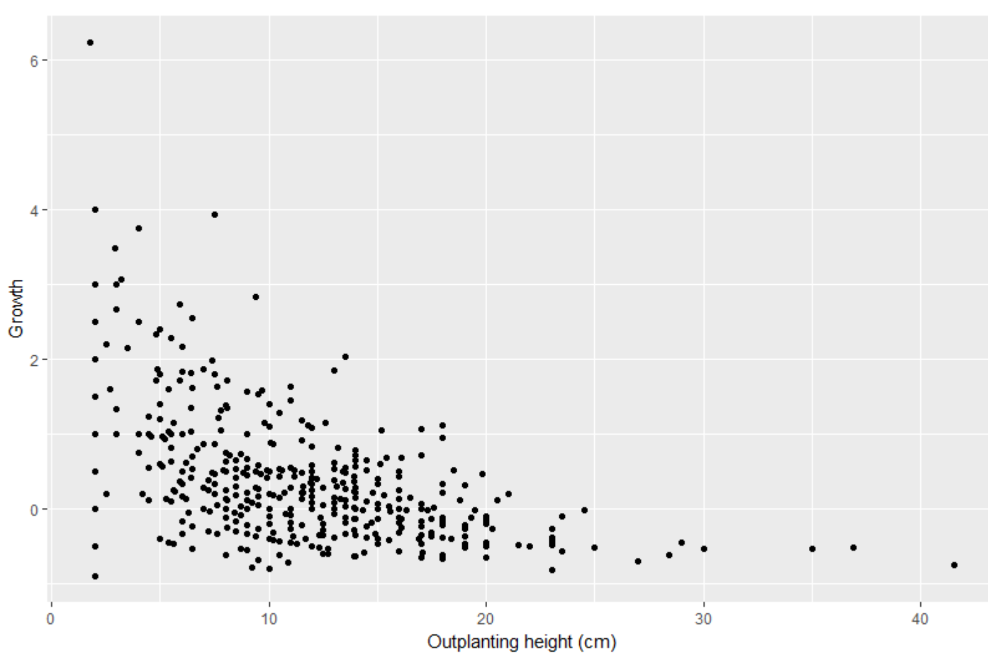

Figure 27: Relationship between outplant height (cm) and growth (one year post outplanting) for *Astragalus michauxii.* Model fit to data was nonlinear least squares. $B_0 = 0.7065$, $B_1 = -0.1749$

Overall population growth rate of reintroduced populations was 0.75, as compared to 0.97 of natural populations. Bootstrapping revealed differences in the transition probabilities between natural and reintroduced populations ($p < 0.05$; see Figure 28). Only six transitions were significantly greater in reintroduced populations than in natural populations: stasis of the small and small-medium size classes and retrogression of the small-medium, medium, and large classes. Survivorship of all size classes was higher in natural than in reintroduced populations ($p < 0.05$; Figure 28). The transition matrix elasticities for the natural and reintroduced populations suggest that the natural populations are most sensitive to changes in survivorship of the small-medium and medium size classes, while the reintroduced populations are most sensitive to changes in the survivorship and size-class shifts (either growth or retrogression) of the small-medium and medium size classes (Figure 29). The life table response experiments comparing the natural and reintroduced transition matrices suggest that the lower population growth rate of the reintroduced populations was mainly driven by the reduced growth of small-medium individuals to the next size class, as well as transition of small individuals into the larger size classes (Figure 30). As the reintroduced populations become more established, it is anticipated that the natural and reintroduced transition matrices will become more similar. As of 2016, the number of established outplants in the four reintroduction sites was 70 (ASMI36A), 57 (ASMI56A), 84 (ASMI59B) and 46 (ASMI60A).

Natural populations

	seedling	**extra-small**	**small**	**small-med**	**medium**	**large**
seedling	0.00	0.00	0.00	0.00	0.04	0.14
extra-small	0.33	0.20	0.05	0.01	0.01	0.01
small	0.00	0.30	0.25	0.15	0.06	0.03
small-med	0.00	0.21	0.33	0.34	0.24	0.14
medium	0.00	0.05	0.27	0.41	0.55	0.43
large	0.00	0.04	0.01	0.06	0.12	0.37
Survivorship	0.33	0.80	0.92	0.96	0.98	0.98

Reintroduced populations

	seedling	**extra-small**	**small**	**small-med**	**medium**	**large**
seeding	0.00	0.00	0.00	0.00	0.04	0.14
extra-small	0.33	0.15	0.04	0.03*	0.03*	0.06*
small	0.00	0.36	0.42*	0.18	0.08	0.04
small-med	0.00	0.00	0.11	0.53*	0.33*	0.16
medium	0.00	0.00	0.00	0.08	0.46	0.44
large	0.00	0.00	0.00	0.00	0.08	0.30
Survivorship	0.00	0.02	0.14	0.46	0.31	0.07

Figure 28: Transition matrices for size classes within natural (top) and reintroduced (bottom) *Astragalus michauxii* populations. Elements in the reintroduced populations matrix identified with an asterisk are significantly higher ($p < 0.05$) than the same elements in the natural populations matrix. Survivorship (proportion) of each size class is shown at the bottom in gray shading.

Natural populations

	seedling	extra-small	small	small-med	medium	large
seedling	0.00	0.00	0.00	0.00	0.00	0.00
extra-small	0.00	0.00	0.00	0.00	0.00	0.00
small	0.00	0.01	0.08	0.04	0.01	0.00
small-med	0.00	0.00	0.06	0.33	0.07	0.01
medium	0.00	0.00	0.00	0.09	0.19	0.03
large	0.00	0.00	0.00	0.00	0.04	0.03

Reintroduced populations

	seedling	extra-small	small	small-med	medium	large
seedling	0.00	0.00	0.00	0.00	0.01	0.00
extra-small	0.01	0.00	0.00	0.00	0.00	0.00
small	0.00	0.01	0.03	0.04	0.03	0.00
small-med	0.00	0.01	0.04	0.09	0.11	0.02
medium	0.00	0.00	0.03	0.12	0.27	0.05
large	0.00	0.00	0.00	0.02	0.06	0.05

Figure 29: Elasticity matrices for natural (top) and reintroduced (bottom) *Astragalus michauxii* populations.

	seedling	extra-small	small	small-med	medium	large
seedling	0.00	0.00	0.00	0.00	0.00	0.00
extra-small	0.00	0.00	0.00	0.00	0.00	0.00
small	0.00	0.00	0.02	0.01	0.00	0.00
small-med	0.00	-0.01	-0.04	0.06	0.03	0.00
medium	0.00	0.00	-0.06	-0.13	-0.04	0.00
large	0.00	0.00	0.00	-0.02	-0.02	-0.01

Figure 30: Results of life table response experiments comparing transition matrices of natural and reintroduced populations of *Astragaluls michauxii*.

Only eleven outplanted individuals flowered over the course of the demonstration: eight in 2015 and five in 2016. Flowering was observed in all four reintroduction sites. Four of these flowering individuals produced fruits in 2015, but only one did in 2016. Although observations of flowering within several years post-outplanting is a positive indication of the populations' viability, we have no observations of *in situ* recruitment within reintroduced populations due to a general lack of reproduction. Reproduction shown in the transition matrix for reintroduced populations was estimated based on data from natural populations.

Supplemental irrigation was provided to 181 individuals within two of our reintroduction sites, but did not enhance *A. michauxii* growth or survival ($p > 0.05$). At a cost of roughly $22 per individual (see Chapter 7, Cost Assessment), we cannot recommend the use of supplemental irrigation within *A. michauxii* reintroduction sites (see Chapter 6, Performance Assessment, PO6).

No *A. michauxii* seedlings recruited into any of our seed-addition plots during any year. Consequently, there is no efficacy or cost-effectiveness in attempting to establish individuals via seed addition versus transplanting individuals.

5.6.3 *Lilium pyrophilum*

We outplanted 670 *L. pyrophilum* individuals across four sites and three years. We collected data on survivorship, growth, and reproduction for these individuals as well as 203 individuals within natural populations. Combined across four years of data collection, our demographic dataset includes 2,610 observations. The size distribution of outplanted individuals was roughly similar across years ($p > 0.05$; Figure 31).

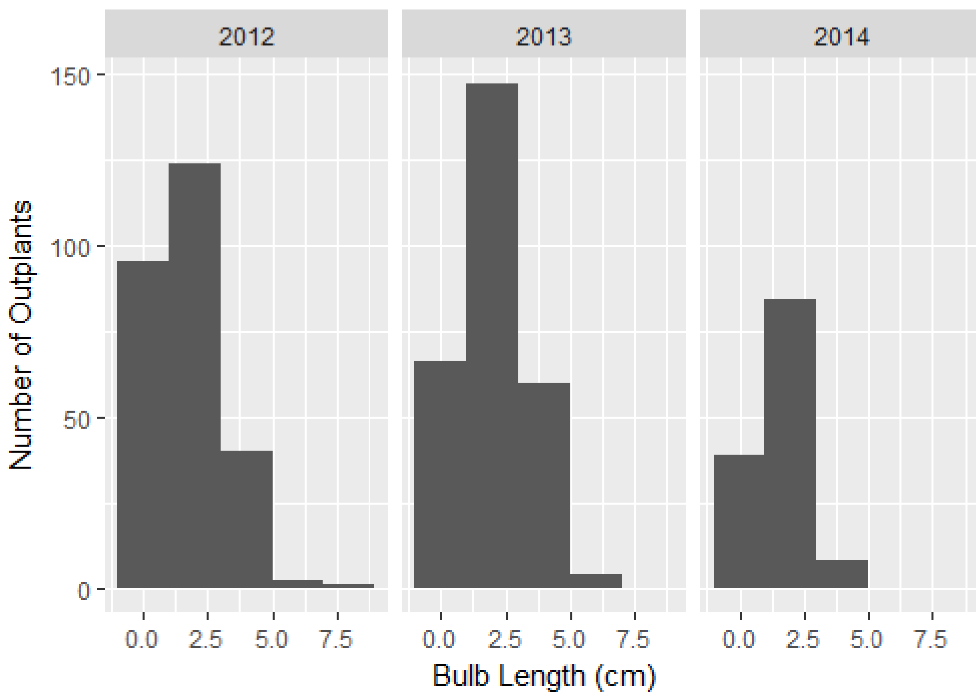

Figure 31: Outplant numbers for *Lilium pyrophilum* by bulb length (cm) and year.

For outplanted *L. pyrophilum* individuals, first-year survivorship was significantly affected by bulb size at outplanting ($p < 0.05$; Figure 32). Interestingly, however, there was a negative correlation, with bulbs shorter than 2 cm at outplanting having approximately 50% survival, while the largest individuals had approximately 25% survival.

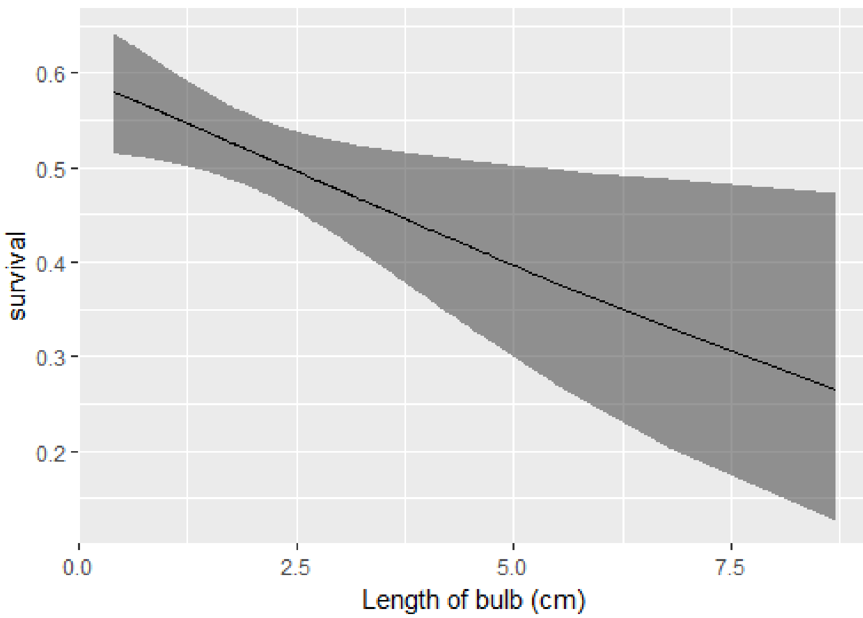

Figure 32: Survival as a function of outplant height (cm). Line represents best fit and dark gray represents 95% confidence intervals for *Lilium pyrophilum*. $B_0 = 0.389$, $B_1 = -0.162$

Stem height of individuals the first growing season post-outplanting was positively correlated with bulb length at outplanting ($p < 0.001$; Figure 33).

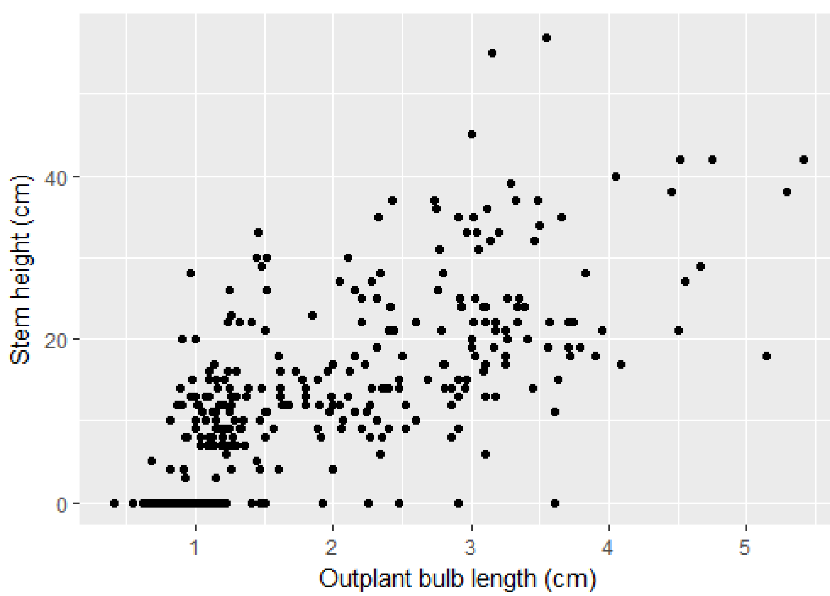

Figure 33: Relationship between bulb length (cm) and stem height (cm) of outplanted *Lilium pyrophilum* individuals the first growing season post-outplanting. Individuals with a stem height of zero represent single-leaved individuals. Data were fit with a simple linear regression model, $B_0 = -1.036$, $B_1 = 7.435$

Overall population growth rate for the reintroduced *L. pyrophilum* populations was 0.98, as compared to the natural population growth rate of 1.02. Bootstrapped population growth rates for the natural and the reintroduced populations did not demonstrate significant differences between the two populations. Transition probabilities differed for only a few transitions between the natural and the reintroduced populations (Figure 34). A significantly greater percentage of the reintroduced *L. pyrophilum* individuals in the small size class remained in the small size class, relative to the natural populations. In addition, the dormancy of small-medium individuals was greater in reintroduced than in natural populations, but the differences may not be biologically significant.

Natural populations

	D Sdlng	Sdlng Yr1	Sdlng Yr2	Sdlng Yr3	single leaf	small	small-med	medium	large	D single leaf	D small	D small-med	D med	D large
D Sdlng	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.62	9.16	0.00	0.00	0.00	0.00	0.00
Sdlng Yr1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr2	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr3	0.00	0.00	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
single leaf	0.00	0.00	0.00	0.80	0.27	0.10	0.03	0.01	0.01	0.78	0.33	0.20	0.25	0.06
small	0.00	0.00	0.00	0.05	0.09	0.08	0.04	0.01	0.03	0.00	0.22	0.20	0.05	0.00
small-med	0.00	0.00	0.00	0.00	0.07	0.15	0.24	0.05	0.05	0.11	0.22	0.60	0.10	0.06
medium	0.00	0.00	0.00	0.00	0.05	0.06	0.19	0.30	0.09	0.00	0.22	0.00	0.50	0.50
large	0.00	0.00	0.00	0.00	0.02	0.04	0.07	0.13	0.49	0.11	0.00	0.00	0.10	0.38
D single leaf	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small-med	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
D large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Survivorship	1.00	0.65	0.87	0.85	0.61	0.63	0.64	0.61	0.79	1.00	1.00	1.00	1.00	1.00

Figure 34: Transition matrices for size classes within natural (this page) and reintroduced (next page) *Lilium pyrophilum* populations. Elements in the reintroduced populations matrix identified with an asterisk are significantly higher ($p < 0.05$) than the same elements in the natural populations matrix. D = dormant; Sdling = seedling. [Figure continues to next page.] Survivorship (proportion) of each size class is shown at the bottom in gray shading.

Figure 34 [concluded].

Reintroduced populations

	D Sdlng	Sdlng Yr1	Sdlng Yr2	Sdlng Yr3	single leaf	small	small-med	medium	large	D single leaf	D small	D small-med	D med	D large
D Sdlng	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.62	9.16	0.00	0.00	0.00	0.00	0.00
Sdlng Yr1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr2	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr3	0.00	0.00	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
single leaf	0.00	0.00	0.00	0.80	0.16	0.04	0.00	0.01	0.01	0.85	0.21	0.00	0.33	0.06
small	0.00	0.00	0.00	0.05	0.08	0.26*	0.13	0.01	0.03	0.08	0.64	0.60	0.00	0.00
small-med	0.00	0.00	0.00	0.00	0.02	0.07	0.21	0.05	0.05	0.08	0.14	0.20	0.67*	0.06
medium	0.00	0.00	0.00	0.00	0.05	0.06	0.19	0.30	0.09	0.00	0.00	0.20	0.00	0.50
large	0.00	0.00	0.00	0.00	0.02	0.04	0.07	0.13	0.49	0.00	0.00	0.00	0.00	0.38
D single leaf	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small-med	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
D large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Survivorship	1.00	0.65	0.87	0.85	0.44	0.53	0.65	0.61	0.79	1.00	1.00	1.00	1.00	1.00

The transition matrix elasticity values suggest that both the natural and reintroduced populations are most sensitive to changes in seedling survivorship, survivorship of the largest individuals, and changes in fecundity for the largest size class (Figure 35). These parameter estimates were leveraged from the natural populations. The lack of a significant difference between the natural and reintroduced population growth rates is a likely consequence. The life table response experiments comparing the natural and reintroduced transition matrices suggest the transition differences between the natural and reintroduced populations, while minimal, were mainly driven by the differences in single-leaf stage survivorship and growth as well as differences in single-leaf dormancy probabilities (Figure 36). As the reintroduced populations become more established, it is anticipated that the natural and reintroduced transition matrices will become more similar. As of 2016, the number of established outplants in the four reintroduction sites was 57 (LIPY11A), 16 (LIPY13X), 57 (LIPY15A) and 13 (LIPY15B).

Only one outplanted individual flowered over the course of the demonstration. Although observations of flowering within several years post-outplanting is a positive indication of population viability, we have no observations of *in situ* recruitment within reintroduced populations due to a general lack of reproduction. Reproduction shown in the transition matrix for reintroduced populations was estimated based on data from natural populations.

Site maintenance was provided to 141 individuals within two of the reintroduction sites. The vegetation-removal treatment applied to *L. pyrophilum* approximately doubled first-year survival (site maintained = 0.38 vs. not removed = 0.18; $p = 1.23$ e-08), but had little effect on growth ($F = 0.245$, $p = 0.63$). Given a cost of approximately $4 per individual to implement vegetation removal, and the extended propagation time required for this species (see Chapter 7, Cost Assessment), we recommend vegetation removal within reintroduction sites (see Chapter 6, Performance Assessment, PO6).

No *L. pyrophilum* seedlings recruited into any of the seed-addition plots during any year. Consequently, there is no efficacy or cost-effectiveness in attempting to establish individuals by seed addition versus transplanting individuals.

Natural populations

	D Sdlng	Sdlng Yr1	Sdlng Yr2	Sdlng Yr3	single leaf	small	small-med	medium	large	D single leaf	D small	D small-med	D med	D large
D Sdling	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.09	0.00	0.00	0.00	0.00	0.00
Sdlng Yr1	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr2	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr3	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
single leaf	0.00	0.00	0.00	0.10	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
small	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
small-med	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
medium	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00
large	0.00	0.00	0.00	0.00	0.03	0.01	0.02	0.03	0.10	0.01	0.00	0.00	0.00	0.01
D single leaf	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small-med	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
D large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00

Figure 35: Elasticity matrices for natural (this page) and reintroduced (next page) *Lilium pyrophilum* populations. D = dormant; Sdling = seedling. [Figure continues to next page.]

Figure 35 [concluded].

Reintroduced populations

	D Sdlng	**Sdlng Yr1**	**Sdlng Yr2**	**Sdlng Yr3**	**single leaf**	**small**	**small-med**	**medium**	**large**	**D single leaf**	**D small**	**D small-med**	**D med**	**D large**
D Sdling	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.09	0.00	0.00	0.00	0.00	0.00
Sdlng Yr1	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr2	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr3	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
single leaf	0.00	0.00	0.00	0.10	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
small	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
small-med	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
medium	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.03	0.01	0.00	0.00	0.00	0.01	0.00
large	0.00	0.00	0.00	0.00	0.03	0.01	0.01	0.03	0.10	0.00	0.00	0.00	0.00	0.00
D single leaf	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small-med	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
D large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00

	D Sdlng	Sdlng Yr1	Sdlng Yr2	Sdlng Yr3	single leaf	small	small-med	medium	large	D single leaf	D small	D small-med	D med	D large
D Sdling	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sdlng Yr3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
single leaf	0.00	0.00	0.00	0.00	-0.02	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00
small	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
small-med	0.00	0.00	0.00	0.00	-0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00
D single leaf	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D small-med	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D medium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D large	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	0.00

Figure 36: Results of life table response experiments comparing transition matrices of natural and reintroduced populations of *Lilium pyrophilum*. D = dormant; Sdling = seedling

5.6.4 *Lysimachia asperulifolia*

We outplanted 710 *L. asperulifolia* individuals across four sites and three years. We collected data on survivorship, growth, and reproduction for these individuals as well as 651 individuals (i.e., "stem-years") within natural populations. Combined across four years of data collection, the demographic dataset includes 2,270 observations for the reintroduced populations. As of 2016, the number of established outplants in the four reintroduction sites was 9 (LYAS30B), 82 (LYAS51A), 53 (LYAS57A), and 28 (LYAS69A). However, the two sites with lower counts (LYAS30B and LYAS69A) burned unexpectedly during the 2016 growing season prior to demographic data collection. Based on counts from 2015 (LYAS30B = 49 and LYAS69A = 48), we anticipate the size of these two populations is actually larger than observed in 2016.

The size distribution of outplanted individuals was relatively consistent across years (Figure 37), although 2013 and 2014 included a number of longer rhizomes.

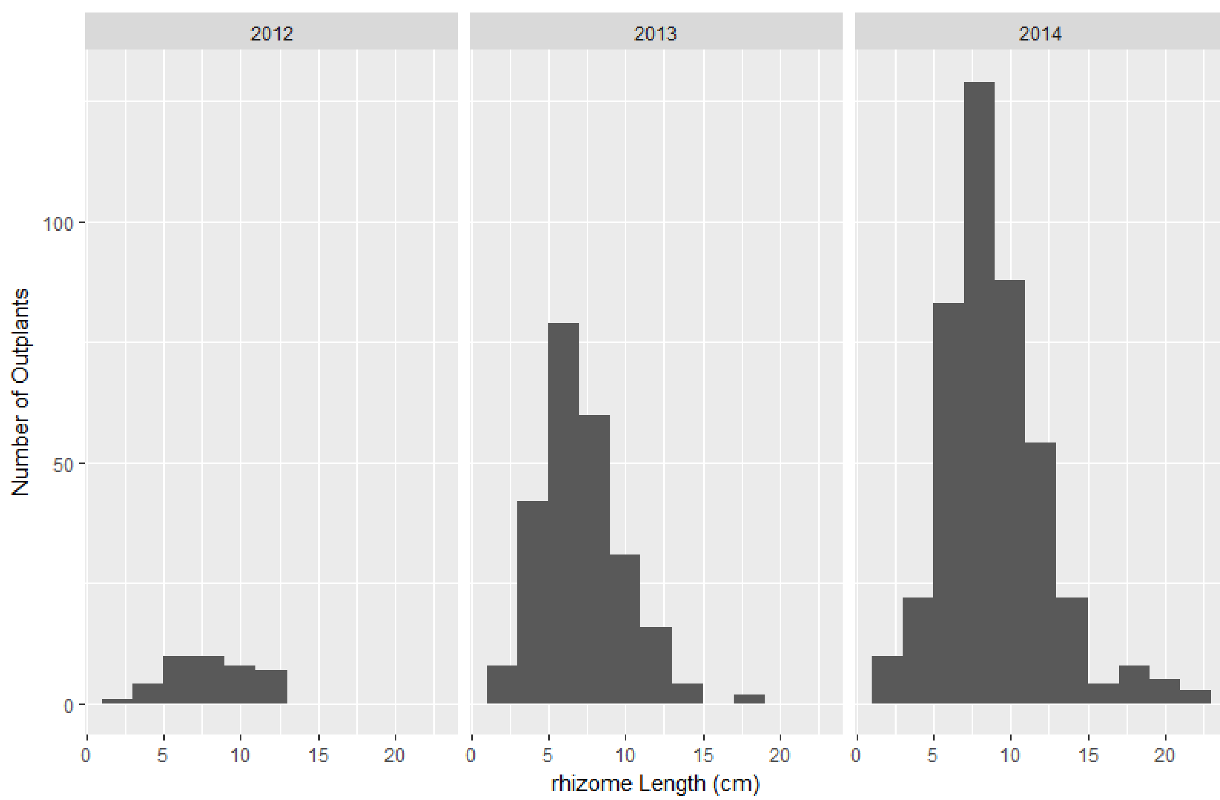

Figure 37. Outplant numbers for *Lysimachia asperulifolia* by rhizome size and year.

For outplanted *L. asperulifolia* individuals, first-year survivorship was not significantly affected by size at outplanting (Figure 38), though this is likely an artifact of small sample size for short and long rhizome lengths. Survivorship increased from a minimum of 0.33 for the shortest rhizomes to 0.39 for the longest rhizomes.

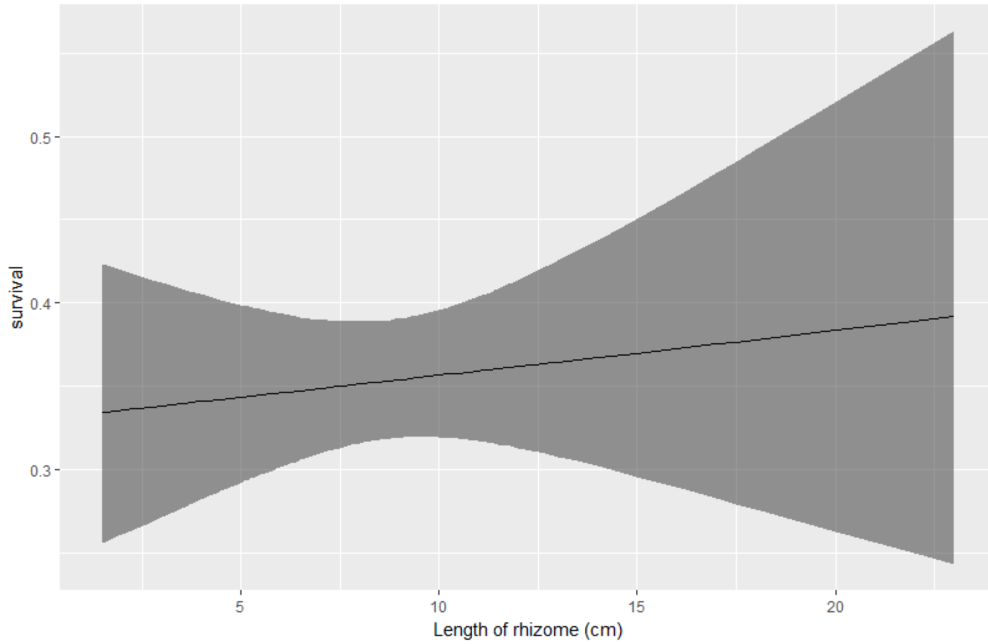

Figure 38: First year survival of *Lysimachia asperulifolia* outplants as a function of rhizome length (cm) at outplanting. Line represents best fit and dark gray represents 95% confidence intervals.

Stem height of individuals one year post-outplanting was positively correlated with rhizome length at outplanting and was marginally significant (p = 0.07; Figure 39), suggesting that the outplanting of longer rhizomes may yield larger individuals in subsequent years. However, the relationship appears to be driven mainly by the largest rhizomes.

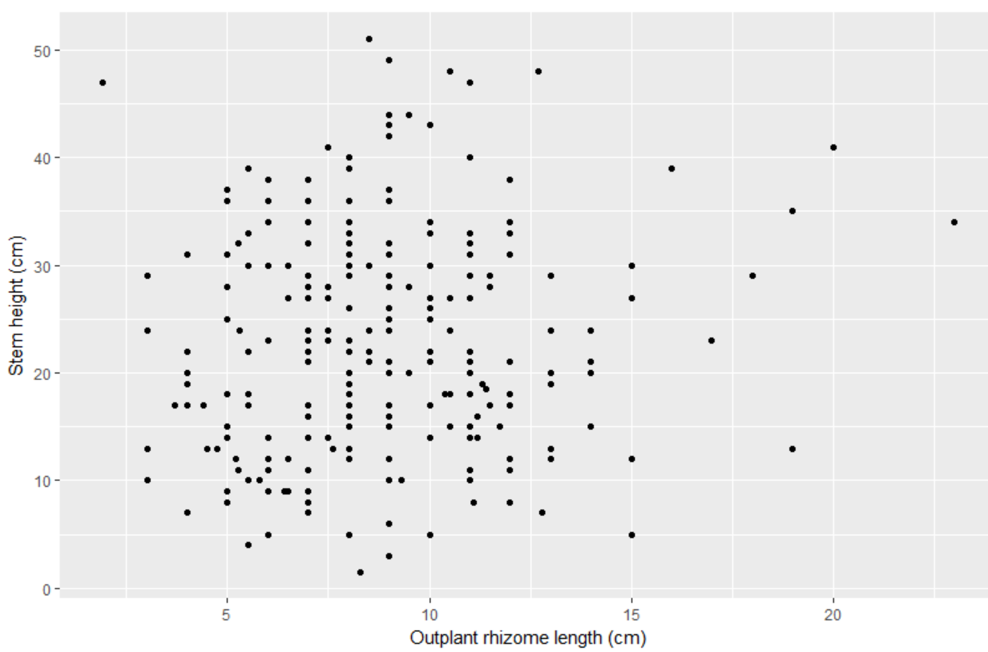

Figure 39: Relationship between rhizome length and stem height of outplanted *Lysimachia asperulifolia* individuals one year post-outplanting.

Mean survivorship of outplanted *L. asperulifolia* individuals was 0.57 and was not correlated with stem height ($p > 0.05$, Figure 40).

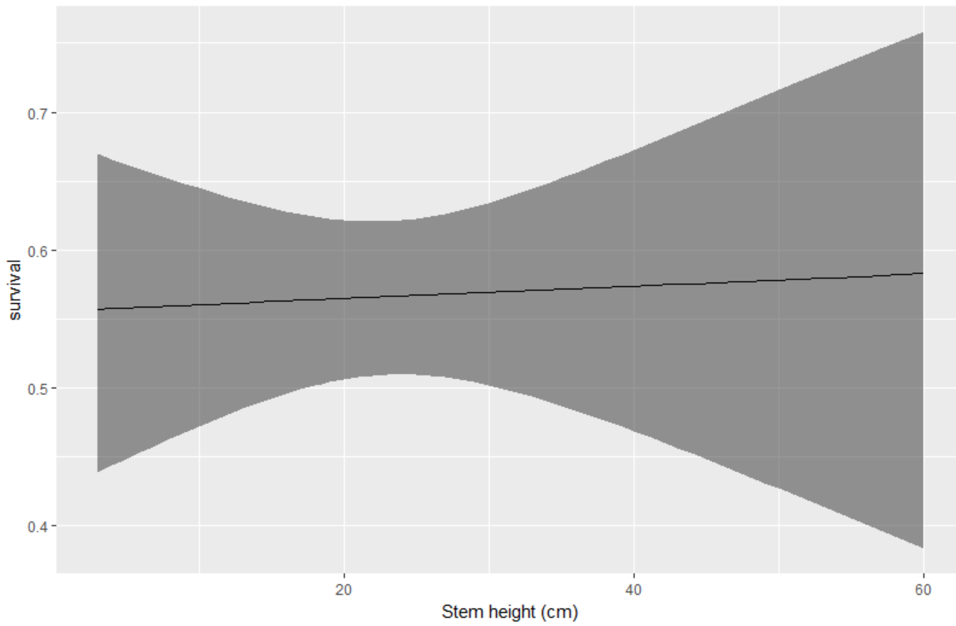

Figure 40: Relationship between stem height (cm) and survivorship of outplanted *Lysimachia asperulifolia* individuals. $B_0 = 0.224$, $B_1 = 0.002$

Growth was negatively correlated with initial height ($p < 0.05$; Figure 41). Unsurprisingly, smaller individuals tended to exhibit greater relative growth compared to larger individuals.

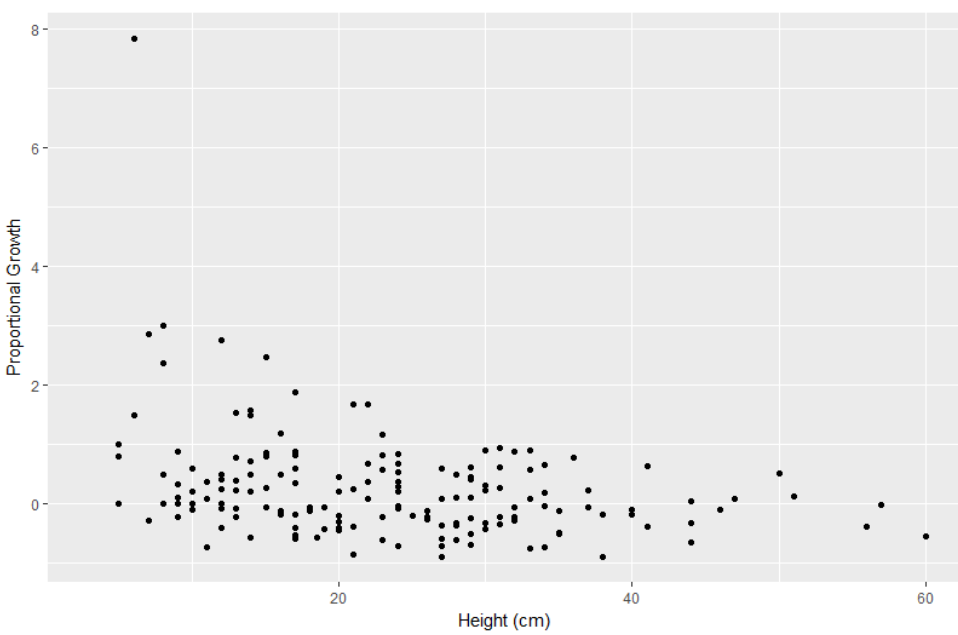

Figure 41: Relationship between growth and stem height (cm) the previous year for *Lysimachia asperulifolia*. Proportional growth is defined as (*size at time* t_1 – *size at* t_0) / *size at* t_0.

Density plots of stem heights within natural and reintroduced *L. asperulifolia* populations from 2012–2016 show there is a greater probability of stems being smaller in reintroduced than in natural populations (Figure 42), but that over time stems of outplanted rhizomes increased in size to more closely match the heights observed in natural populations. The persistent prevalence of small stems in 2016 may be attributable to growing-season fires within two of the reintroduced populations.

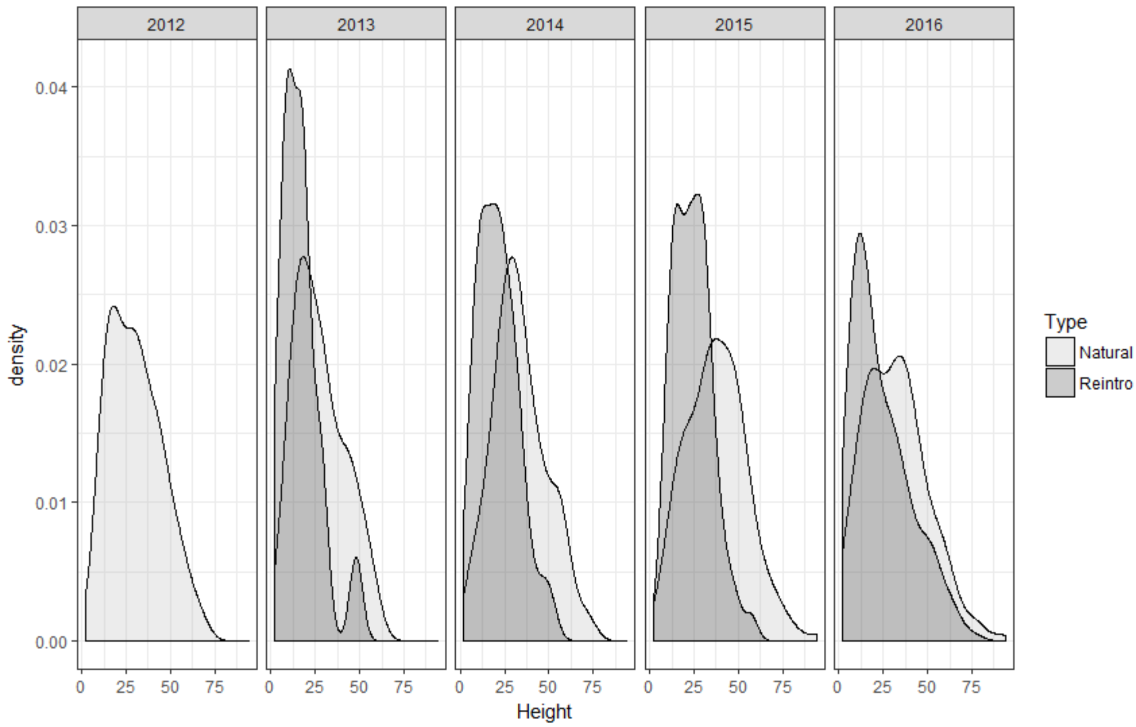

Figure 42: Density plots of individual stem heights (cm) within natural and reintroduced *Lysimachia asperulifolia* populations from 2012–2016.

Although no *L. asperulifolia* flowered in any of the reintroduced or natural populations during our demonstration, we did document vegetative recruitment within our reintroduced populations (Table 8). Comparing the observed rates of vegetative reproduction in reintroduced populations with vegetative reproduction in natural populations was limited by our ability to identify individuals in the latter. The wide spacing of outplanted rhizomes allowed us to identify when multiple stems originated from single individuals, but this was not possible in natural populations, which had many closely spaced stems.

Table 8: Number of *Lysimachia asperulifolia* stems vegetatively reproducing by year within reintroduced populations.

Year	Number	Reproduction	Proportion
2013	18	3	0.17
2014	73	1	0.01
2015	234	15	0.06
2016	175	0	0.00

6. PERFORMANCE ASSESSMENT

Power analyses were conducted to ensure adequate sample sizes to detect differences in the vital rates between natural and reintroduced populations using the software program G*Power 3.1 (Faul et al. 2007). For survivorship, we assumed a logistic regression with a binomial distribution. Effect size was estimated as the log of the odd ratio (2.47) divided by 1.81 for the appropriate effect size (Chinn 2000). Power analysis for growth was calculated using the statistical test "ANOVA: Fixed effects, special, main effects and interactions" with the numerator degrees of freedom set at 1. For reproduction, we selected the statistical test Poisson regression with $Exp(\beta_1)$ set to 1.15. This value corresponds to an effect size of 0.5 and represents the power to detect a 15% difference in reproduction between reintroduced and natural populations. All power analyses for the vital rates suggest that our sample sizes were large enough to detect differences (Table 9). Because the transition probabilities of the different stages were calculated from the vital rates and confidence intervals were generated through bootstrapping methods, we did not perform power analyses for these proposed statistical tests.

Table 9: Results of power analyses assessing adequate sample size.

Vital Rate	Test	α	Power (1-β)	Effect Size	Sample Size
Survivorship	Logistic regression	0.05	0.80	0.5	249
Growth	ANOVA: Fixed effects	0.05	0.95	0.5	54
Reproduction	Poisson regression	0.05	0.8	0.5	370

Because a priori analyses of data collected from natural populations of the target species suggested that the random effects of population and year are small, we used generalized linear models for most analyses.

PO1: Gain user acceptance

We analyzed the data collected for PO1 by calculating the average score on the graded surveys administered to potential users. For each species except *P. brevifolia* we asked (1) whether land managers intended to implement our demonstrated reintroduction technologies and (2) whether they intended to incorporate the reintroduction technologies within the next revision of their Endangered Species Management Plan, Integrated Natural Resources Management Plan, or other relevant management plan. This question is relevant because having management actions within an approved plan is often a necessary first step, before any funding can be secured or management actions can proceed. Potential responses were "strongly disagree", "disagree", "neither agree nor disagree", "agree', "strongly agree", and "not applicable." The first five were assigned values 1–5 in the given order. Our criterion for success was an average score ≥4.0 on a 1–5 scale. We also posed two follow-up, but unscored questions related to the first question. First, if managers did not intend to implement species reintroduction technologies, we asked them to identify and rank reasons why not (e.g., lack of funding, lack of interest, land use conflicts, etc.). Second, if they did intend to implement species reintroduction technologies, we asked them to identify the locations and anticipated timelines for implementation.

Four of fifteen contacted land managers responded to our survey, equaling a response rate of approximately 27%. The average score for the first question was ≥4 for all species except *L.*

pyrophilum (Table 10). Lack of staff and funding (in rank order) were the cited reasons for not planning to implement the technologies for this species. A total of eight properties were identified by land managers as likely reintroduction sites for the four species. In regard to including reintroduction technologies within management plans, the average score for the second question was ≥4 for all species (Table 10). Fort Bragg has already incorporated the reintroduction technologies within the latest revision of its INRMP, which is pending final approval. In summary, this PO1 was largely met for all species except *P. brevifolia*.

Table 10: Average scores of survey questions for each species

	Question	
Species	**(1) Intend to implement reintroduction technologies?**	**(2) Intend to include reintroduction technologies within next revision of relevant management plan(s)?**
Amorpha georgiana	4.3	4.3
Astragalus michauxii	4.0	4.0
Lilium pyrophilum	3.5	4.25
Lysimachia asperulifolia	4.5	4.5

PO2: Establishment of four self-sustaining viable populations

For PO2, we compared the population growth rates of the reintroduced populations and the natural populations. We began by classifying individuals into size classes using the pooled multiyear datasets and ensuring size classes were biologically meaningful and represented by an adequate number of individuals. Size classes were based on maximum stem height (Table 11). One of the four target species (*A. michauxii*) has previously been modeled in this way (Wall et al. 2012). We included a single age class for seedlings in their first year for all the target species. For *L. pyrophilum*, we also included dormancy classes for each size class (see Figure 34).

Table 11: Size classes for the five target species.

Species	**Size class delineation**
Amorpha georgiana	seedling, small (1-10 cm), small-medium (10-25 cm), medium (25-50 cm), large (50-75 cm) and extra large (>75 cm).
Astragalus michauxii	seedling, extra small (1-10 cm), small (10-20 cm), small-medium (20-40 cm), medium (40-80), large (>80 cm)
Lilium pyrophilum	single leaf, small (1-20 cm), small-medium (20-40 cm), medium (40-100 cm), and large (>100 cm)
Lysimachia asperulifolia	small (1-10 cm), medium (11-20 cm), medium large (21-40 cm), large (>40 cm)

Transition matrix models were estimated from the vital rates using a Lefkovitch matrix model approach (Lefkovitch 1965; Caswell 2001). To avoid "immortal" size classes, we estimated survivorship for the size classes with no observed mortality based on neighboring size classes for each of the target species. For natural populations of *A. georgiana*, *A. michauxii*, and *L. pyrophilum* we estimated annual transition matrices for the pooled populations, as rare plant populations generally do not have enough individuals to estimate vital rates with enough precision. Transition matrices could not be generated for *L. asperulifolia*, due to an inability to identify individuals of this rhizomatous species in natural populations. We estimated annual transition matrices for each of the reintroduced populations of the three species considered individually and pooled together. However, we present the pooled matrices (section 5.6), given that one or more reintroduced population of all species had low numbers of individuals, and that data from natural populations were pooled. Population growth rates were calculated for all estimated transition matrices. The population growth rates from the pooled reintroduced population transition matrices were compared to the population growth rates from the natural populations using confidence intervals. Confidence intervals were generated for each population growth rate by resampling the original data with replacement (Efron and Tibshirani 1986).

Population growth rates for natural and reintroduced populations are shown in Table 12. Both *A. georgiana* and *L. pyrophilum* exhibited positive population growth rates within natural populations, but none of the individual or pooled growth rates of reintroduced populations exceeded the growth rates of natural populations. Consequently, our success criterion for this PO was not met for any of the species. However, it may be unrealistic to expect positive population growth rates within such a short timeframe. Many plant reintroduction efforts take a number of years to reach positive growth rates (Guerrant and Kaye 2007), and while the population growth rates for our reintroduced populations were less than one, stage distributions were shifting toward the larger size classes, suggesting growth, and there was evidence of reproduction. Both of these observations suggest that the trajectories of the reintroduced populations are moving in the right direction. As has been recommended (Godefroid et al. 2011), monitoring over a longer time span will be needed to determine the ultimate success of these reintroduced populations.

Table 12: Pooled population growth rates (λ) for natural and reintroduced populations.

Species	Natural	Reintroduced
Amorpha georgiana	1.06	0.80
Astragalus michauxii	0.97	0.75
Lilium pyrophilum	1.02	0.98

PO3: Equivalent or better *in situ* seedling recruitment in reintroduced vs. natural populations

PO3 was evaluated by comparing the number of seedlings per extant individual scaled by the number of seeds produced. Because the seeds of the target species all lack significant dispersal mechanisms, we assumed that the nearest individual was the parent plant. We marked all identified new seedlings in natural populations, but no seedling recruitment was observed in reintroduced populations of *A. georgiana*, *A. michauxii*, or *L. pyrophilum*.

In the case of *L. asperulifolia*, *in situ* recruitment was based on number of new stems. New stems (vegetative reproduction) were observed in both natural and reintroduced populations (section 5.6.4), but we were unable to distinguish individuals in the former. In the latter, we analyzed *L. asperulifolia* vegetative recruitment by simply calculating the proportion of outplanted individuals that vegetatively reproduced (i.e. produced more than a single stem; Table 8). There was great variability in the data, which is in line with our observations of high annual variability in numbers of *L. asperulifolia* stems in natural populations.

This PO was not met for *A. georgiana*, *A. michauxii*, or *L. pyrophilum* due to a general lack of reproduction within reintroduced populations and concomitant absence of recruitment. The PO also was not met for *L. asperulifolia*, despite documented reproduction within reintroduction sites.

PO4: Equivalent or better survivorship and transition probabilities in reintroduced vs. natural populations for all size classes

Data collected to evaluate PO4 were analyzed in three separate analyses. First we compared the survivorship (as the response variable) of reintroduced and natural populations using a generalized linear model with a binomial distribution and a logit link, which is defined as $\log(p/(1-p))$, where p is the probability of one of the two possible outcomes. Population type (reintroduced or natural) and size of individual (as a continuous variable) were considered fixed effects. Treating size as a continuous variable, rather than binned size classes, allowed for a more sensitive analysis of the data. To analyze survivorship for each size class of the target populations, we used a generalized linear model with population type and size class as categorical variables and survivorship as the response variable (McCullagh and Nelder 1989). Finally, differences in transition probabilities between reintroduced and natural populations were evaluated by calculating confidence intervals for each of the transition probabilities by bootstrapping the original data to create 1,000 matrices.

Results of analyses showed that only the survivorship of outplanted medium sized *L. pyrophilum* individuals was greater than the natural populations. For all other species and size classes, the survivorship of reintroduced individuals was the same or significantly less than survivorship in the natural populations (Figure 43). Note that the survivorship estimates shown in Figure 43 do not leverage information from natural populations for size classes not represented in the reintroduced populations, as was done for the transition matrices presented in section 5.6. Hence, the small discrepancies in the survivorship estimates of size classes presented here and previously.

No transition probabilities representing growth were significantly greater in the reintroduced populations than the corresponding transition probabilities in the natural populations for any species or size class. (See Chapter 6 for additional details). Consequently, this PO was not met for any of the four species.

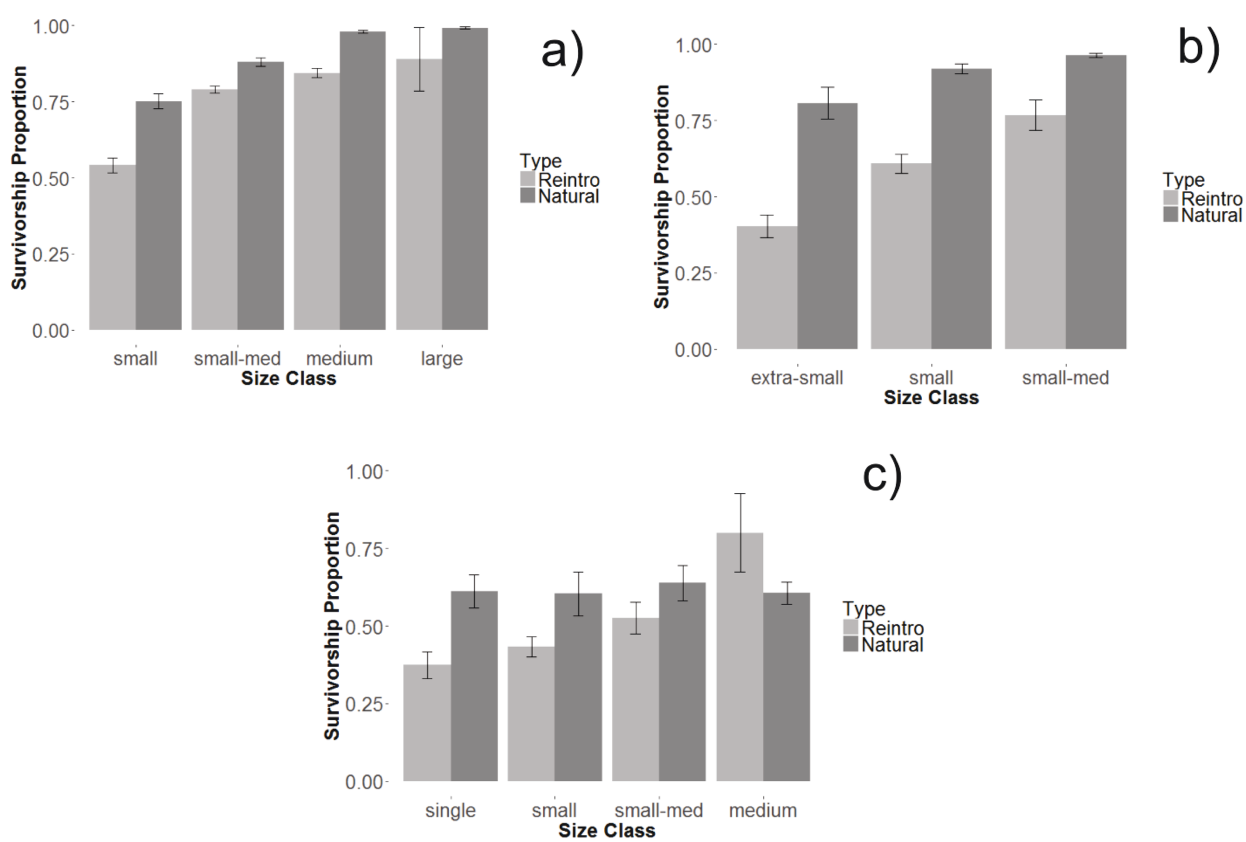

Figure 43: Survivorship by size class for *Amorpha georgiana* (a), *Astragalus michauxii* (b), and *Lilium pyrophilum* (c) in the natural and reintroduced populations.

PO5: Meet maximum cost threshold for growing and out-planting individuals of different size class to achieve positive population growth rate

PO5 was the threshold cost of $175 for the successful establishment of outplanted individuals. Although λs greater than one in reintroduced populations were not achieved, we estimated the costs associated with growing and outplanting individuals accounting for survivorship. For *A. georgiana, A. michauxii*, and *L. pyrophilum* cost was calculated by dividing the cost of propagation and out-planting cohorts by the survivorship of the outplanted size classes representing cohorts (as calculated for PO4).

In the case of *L. asperulifolia*, little relationship between size and survivorship was observed in reintroduced populations (Figure 38). Given that we propagated and outplanted a total length of 6,270 cm of *L. asperulifolia* rhizome for $10,574 (Table 16), the estimated cost per cm is $1.69. Outplanted rhizomes of approximately 8 cm in length cost $13.49. Using survivorship estimates for rhizomes of this length (0.35) we estimate an establishment cost of $38.55 per individual.

Results of analyses showed that establishment costs were lower than the $175 threshold and somewhat surprisingly that the most cost-effective cohort of all species was the youngest/smallest (Table 13). Differences in survivorship were not as great as the differences in propagation and reintroduction costs among cohorts.

Consequently, this PO was met for all four species.

Table 13: Survivorship by cohort for *Amorpha georgiana, Astragalus michauxii,* and *Lilium pyrophilum* along with associated costs.

Species	Cohort	Propagation and reintroduction cost per individual ($)	Mean survivorship (SD)	Establishment costs per individual ($)
A. georgiana	1	8.48	0.681 (0.128)	12.45
A. georgiana	2	7.62	0.561 (0.066)	13.59
A. georgiana	3	6.52	0.544 (0.023)	11.99
A. michauxii	1	12.83	0.527 (0.122)	24.34
A. michauxii	2	11.08	0.485 (0.105)	22.85
A. michauxii	3	8.26	0.451 (0.088)	18.32
L. pyrophilum	1	29.12	0.610 (0.120)	47.73
L. pyrophilum	2	22.43	0.523 (0.109)	42.89
L. pyrophilum	3	13.52	0.412 (0.115)	32.82

PO6: Demonstrate improved cost-effectiveness of watering (*A. georgiana* and *A. michauxii*) or competition reduction (*L. pyrophilum*) on transplant survival

For PO6 the threshold was that the cost of watering or site maintenance of individuals is less than or equal to the cost of establishing individuals without supplemental treatments. PO6 was similar to PO5, but considers the cost of supplemental watering or maintenance and the survivorship of the subset of individuals that received either treatment.

Results for supplemental irrigation of *A. georgiana* and *A. michauxii* showed that survival was not significantly increased by watering (section 5.6). It is likely that the supplemental irrigation of outplants was not effective because the sandy soils are excessively well-drained and water cannot be provided at the volume needed to substantially affect individual survivorship and growth, thus not translating into improved survival. At a cost of $19.83 per individual *A. georgiana* and $21.92 per individual *A. michauxii* (see Chapter 7, Cost Assessment), there is no cost-effectiveness in providing supplemental irrigation.

The vegetation-removal treatment applied to *L. pyrophilum* approximately doubled first-year survival but had little effect on growth (section 5.6). The cost of this maintenance treatment was estimated at only $3.87 per individual compared to the much higher propagation and reintroduction cost (Table 13). Consequently, increased survivorship associated with vegetation clearing was cost-effective.

In summary, this PO was met for *L. pyrophilum*, but not met for *A. georgiana* or *A. michauxii*.

PO7: Optimize cost for establishment of self-sustaining populations

PO7 involves optimizing the cost of establishment of new populations by identifying the transplant size classes that are the most cost-effective for establishing populations with population growth rates greater than one. None of the reintroduced populations of our target species exhibited growth rates greater than one, so technically this PO was unmet for the established criterion.

However, we conducted an alternative evaluation to support our Cost Analysis and Comparison example (see section 7.3), which identifies the number and cost associated with establishing 100 individuals in a reintroduced population five years post-outplanting. Establishment of 50 individuals is a common metric of success in plant reintroduction efforts, but larger numbers have also been suggested (Godefroid et al. 2011).

For *A. georgiana* and *A. michauxii* analyses included calculating vital rates and transition probabilities for the various outplanted size classes (see description in PO2 above), modeling population growth rates by systematically varying starting numbers of size classes, incorporating the cost per size class, and then using simulation methods to arrive at one or more sets of numbers of individuals and size classes that does not exceed the established threshold of $10,000. The size/stage distribution by cohort for *A. georgiana* and *A. michauxii* is shown in Table 14.

Table 14: Stage distribution by cohort for *Amorpha georgiana* and *Astragalus michauxii*. Note only data from 2014 outplants are shown for *A. michauxii*.

Species	Cohort	Stage	Number	Total	Proportion
A. georgiana	1	small	454	994	0.46
A. georgiana	1	small-med	468	994	0.47
A. georgiana	1	medium	72	994	0.07
A. georgiana	2	small	953	1032	0.92
A. georgiana	2	small-med	78	1032	0.08
A. georgiana	2	medium	1	1032	0.00
A. georgiana	3	small	1028	1037	0.99
A. georgiana	3	small-med	9	1037	0.01
A. michauxii	1	extra-small	180	395	0.46
A. michauxii	1	small	183	395	0.46
A. michauxii	1	small-med	31	395	0.08
A. michauxii	1	medium	1	395	0.00
A. michauxii	2	extra-small	206	338	0.61
A. michauxii	2	small	128	338	0.38
A. michauxii	2	small-med	4	338	0.01
A. michauxii	3	extra-small	117	153	0.76
A. michauxii	3	small	36	153	0.23

Results of our optimization, which identified the number of each cohort needed to establish 100 individuals in a reintroduced population of *A. georgiana* and *A. michauxii* five years post-outplanting and the associated costs are shown in Table 15. For *A. geogiana* cohort 1 (largest, 8 month) was identified as the most cost-effective, and satisfied the $10,000 threshold. In contrast, the youngest/smallest cohort was identified to be the most cost-effective for *A. michauxii*, and was the only cohort estimated to meet the $10,000 threshold.

Table 15: Outplant numbers needed by cohort for *Amorpha georgiana* and *Astragalus michauxii* to establish 100 individuals five years post-outplanting and estimated costs.

Species	Cohort	Number needed	Propagation and reintroduction cost per individual ($)	Estimated cost ($)
A. georgiana	1	396	8.48	3,358.08
A. georgiana	2	545	7.62	4,152.90
A. georgiana	3	574	6.52	3,742.48
A. michauxii	1	905	12.83	11,611.15
A. michauxii	2	1,080	11.08	11,966.40
A. michauxii	3	1,184	8.26	9,779.84

PO8: Demonstrate efficacy and cost-effectiveness of establishment of individuals via seed-addition versus transplanted individuals

PO8 evaluated the efficacy and cost-effectiveness of establishment of individuals via seed-addition plots compared to transplanted individuals for *A. georgiana* and *L. pyrophilum* (Table 2). No seedlings recruited into seed-addition plots during the course of the demonstration. Consequently, seed-addition is neither effective nor cost-efficient relative to transplanting for these species.

It is well-documented that for most species seeds provide lower rates of establishment than transplants (Godefroid et al. 2011). Although this performance objective was technically unmet as laid out in our demonstration, had we reversed the objective to match our anticipated outcome we would have successfully demonstrated that transplanting is more efficient and cost-effective than seed-addition.

PO9: Cost-effectiveness of watering seed-addition plots

PO9 addressed whether watering *A. georgiana* seed-addition plots would improve the cost-effectiveness of establishing individuals compared to no supplemental watering (Table 2). No seedlings recruited into irrigated or non-irrigated seed-addition plots during the course of the demonstration. Consequently, watering seed-addition does not improve the cost-effectiveness of establishing *A. georgiana* individuals compared to no supplemental watering. The success criterion for this PO was not met.

7. COST ASSESSMENT

For purposes of cost assessment, life-cycle costs (LCC) typically are divided into four components: (1) research and development, (2) investment (i.e. production and deployment), (3) operations and support, and (4) disposal. This project was focused on demonstrating and validating rare-plant population reintroduction technologies at operational scales. Consequently, our cost assessment emphasized costs associated with production and deployment. Life-cycle costs associated with disposal were not applicable to the technology being demonstrated since the intended purpose was to establish viable populations by permanently leaving transplants in place. Life-cycle costs associated with operations and support were also expected to be minimal. While we did not originally expect that ongoing demographic monitoring would be a necessary cost associated with the technologies, the population growth rates estimated to date suggest additional monitoring will be necessary to document demographic changes that would increase population trajectories. Although we have limited ability to predict the number of years needed to achieve self-sustaining populations, five additional years should be sufficient. If conditions do not change, population growth rates documented during this post-establishment phase (as opposed to early deployment) should largely remain constant. Other potential operations and support costs (e.g., invasive plant control, prescribed fire) are not specific to the technologies being demonstrated, but rather typical expenses associated with rare-plant conservation and management.

For our cost assessment we employed a combination of estimating techniques, including actual cost and engineering estimates for the various cost elements. To the extent possible we attempted to use cost data that are broadly representative of the current industry rates.

7.1 COST MODEL

Our overall cost model for production and deployment included seven cost elements (Table 16), and can be expressed as:

$$\text{Overall cost} = \text{SC} + \text{Pr} + \text{StP} + \text{OPl} + \text{StM} + \text{DM} + \text{DMo}.$$

Cost elements were broken down into multiple common sub-elements to facilitate scaling technology during future deployment.

Table 16: Cost elements and data tracked during demonstration. Calculated totals are rounded to the nearest dollar. [Continued to next two pages.]

Cost Element	Data Tracked	Formula	Estimated Costs			
			Amorpha georgiana	*Astragalus michauxii*	*Lilium pyrophilum*	*Lysimachia asperulifolia*
1. Propagule Collection (PC) Labor Transportation	Labor rate (L), hours (H), and Miles travelled (M) and cost/mile (MC)	PC= L * H + M * MC	43 hrs NCBG professional @ \$25.80/hr = \$1,109; 750 miles @ \$0.41/mile = \$310 Total: \$1,419 Cost/pop: \$355 Cost/ind.: \$0.46	48 hrs NCBG professional @ \$25.80/hr = \$1,238; 600 miles @ \$0.41/mile = \$248 Total: \$1,485 Cost/pop: \$371 Cost/ind.: \$0.78	19 hrs NCBG professional @ \$25.80/hr = \$490; 450 miles @ \$0.41/mile = \$186 Total: \$676 Cost/pop: \$169 Cost/ind.: \$1.01	24 hrs NCBG professional @ \$25.80/hr = \$619; 450 miles @ \$0.41/mile = \$186 Total: \$805 Cost/pop: \$201 Cost/ind.: \$1.13
2. Propagation (Pr) Labor Supplies Facility costs	Labor rate (L), hours per individual (HI), number of individuals (I), cost of purchased supplies (S), facility cost per area (F), area used (A)	Pr = L * HI * I + S + F * A	284 hrs Technician @ \$12/hr = \$3,408; Supplies = \$539; 819 ft^2 @ \$4.07/ft^2 = \$3,333 Total: \$7,280 Cost/pop: \$1,820 Cost/ind.: \$2.37	696 hrs Technician @ \$12/hr = \$8,352; Supplies = \$353; 601 ft^2 @ \$4.07/ft^2 = \$2,444 Total: \$11,150 Cost/pop: \$2,787 Cost/ind.: \$5.83	308 hrs Technician @ \$12/hr = \$3,696; Supplies = \$110; 1,800 ft^2 @ \$4.07/ft^2 = \$7,328 Total: \$11,134 Cost/pop: \$2,783 Cost/ind.: \$16.62	297 hrs Technician @ \$12/hr = \$3,564; Supplies = \$74; 531 ft^2 @ \$4.07/ft^2 = \$2,163 Total: \$5,801 Cost/pop: \$1,450 Cost/ind.: \$8.17
3. Site Preparation (StP) Labor Supplies Transportation	Labor rate (L), hours (H), cost of purchased supplies (S), miles travelled (M), and cost/mile (MC)	StP = L * H + S + M * MC	4 hrs NCBG professional @ \$25.80/hr = \$103; 42 hrs Technician @ \$12/hr = \$607; Supplies = \$417; 250 miles @ \$0.41/mile = \$103 Total: \$1,127 Cost/pop: \$282 Cost/ind.: \$0.94	4 hrs NCBG professional @ \$25.80/hr = \$103; 25 hrs Technician @ \$12/hr = \$403; Supplies = \$347; 550 miles @ \$0.41/mile = \$227 Total: \$978 Cost/pop: \$245 Cost/ind.: \$0.98	4 hrs NCBG professional @ \$25.80/hr = \$103; 30 hrs Technician @ \$12/hr = \$463; Supplies = \$233; 450 miles @ \$0.41/mile = \$186 Total: \$882 Cost/pop: \$221 Cost/ind.: \$1.32	4 hrs NCBG professional @ \$25.80/hr = \$103; 70 hrs Technician @ \$12/hr = \$943; Supplies = \$247; 600 miles @ \$0.41/mile = \$248 Total: \$1,438 Cost/pop: \$360 Cost/ind.: \$2.03

Cost Element	Data Tracked	Formula	Estimated Costs			
			Amorpha georgiana	*Astragalus michauxii*	*Lilium pyrophilum*	*Lysimachia asperulifolia*
4. Outplanting (OPl) Labor Supplies Transportation	Labor rate (L), hours (H), cost of purchased supplies (S), miles travelled (M), and cost/mile (MC)	OPl = L * H + S + M * MC	734 hrs Technician @ $12/hr = $8,808; Supplies = $2,045; 2,250 miles @ $0.41/mile = $930 Total: $11,783 Cost/pop: $2,946 Cost/ind.: $3.84	375 hrs Technician @ $12/hr = $4,500; Supplies = $1,277; 2,250 miles @ $0.41/mile = $930 Total: $6,707 Cost/pop: $1,677 Cost/ind.: $3.50	304 hrs Technician @ $12/hr = $3,648; Supplies = $447; 2,100 miles @ $0.41/mile = $868 Total: $4,516 Cost/pop: $1,129 Cost/ind.: $6.74	185 hrs Technician @ $12/hr = $2,220; Supplies = $474; 750 miles @ $0.41/mile = $310 Total: $2,530 Cost/pop: $633 Cost/ind.: $3.56
5. Site Maintenance Treatments (StM) Labor Supplies Transportation	Labor rate (L), hours (H), cost of purchased supplies (S), miles travelled (M), and cost/mile (MC)	StM = L * H + S + M * MC	147 hrs Technician @ $12/hr = $1,764; Supplies = $963; 5,400 miles @ $0.41/mile = $2,232 Total: $~~4,959~~ Cost/ind.: $~~19.83~~	147 hrs Technician @ $12/hr = $1,764; Supplies = $963; 3,000 miles @ $0.41/mile = $1,240 Total: $~~3,967~~ Cost/ind.: $~~21.92~~	30 hrs Technician @ $12/hr = $360; 450 miles @ $0.41/mile = $186 Total: $545 Cost/ind.: $3.87	NA
6. Demographic Monitoring (DM) Labor Supplies Transportation	Labor rate (L) and hours (H) Cost of purchased supplies (S) Miles travelled (M) and cost/mile (MC)	DM = L * H + S + M * MC	400 hrs NCBG professional @ $25.80/hr = $10,320; Supplies = $94; 1,746 miles @ $0.41/mile = $722 Total: $11,136 Cost/pop: $2,784 Cost/ind.: $0.73	367 hrs NCBG professional @ $25.80/hr = $9,469; Supplies = $94; 3,005 miles @ $0.41/mile = $1,242 Total: $10,805 Cost/pop: $2,701 Cost/ind.: $1.41	227 hrs NCBG professional @ $25.80/hr = $5,857; Supplies = $94; 1,724 miles @ $0.41/mile = $713 Total: $6,569 Cost/pop: $1,642 Cost/ind.: $2.37	168 hrs NCBG professional @ $25.80/hr = $4,334; Supplies = $94; 1,760 miles @ $0.41/mile = $727 Total: $5,062 Cost/pop: $1,266 Cost/ind.: $2.23

Cost Element	Data Tracked	Formula	Estimated Costs			
			Amorpha georgiana	***Astragalus michauxii***	***Lilium pyrophilum***	***Lysimachia asperulifolia***
7. Demographic Modeling (DMo) Labor	Labor rate (L) and hours (H)	DMo = L * H	40 hrs ERDC professional @ $40.00/hr = $1,600; Total: $1,600 Total/pop: $400 Total/ind.: $2.36	40 hrs ERDC professional @ $40.00/hr = $1,600; Total: $1,600 Total/pop: $400 Total/ind.: $6.23	40 hrs ERDC professional @ $40.00/hr = $1,600; Total: $1,600 Total/pop: $400 Total/ind.: $9.94	40 hrs ERDC professional @ $40.00/hr = $1,600; Total: $1,600 Total/pop: $400 Total/ind.: $9.30
Total Cost			Total: $34,345 Total/pop: $8,586 Total/ind.: $11.21	Total: $32,725 Total/pop: $8,181 Total/ind.: $17.10	Total: $25,923 Total/pop: $6,481 Total/ind.: $38.69	Total: $17,236 Total/pop: $4,309 Total/ind.: $24.28

Cost Element 1: Propagule Collection (PC)

The propagule collection cost element included the cost associated with obtaining necessary permits, collecting propagules (i.e., seeds and rhizomes), and post-processing prior to storage or use in propagation for the target species over three years. Costs can be divided into labor rate, hours, and travel costs. The hourly labor rate for this cost element was based on NCBG professional labor ($25.80). Data on travel costs included mileage and the NCBG motor fleet rate ($0.4133 per mile). These figures support an estimate for future implementation but, as discussed previously, travel costs will be highly variable depending on distances to propagule source populations. Given the ease of collection for all species except *L. asperufolia,* it seems likely managers of the collection site(s) would be willing to collect and mail seeds to the propagation facility. Supplies costs were not tracked since only a few readily available items of minimal expense (e.g., paper bags and several shovels) were needed. No scaling issues were applicable and the data were interpreted as calculated.

Cost Element 2: Propagation (Pr)

The propagation cost element was the cost associated with germination and growing the individual plants in the greenhouse for outplanting into the selected reintroduction sites. Data tracked for the propagation cost element included labor, supplies, and facility costs. Labor was calculated as number of hours multiplied by our technician rate ($12.00/hr) and was tracked for the different size classes propagated for each of the target species. Supplies included items such as plug trays and soil. Facility costs varied by species and cohort due to differing space requirements and growing durations. No scaling issues were applicable and the data were interpreted as calculated.

Total labor, supplies and facility costs were divided by the number of individuals of each species surviving until outplanting. Costs reported reflect cost per individual to allow estimation of future implementation costs.

Cost Element 3: Site Preparation (StP)

Site preparation (StP) costs included labor, travel expenditures, and supplies (e.g., measuring tapes, chain pins, pin flags, loppers, gloves, etc.) associated with site selection and preparation prior to outplanting, but not our irrigation or vegetation removal treatments (see Cost Element 4 below). Labor for site selection was calculated as number of hours multiplied by our NCBG professional rate ($25.80/hr) and was tracked for each of the target species. Labor for site preparation was calculated as number of hours multiplied by our technician rate ($12.00/hr) and was also tracked for each of the target species. Travel costs were estimated using the NCBG motor fleet rate ($0.4133 per mile).

Once a site is chosen, the amount of preparation required is a function of the condition and size of the site. Condition refers to the degree to which the structure and composition of the plants at the site conform to plant communities known to be occupied by the target species. To a lesser degree it also refers to the amount of above-ground biomass that might need to be removed to facilitate planting. A growing-season burn prior to outplanting will eliminate the need to clear biomass in most communities. The size of reintroduction sites will be determined by the number and spacing of plants, the outplanting schedule, and the species life cycle. For all species, we spaced plants at 1–2 m using a regular grid. We also outplanted over three successive years to facilitate a variable age/size structure and in anticipation of inter-year, weather-induced, variation in survivorship. For species not known to exhibit dormancy (e.g., *A. georgiana* and *A. michauxii*), we were able to replant into the locations of plants that had died in the prior year, thus reducing the need to expand the size of the reintroduction sites during successive years of outplanting.

Total costs of labor, travel, and supplies were divided by the total number of *L. pyrophilum* (n = 670) and *L. asperulifolia* (n = 710) individuals outplanted over three years, and by the maximum number of *A. georgiana* (n = 1,200) and *A. michauxii* (n = 1,000) individuals outplanted during any year. Costs reported reflect cost per individual to facilitate cost estimates during future implementation.

Cost Element 4: Site Maintenance (StM)

Site maintenance costs included labor, supplies, and travel expenditures associated with the irrigation and competition reduction treatments applied to several species. Labor was calculated as number of hours multiplied by our technician rate and was tracked for each of the target species. Travel costs were estimated using the NCBG motor fleet rate and tracked for each species. Supply costs were incurred for the irrigation treatment and included items such as tubing, connectors, spigots, rain barrels, and timers. No new supplies beyond those used for site preparation were needed for the competition reduction treatment.

Irrigation treatments applied to *A. georgiana* had negligible impact on reintroduction success; survivorship of irrigated plants was marginally greater than non-irrigated plants (79% vs. 70%, respectively; $p > 0.05$). At an estimated cost of roughly $20 per individual (Table 10), we cannot recommend the use of supplemental irrigation within *A. georgiana* reintroduction sites. Similarly, irrigation did not enhance *A. michauxii* growth or survival ($p > 0.05$), and cannot be recommended (see section 5.6, PO6). Consequently, we provide an estimate for this cost element in Table 16, but it was not included in our total costs. The vegetation-removal treatment applied to *L. pyrophilum* approximately doubled first-year survival but had little effect on growth. Given the extended propagation phase required for this species, we recommend this treatment and have included the associated cost in our overall cost estimates.

After observing herbivory of *L. pyrophilum* vegetative growth and excavation of bulbs, we also added an enclosure treatment. Exclosures were constructed from 1.6 m^2 of 19-gauge hardware cloth at a cost of approximately $10 each, and they were easily positioned during outplanting. However, these exclosures did not confer a statistically significant increase in outplant survivorship (67% cage vs 48% no cage; $p > 0.05$) or growth ($p > 0.05$) (see Section 5.6). Therefore, we have not included the cost of this treatment within our overall cost estimates for this species.

Total costs of labor, travel, and supplies were divided by the number of individuals receiving each treatment. Irrigation was provided to 250 *A. georgiana* and 181 *A. michauxii* individuals.

Competition reduction treatments were applied to 141 *L. pyrophilum* individuals within two sites. Costs reported reflect the costs of applying the effective treatments on a cost per individual basis, allowing easy estimation during future implementation.

Cost Element 5: Outplanting (OPl)

Outplanting costs included labor, supplies, and travel expenditures incurred during the three years that plants were reintroduced into our demonstration sites. Labor for outplanting was calculated as number of hours multiplied by the specified technician rate and was tracked for each of the target species. Included in the labor estimate are hours spend planning, transporting plants, and actual outplanting. Supply costs included various items such as aluminum identification tags, hand tools for planting, auger bits, and backpack sprayers. Travel costs were estimated using the specified NCBG motor fleet rate.

Total costs of labor, travel, and supplies were divided by the total number of individuals outplanted for each species (3,065 *A. georgiana*; 1,914 *A. michauxii*; 670 *L. pyrophilum*; 710 *L. asperulifolia*) over three years to estimate the cost per individual. Outplanting costs did not differ among cohorts.

Cost Element 6: Demographic Monitoring (DM)

Demographic monitoring costs included labor, supplies, and travel expenditures incurred during the four years that data were collected on plant survival, growth, and reproduction within our reintroduction sites and within natural populations. Labor for monitoring was calculated as number of hours multiplied by the NCBG professional rate and was tracked for each of the target species. Included in the labor estimate are hours spend planning, traveling to natural and reintroduced populations, collecting data in the field, and entering data into spreadsheets. Supply costs included items such as calipers, retractable measuring tapes, and field notebooks. Travel costs were estimated using the NCBG motor fleet rate.

Total costs of labor, travel, and supplies for each species were divided by the total number of monitoring observations made within reintroduced and natural populations (Table 17) over four years to estimate cost per individual. Monitoring costs did not differ among cohorts.

Table 17: Numbers of monitoring observations collected for each species within natural and reintroduced populations over five years (2012-2016).

Species	Natural populations	Reintroduced populations
A. georgiana	3,812	11,420
A. michauxii	2,554	5,116
L. pyrophilum	620	2,147
L. asperulifolia	651	1,619

Cost Element 7: Demographic Modeling (DMo)

Demographic modeling costs included labor rate multiplied by hours. Labor rate for the demographic modeling were calculated based on the mean ERDC-CERL professional labor rate

($40/hr). Total cost of labor was divided by the total number of individuals established for each species (677 *A. georgiana*; 257 *A. michauxii*; 161 *L. pyrophilum*; 172 *L. asperulifolia*) over four years to estimate the cost per individual.

7.2 COST DRIVERS

The three primary cost drivers to be considered when implementing the technology are propagation facility costs, labor, and travel costs. Propagation facility costs are highly variable, so particular attention should be paid to this cost during future implementation. During our demonstration we made extensive use of a volunteer-labor base, which has been developed over many years by NCBG. Although this volunteer labor was free to the demonstration project, we applied a technician rate of $12/hour in our cost estimates. It is expected that with some effort, future implementation of the reintroduction technologies elsewhere could also make use of volunteer labor that would be cost-free.

All aspects of population reintroduction (phase 2) and the monitoring component of comparative demographic analysis (phase 3) are inherently site-specific. Consequently, cost elements related to site preparation (StP), outplanting (OPl), site maintenance (StM), and demographic monitoring (DM) are likely to vary across sites and affect overall implementation costs. It is the labor and travel cost elements that are primarily affected by site location. For example, our demonstration was conducted in a location having convenient access to many nearby natural populations that could serve as comparisons when evaluating the success of our reintroduced populations. Unfortunately, many rare species have suffered extensive loss of populations and habitat across their ranges. This factor has the potential to increase implementation costs. The impact on implementation costs will be greatest when conservation goals focus on increasing representation of species across their former ranges, but those historical ranges have been greatly diminished. An example of this scenario among our species is *A. georgiana*, which is currently known from nine counties in North Carolina, South Carolina, and Georgia, but had a historical distribution that spanned at least 14–16 counties (Straub et al. 2009). Several of these counties are isolated (e.g., >115 miles) from currently extant populations, which would likely increase travel time and inflate reintroduction and demographic monitoring costs. Partnering with others who are near either the reintroduced or comparison natural populations, and who could assist with monitoring would help to abate costs.

7.3 COST ANALYSIS AND COMPARISON

We effectively conducted an operational implementation during our demonstration, which included both production scale propagation of outplants and reintroduction of populations at multiple sites. However our reintroduced populations were not optimized for cost or success, due to our test design which evaluated variable efficacy and cost-efficiency of different age/size cohorts. Therefore, here we provide a cost analysis and comparison example that utilizes our "best" size cohorts. This example would be suitable for reintroductions at multiple public and private conservation lands near Fort Bragg that host suitable habitats for one or more of the species, such as Carver's Creek State Park, Sandhills Game Lands, and the Calloway Forest Preserve. As was described for PO7 in Chapter 6, we identified the number of individuals of each cohort required to establish 100 surviving individuals five years post-outplanting and the associated cost for propagation and reintroduction. For this example, we (1) used the transition matrices generated for *A. georgiana*, and *A. michauxii*, (2) applied the costs of the best performing cohort estimated in our demonstration cost assessment, (3) modeled outplanting during a single year, as opposed to multiple years, and (4) excluded monitoring costs. Thus, it assumes the same vital rates, as well as propagation and reintroduction costs as those documented in our cost optimization (Table 15).

For *A. michauxii*, the cost of establishing a population of 100 individuals five years post-outplanting varied from $3,338 for the 8 month cohort to $4,153 for the 6 month cohort. Consequently, there is a potential $795 per population cost avoidance when using the most cost-effective cohort. For *A. georgiana*, the cost of establishing a population of 100 individuals five years post-outplanting ranged from $9,780 for the 3 month cohort to $11,611 for the 6 month cohort. Consequently, there is a potential $2,187 per population cost avoidance when using the most cost-effective cohort.

8. IMPLEMENTATION ISSUES

Many factors can potentially influence rare plant propagation and reintroduction success. Some of these factors can be anticipated and planned for, while others will be unpredictable (e.g., flooding, drought, or wildfires). Maschinski and Haskins (2012) provide a comprehensive, up-to-date summary of plant reintroduction science and practice, which identifies many potential challenges and pitfalls practitioners may encounter. This authoritative resource should be thoroughly reviewed before embarking on any effort to propagate or reintroduce the species included in this demonstration. In addition to the present Final Report, we also recommend that practitioners reference our separate Reintroduction Guidelines Manual (Appendix C) for a detailed summary of propagation and reintroduction protocols for each species.

Our test design allowed us to empirically evaluate several important implementation considerations, which serve as valuable lessons learned. As highlighted in section 5.6 (Sampling Results) and observed by others (Guerrant 1996), size at outplanting is a critical determinant of outplant survival and establishment. We anticipated greater survival of larger individuals, but explored the efficacy and cost-efficiency of using smaller size classes in our demonstration. This depressed the vital rates and growth rates of our reintroduced populations. However, we also learned that these smaller size classes were the most cost-efficient to establish (Table 13). Future reintroduction efforts should utilize the optimal size of outplants identified by this demonstration.

We also learned that irrigation, as provided by a gravity drip system, had negligible effect on reintroduction success. For example, survivorship of irrigated *A. georgiana* plants was only marginally greater than non-irrigated plants (79% vs. 70%, respectively; $p > 0.05$). We speculate that the water delivery rate was insufficient given the sandy soils, or that established perennials near *A. georgiana* and *A. michauxii* outplants were preferentially able to utilize the additional water, which resulted in increased competition that negatively affected outplants. If the first growing season post-outplanting happens to coincide with drought (Stahle et al. 1988), however, it is possible that occasional supplemental water may increase survival.

Additionally, our test design showed that site maintenance (i.e., vegetation removal) can improve reintroduction success. For example, *L. pyrophilum* survival approximately doubled when above-ground vegetation was cut within 1 m of outplanted bulbs ($p = 1.23$ e-08). Although, we initially thought vegetation removal would positively affect success by reducing above-ground competition, we found no increase in growth under this treatment compared to control plants ($F = 0.245$, $p = 0.63$). It is possible vegetation clearing and prescribed fire both affect *L. pyrophilum* survivorship indirectly, by influencing rodent abundance and foraging behavior within inhabited communities (Krall et al. 2014).

Although not part of our original test design, observations of herbivory and bulb predation during the first year of our demonstration prompted us to erect exclosures around *L. pyrophilum* outplants. These exclosures did not confer a statistically significant increase in outplant survivorship (67% cage vs 48% no cage; $p > 0.05$) or growth ($p > 0.05$). However, deer are known to browse *Lilium* spp., halting growth and reproduction during that growing season (Fletcher et al. 2001). For *L. pyrophilum*, we documented browsing on 12.5% of plants (n = 706) over eight years across monitored natural populations (Wall et al. unpublished data) and 1% of outplants within four reintroduction sites over four years. Rodents (e.g., mice, squirrels and chipmunks) can potentially have greater negative impacts on reintroduction success, as they are known to dig up and consume *Lilium* spp. bulbs (Fletcher et al. 2001). Some success in protecting outplants from these herbivores can be achieved by erecting exclosures. Given the time needed to propagate bulbs and the minimal cost of the exclosures, practitioners may want to use exclosures to protect *L. pyrophilum* outplants during at least the first year when implementing *L. pyrophilum* population reintroductions.

Implementing the reintroduction technologies will in most cases require obtaining relevant permits for working with the species, and permission from owner of the property where the reintroduction takes place. Various permits for working with the species (e.g., possessing, collecting, transporting, or propagating) may be required, depending on federal and state protection status (Table 1). Research permits should be sought from the USFWS for species federally listed under the ESA, and comparable permits should be sought from appropriate State agencies for state-protected species. For example, the NCBG has a federal research permit for *Lysimachia asperulifolia*. We also obtained a permit from the Plant Conservation Program (North Carolina Department of Agriculture) to work on the five target species. The Plant Conservation Program is the state-level regulatory agency in North Carolina responsible for issuing permits to perform research on state-listed plant species. In addition, we obtained a site-specific research permit for Weymouth Woods Sandhills NP. Although all of these entities supported our efforts and granted permits, it is possible that regulators in other states or USFWS field offices may be less receptive to reintroduction efforts.

End-user concerns identified during our workshop included lack of funding, limitations on seed availability, uncertainty about choosing a propagation facility for future implementation, potential damage to reintroduced populations by wild hogs, site-specific fire behavior within reintroduced populations, and poaching of *L. pyrophilum* outplants.

Use of reintroduction as a strategy for rare-plant conservation and mitigation has been shown to have variable success. In a review of reintroduction success that examined data from 249 different plant species, Godefroid et al. (2011) estimated that mean survival was approximately 20% for individuals three years post-outplanting. We documented comparable rates of survival for three of our target species (i.e. *A. georgiana*, *L. pyrophilum*, and *L. asperulifolia*), while the survival rates of the other two species (*A. michauxii* and *P. brevifolia*) were decreased by small outplant size during one or more years. Additional monitoring will be needed to determine the ultimate fate of the populations reintroduced during this demonstration. Although many of our performance objectives were unmet, we remain optimistic about the expanded conservation strategies and new opportunities to share conservation responsibility with partner agencies and organizations that are made possible by the propagation and reintroduction protocols. However, use of the demonstrated technologies should not be a substitute for active conservation of the remaining natural populations of the five species, especially given the modest success of our reintroduction efforts compared to the effort invested.

9. FUTURE RESEARCH NEEDS AND RECOMMENDATIONS

We recommend several future studies that can directly or indirectly inform the operational-scale implementation of the reintroduction technologies, including studies focused on species metapopulation processes, distribution modeling, and functional traits.

Many natural populations of plants in modern landscapes are threatened by their vast isolation from other populations of the same species (Ellstrand and Elam 1993; Hanski 1998; Fischer and Lindenmayer 2007). Fort Bragg and many other military lands are exceptions to this trend because they offer large and relatively intact landscapes where multiple populations of a species can persist sufficiently near each other to allow genetic exchange, provide a source of propagules that can rescue declining populations, and permit (re)colonization of suitable habitat. These interactions among interconnected populations, or *metapopulations*, are widely assumed to promote the local and regional viability of rare species (Hanski 1998). Explicit characterization of species' metapopulation structure (i.e. population size, separation distance, and connectivity) would be helpful for guiding current and future population reintroduction and augmentation efforts, as well as habitat management to enhance population connectivity.

Land-management agencies increasingly recognize the utility of species distribution modeling (SDM) for rare-plant conservation planning and management (e.g., Elith and Burgman 2002; Williams et al. 2009; Gogol-Prokurat 2011). Knowledge about species habitat requirements and the spatial distribution of available habitat are critical for designing and implementing many conservation actions, but oftentimes this knowledge is incomplete for rare plants. SDM can provide insights about habitat requirements and availability, which in turn can be used to (1) confidently select sites suitable for population reintroductions and augmentation, (2) prioritize land management and acquisition initiatives to better support species conservation and recovery, and (3) foster additional opportunities for sharing conservation responsibilities among Federal, state, and nongovernmental organization (NGO) land-management partners. To date, species-distribution models have not been developed for the five species addressed in this study.

Functional traits, which are measurable properties of individuals that are related to performance, are increasingly being utilized by community ecologists as an alternative to species-centered approaches (e.g., Keddy 1992; McGill et al. 2006; Westoby and Wright 2006). Ames et al. (2017) recently showed that rare plants in the North Carolina Sandhills occupy a subset of the trait space occupied by more common community associates. Based on ecological niche theory, this finding suggests that functional similarity may be a primary factor responsible for determining the success (i.e., growth, survival, and reproduction) of reintroduced individuals. Despite having important implications for the allocation of limited conservation resources, the degree to which functional similarity affects the success of reintroduced plants relative to abiotic or biotic conditions is poorly understood. Insights gained from studies of functional traits could be incorporated into microsite selection, which would complement species distribution modeling and could improve reintroduction success.

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APPENDICES

APPENDIX A: POINTS OF CONTACT

POINT OF CONTACT Name	ORGANIZATION Name Address	Phone Fax Email	Role in Project
Matthew Hohmann	US Army ERDC-CERL 2902 Farber Dr. Champaign, IL 61822	217-373-5863 217-373-7266 matthew.g.hohmann@ us.army.mil	project management, demographic monitoring, cost analyses report writing
Dr. Wade Wall	US Army ERDC-CERL 2902 Farber Dr. Champaign, IL 61822	217-373-7320 217-373-7266 wade.a.wall@us.army.mil	project management, demographic monitoring, demographic analysis, report writing
Michael Kunz	North Carolina Botanical Garden University of North Carolina at Chapel Hill CB#375, Totten Center Chapel Hill, NC 27599	919-962-0522 919-962-3531 mkunz@email.unc.edu	plant propagation, demographic monitoring, cost analyses technology end-user
Dr. Johnny Randal	North Carolina Botanical Garden University of North Carolina at Chapel Hill CB#375, Totten Center Chapel Hill, NC 27599	919-962-0522 919-962-3531 jrandall@email.unc.edu	technology end-user
Janet Gray	Department of the Army Public Works Business Center ATTN: AFZA-PW-NE HQ, Fort Bragg Garrison Command (Abn) Fort Bragg, NC 28310	910-396-2544 910-432-7776 janet.bracey.gray@us.arm y.mil	demonstration site liaison, demographic monitoring, technology end-user
Nancy Williamson	Weymouth Woods Sandhills Nature Preserve 1024 Ft. Bragg Rd Southern Pines, NC 28387	910-692-2167 910-692-8042 nancy.williamson@ ncparks.gov	demonstration site liaison, technology end-user
Dale Suitor	U.S. Fish and Wildlife Service P.O. Box 33726 Raleigh, NC 27636-3726	919-856-4520 919-856-4556 dale_suitor@fws.gov	USFWS liaison, technology end-user
Dr. William Hoffmann	Department of Plant Biology North Carolina State University Raleigh, NC 27695-7612	919-513-7668 919-515-3436 william_hoffmann@ ncsu.edu	demography expert

APPENDIX B: MAPS OF DEMONSTRATION SITES

Key to map designations.

Figure A	AMGE010B
Figure B	AMGE012E
Figure C	AMGE015B
Figure D	AMGE017C
Figure E	ASMI036A
Figure F	ASMI056A
Figure G	ASMI059A
Figure H	ASMI060A
Figure I	LIPY011A
Figure J	LIPY015B
Figure K	LIPY015C
Figure L	LIPY013 (Weymouth Woods)
Figure M	LYAS030B
Figure N	LYAS057A
Figure O	LYAS068A
Figure P	LYAS069A
Figure Q	PYBR035A
Figure R	PYBR046G
Figure S	PYBR065G
Figure T	PYBR068A

AMGE010B

Figure A: ***A. georgiana*** **010B**

AMGE012E

Figure B: ***A. georgiana*** **012E**

AMGE015B

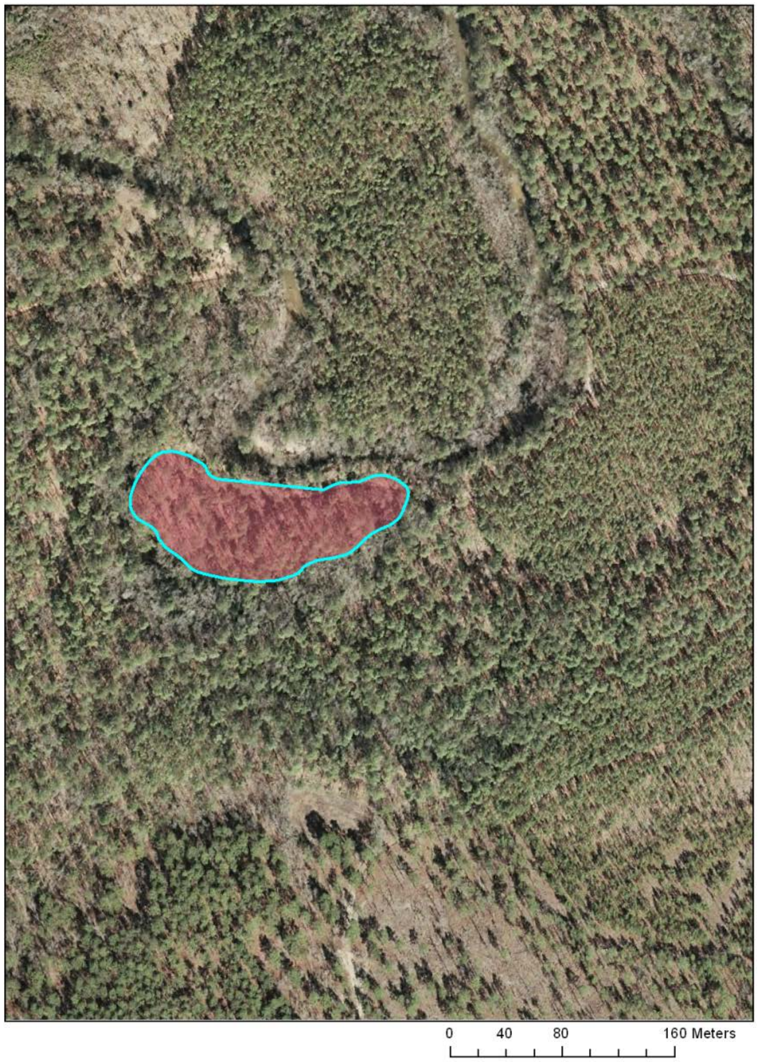


Figure C: ***A. georgiana*** **015B**

AMGE017C

Figure D: ***A. georgiana*** **017C**

ASMI036A

Figure E: ***A. michauxii*** **036A**

ASMI056A

Figure F: ***A. michauxii*** **056A**

Figure G: ***A. michauxii*** **059A**

Figure H: ***A. michauxii*** **060A**

LIPY011A

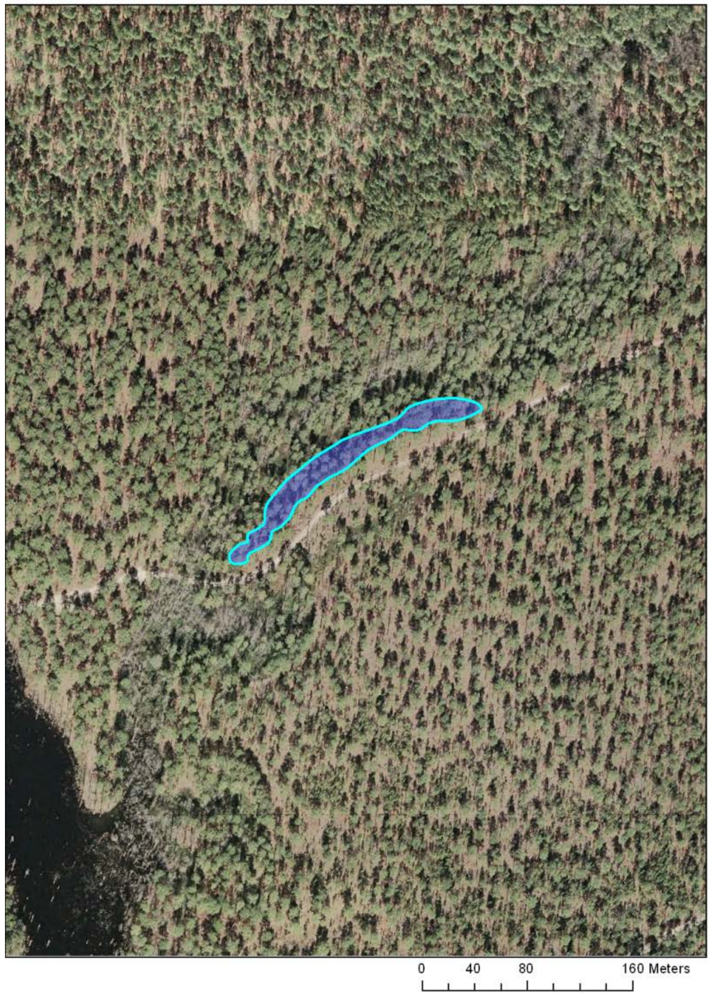


Figure I: ***L. pyrophilum*** **011A**

LIPY015B

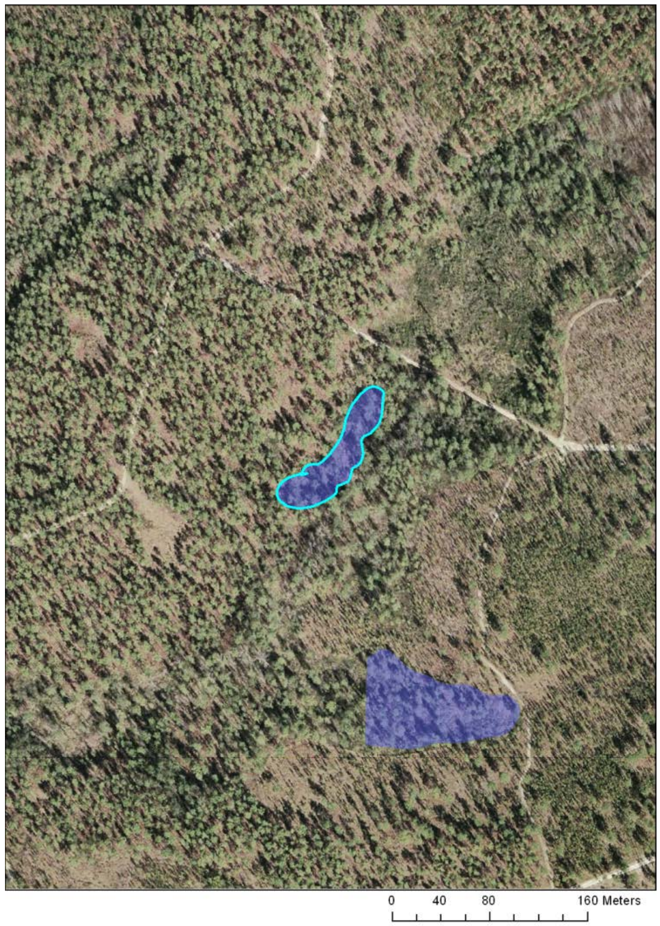


Figure J: ***L. pyrophilum*** **015B**

Figure K: ***L. pyrophilum*** **015C**

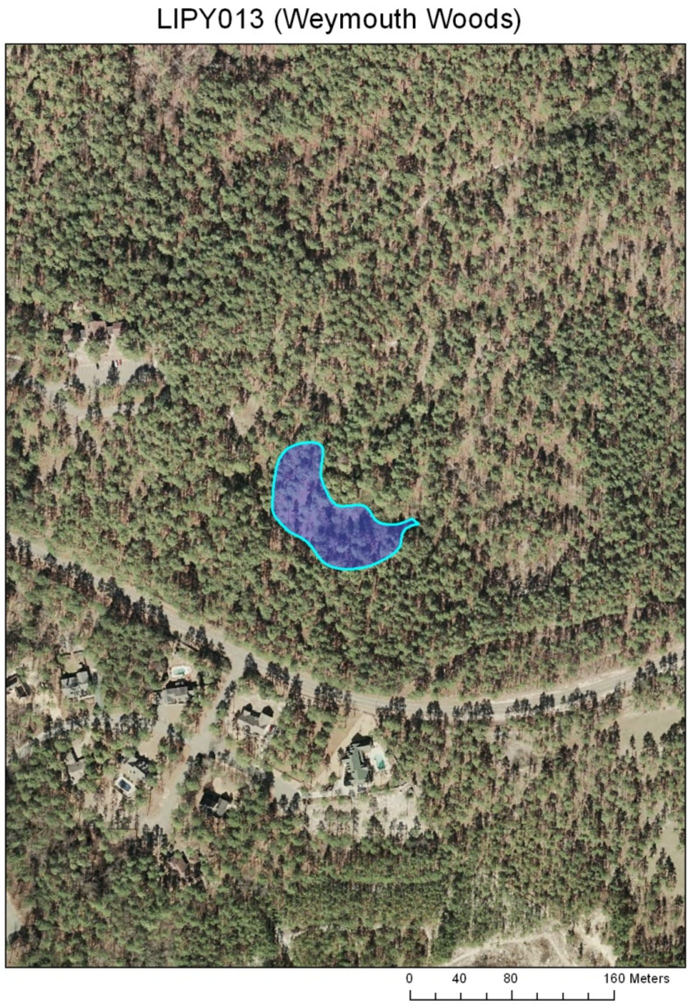


Figure L: ***L. pyrophilum*** **013 (Weymouth Woods)**

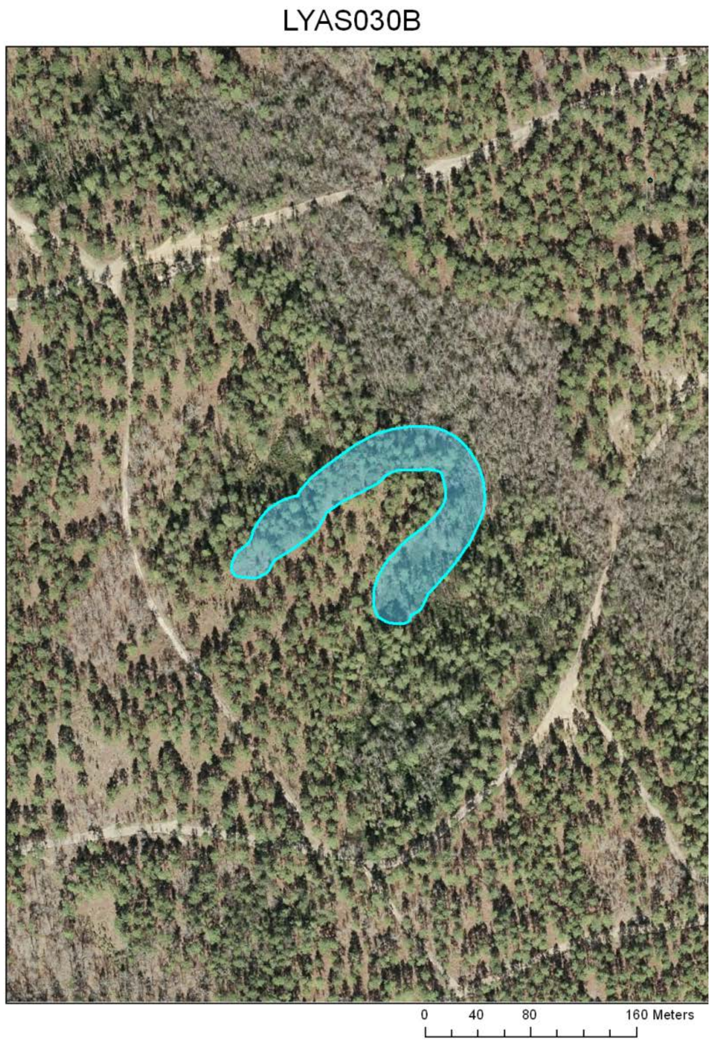


Figure M: ***L. asperulifolia*** **030B**

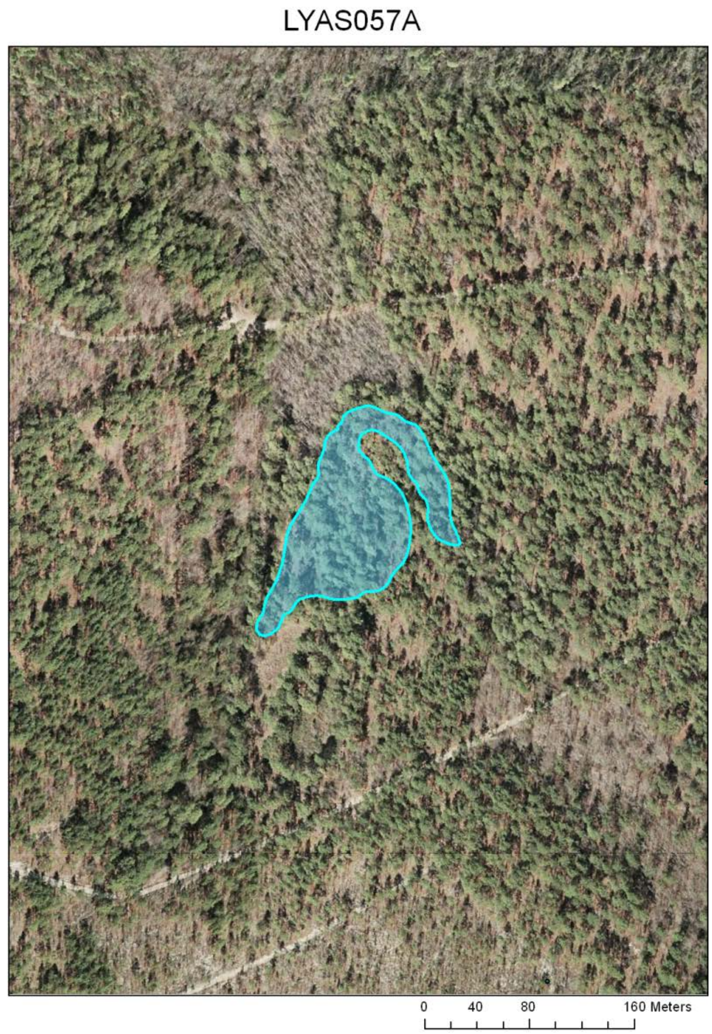


Figure N: ***L. asperulifolia*** **057A**

LYAS068A

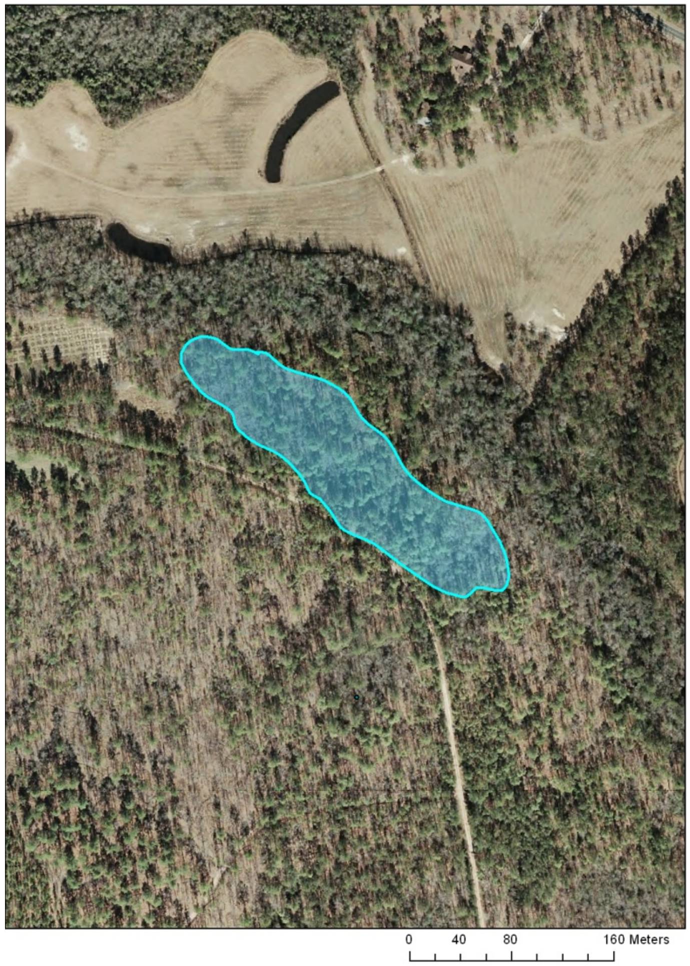


Figure O: ***L. asperulifolia*** **068A**

LYAS069A

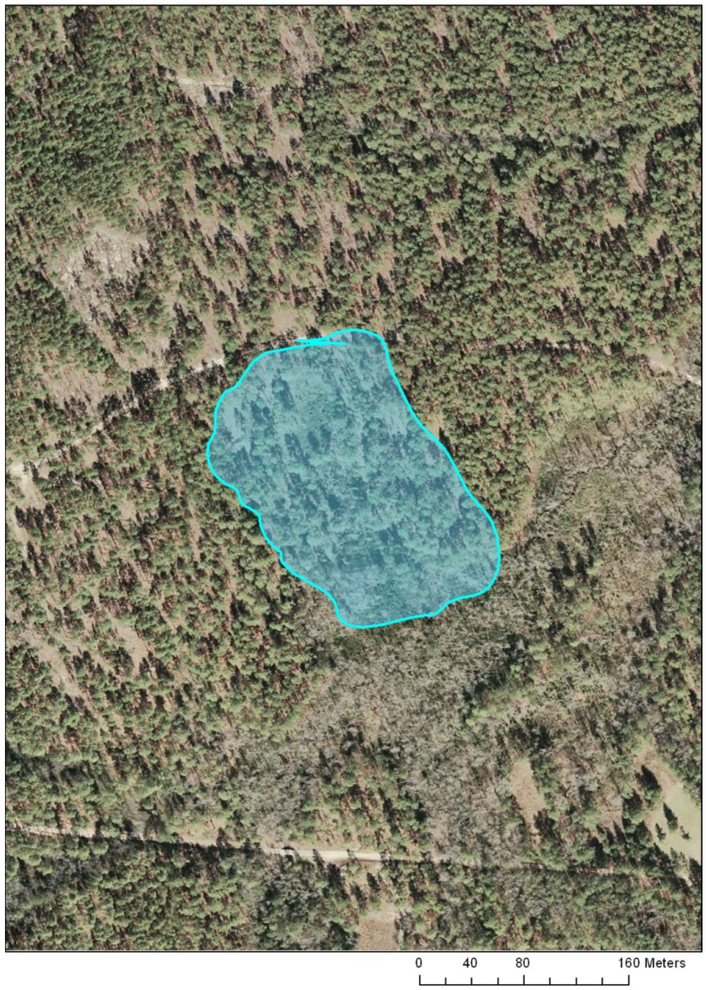
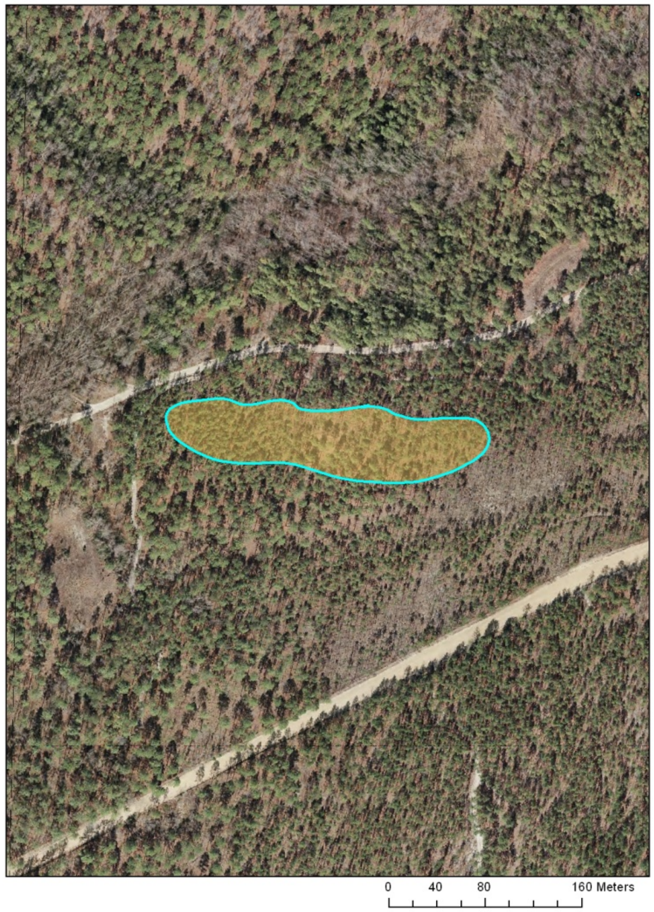


Figure P: ***L. asperulifolia*** **069A**

Figure Q: ***P. brevifolia*** **035A**

PYBR046G



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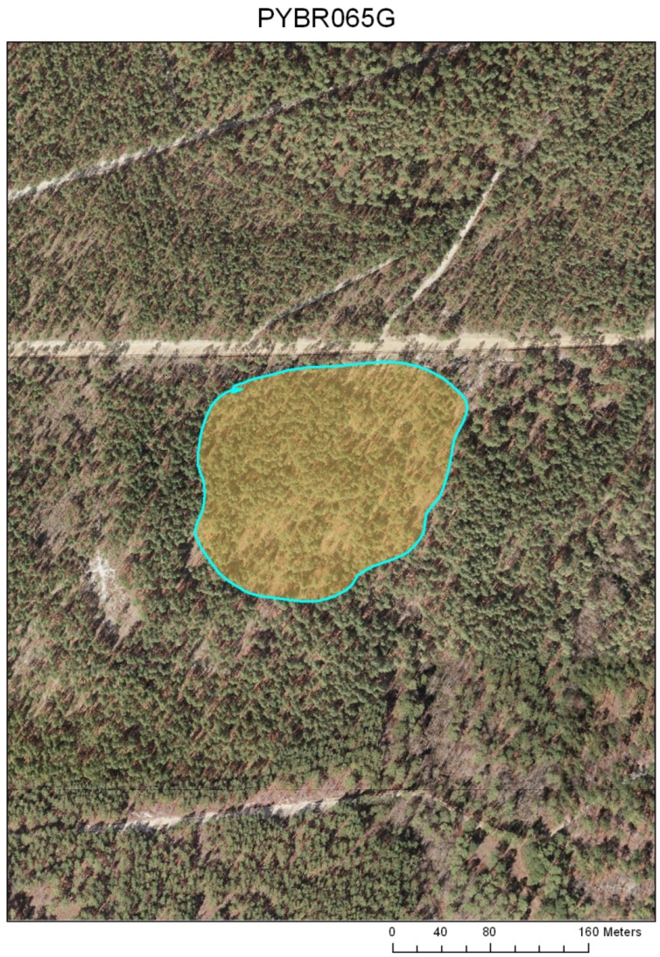

Figure S: ***P. brevifolia*** **065G**

Figure T: ***P. brevifolia*** **068A**

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Reintroduction Guidelines Manual for *Amorpha georgiana* (Georgia indigobush), *Astragalus michauxii* (Sandhills milkvetch), *Lilium pyrophilum* (Sandhills lily), and *Lysimachia asperulifolia* (rough-leaved loosestrife)

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Introduction

Approximately 31% of the 18,804 estimated native plant species in the United States are considered at risk of extinction (i.e., G1 [critically imperiled] or G2 [imperiled]), but only 11% receive protection under the Endangered Species Act of 1973 (Negron-Ortiz 2014). Most of these species would likely benefit from a diverse suite of conservation strategies, such as protecting populations, restoring degraded habitats, eliminating threats (e.g., grazing, invasive species), implementing *ex situ* specimen or propagule conservation, translocating populations slated for eminent destruction, and reintroducing populations.

The objective of this manual of guidelines is to provide specific information about the propagation and reintroduction protocols for *Amorpha georgiana* (Georgia indigobush), *Astragalus michauxii* (Sandhills milkvetch), *Lilium pyrophilum* (Sanhills lily), and *Lysimachia asperulifolia* (rough-leaved loosestrife). All four species are endemic to the Fall-line Sandhills or the Atlantic Coastal Plain of the Carolinas or Georgia (Sorrie and Weakley 2001, Weakley 2010).

Population reintroduction is defined as the establishment of plant populations in currently unoccupied historical locations using source material from natural populations (Maunder 1992, Falk et al. 1996, Guerrant 2013), but the protocols are equally appropriate for population augmentation or introduction. Maschinski and Haskins (2012) provide a comprehensive and up-to-date summary of plant reintroduction science and practice, so we do not try to replicate that information here. The guidelines are organized into six common steps: site selection, propagule collection, propagation, reintroduction, post-reintroduction maintenance, and population monitoring. It is hoped that the availability of these guidelines will promote additional proactive cooperative conservation efforts for these species.

Amorpha georgiana (Georgia indigobush)

Site Selection

Amorpha georgiana is a rare subshrub endemic to the fall-line Sandhills and Coastal Plain of North Carolina, South Carolina and Georgia (Weakley 2010). The habitat of *A. georgiana* is wet and mesic pine flatwoods on ancient river terraces and along swamp margins associated with longleaf pine savannas (Wilbur 1975, Schafale and Weakley 1990, Sorrie 1995, Sorrie et al. 2006). Low shrub cover and an open canopy (<50% cover) appear necessary for successful recruitment. Frequent fires having an expected fire return interval of 1–3 years (Waldrop et al. 1992, Frost 1998, Stambaugh et al. 2011) will maintain a largely herbaceous ground cover and open canopy in these fire-dependent habitats. Site selection is a critical component of reintroduction and can have significant effects on individual survivorship. Species experts should be consulted or habitat parameters should be quantified to match natural populations.

Propagule Collection

Sourcing Considerations – Straub and Doyle (2009) found that *A. georgiana* exhibits high levels of genetic diversity despite its rarity, and that most of the genetic variation occurs within, rather than between, populations. They also recommend that four geographic regions of occurrence (one in the North Carolina Sandhills, one in the North Carolina Coastal Plain, one in South Carolina, and one in Georgia) should be treated as separate management units for *in situ* conservation. Seeds and plants should not be moved between these regions.

Propagule Availability – In suitable habitat *A. georgiana* can produce many fruits (x = 14.2 racemes per plant; x = 65 seeds/raceme) when reproductive. However, numbers will be reduced during the first year post-fire. Fruits mature and disperse from July through October, but occasionally remain on the plant into winter (Weakley 2010) (Figure 1). Ripe pods can be easily stripped from racemes by hand and should be thoroughly dried before storage. Collections from wild individuals should not exceed 10% of the year's total seed set, and occur no more frequently than every 10 years to ensure protection of natural populations (Gurrant et al. 2004).

Figure 1. Mature pods of *Amorpha georgiana*.

Processing for Storage – Prior to storage, pods containing inviable seeds can be separated from pods with viable seeds using a commercial seed blower (e.g., Clipper Office Tester, A.T. Ferrell Company, Inc.) (Figure 2). Pods are slightly woody in consistency and can be removed by lightly crushing. No specific information is available about *A. georgiana* seed viability in storage, but other *Amorpha* spp. exhibit at least modest storage capacity (3–5 years, or longer) when kept sealed, dry, and maintained at low temperatures (Brinkman 1974, Bonner 2008).

Propagation

Germination – Marchin et al. (2009) showed that *A. georgiana* germinates after scarification (nicking the seed coat with a razor blade). Kunz et al. (in prep) evaluated the role of the pod, as well as two additional seed pretreatments (cold stratification and heat shock), on germination rate and mean time to germination. Results suggest a potentially important role for fire (heat shock) in breaking *A. georgiana* dormancy *in situ*. Although implementing a heat shock pretreatment for propagation is a simple exercise (soak in 93 ^{o}C water for 15 min), an apparent inhibitory effect also necessitates removal of the seed pod.

Figure 2. *Amorpha georgiana* seed (left) and pod (right). Units on the scale bar are mm.

For container production of *A. georgiana* in a greenhouse, cleaned, scarified, or heat shocked *Rhizobium* inoculated seeds can be sown directly into 38 cell deep-plug trays filled with a medium of 2:2:1 sand, peat, and sieved pine bark. *Rhizobium* specific for *Amorpha* can be sourced from Prairie Moon Nursery (Winona, MN, USA) and should be mixed into dampened seed just before planting. At 30^{o}/20^{o} C (12h/12 h), germination will begin in 24 hours and is complete in seven days, with germination rates of approximately 90%.

Production – Plants may be grown in a greenhouse or outdoors when overnight temperatures remain above 50 ^{o}F. Plants should be watered every other day and foliar fertilized biweekly at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters Inc, Allentown, Pennsylvania). After one month of growth, plants should receive 100 day slow-release fertilizer (13N:13P_2O_5:13K_2O Nutricote Total, Type 100; Arystra Life-Science America Inc., New York, New York) at approximately 20 g/0.09 m^2. At 8–9 months and approximately 20 cm tall, plants are large enough for fall outplanting or for transplanting to larger containers (Figure 3). First-year post-outplanting survival of plants of this size should be >90%.

Reintroduction

Site Preparation – Site preparation prior to outplanting is important for reintroduction success. Sites should be prepped with a growing season prescribed burn prior to fall outplanting. This will not only reduce aboveground competition, but also give plants sufficient time (i.e., one or more growing seasons) to establish prior to being exposed to prescribed fire. Mechanical removal of woody vegetation may also increase success.

Figure 3. *Amorpha georgiana* growing in a shade house.

Transplant Hardening – If grown in a greenhouse, transplants should be hardened off outdoors for two weeks prior to outplanting in the fall. Plugs should be maintained completely weed free and any weeds must be removed prior to outplanting. When ready to outplant, transport the plug trays to reintroduction sites using an enclosed cargo space.

Outplanting – Plugs can be planted in transects or in a grid pattern to facilitate future monitoring. Spacing plugs at >1 m will facilitate movement between plants during monitoring and provide room for recruitment of second generation seedlings. If available, a power auger will aid outplanting. Drill a hole to the depth of the plugs using an auger bit about the same diameter as the plugs (Figure 4). Place the plugs in the holes, gently pack in soil, and water in. If plants have already senesced at outplanting, no additional care should be required until site maintenance is determined necessary.

Not all outplants will survive. Mean first-year survival within four reintroduction sites on Fort Bragg over three years was approximately 60%. To establish a population having >100 individuals five years post-reintroduction (Pavlik 1996), it will be necessary to propagate and outplant approximately 396 plants at the recommended size. Smaller plants will have lower survivorship. Outplanting over successive years can help minimize impacts of annual climatic variability.

Figure 5. Using a power auger to prepare holes for *A. georgiana* outplants.

Post-reintroduction Maintenance

Amorpha georgiana reintroduction sites should be subjected to regular prescribed fires and/or other treatments to maintain an open canopy and understory. Growing season burns are recommended for hardwood and shrub management. Additionally, with greater awareness of potential for fire-related mortality of resprouting perennials (e.g., Thaxton and Platt 2006, Gagnon et al. 2012), there may be some benefit of removing woody debris and pine cones away from established outplants prior to scheduled burns.

Supplemental irrigation has had negligible impact on reintroduction success; survivorship of irrigated plants was marginally greater than non-irrigated plants (79% vs 70%, respectively; $p > 0.05$) (Wall et al. in prep). If the first growing season post-outplanting happens to coincide with drought (Stahle et al. 1988), it is possible that occasional supplemental watering may increase survival.

Population Monitoring

Monitoring outplants for survival, growth, and reproduction for a minimum of five years can help document reintroduction success and inform adaptive management. Outplants can be annually relocated and monitored if marked at time of outplanting with unique identifiers. One common approach is to affix aluminum tree tags (Ben Meadows or Forestry Suppliers) to pin flags positioned next to the base of plants (Figure 5).

Figure 5. *Amorpha georgiana* transplant (foreground) marked with a pin flag and aluminum tree tag to allow future monitoring of survival, growth, and reproduction.

Astragalus michauxii (Sandhill milkvetch)

Site Selection

Astragalus michauxii (Figure 6) is endemic to the Fall-line Sandhills of North Carolina, South Carolina, and Georgia (Sorrie and Weakley 2001), where it occurs in xeric sandhill scrub and pine-scrub oak sandhill communities (Schafale and Weakley 1990, Sorrie et al. 2006, Weakley 2010). The largest extant populations in North Carolina are found in the loamy soil variant of the latter community type, which generally has soils with higher pH and nutrient availability. Most populations are additionally associated with small topographic depressions having elevated nutrient and moisture availability due to higher loam content of the soils. These topo-edaphic areas are known locally as ‘‘pea swales’’ or ‘‘bean dips’’ because of their high diversity of Fabaceae species (James 2000). A habitat characterization and modeling effort at the Savannah River Site, South Carolina, Sharitz et al. (2009) estimated 17% canopy closure in *A. michauxii* populations. North Carolina populations occur at sites with comparably low canopy closure. If population reintroduction is pursued, care should be taken to target sites that have suitable vegetative communities, are protected and subjected to regular prescribed fires, and are within the species’ historic range (Fiedler and Laven 1996).

Figure 6. *Astragalus michauxii* plant in flower at Fort Bragg, NC.

Propagule Collection

Sourcing Considerations – In an assessment of population genetic diversity and structure, Wall et al. (2014) found that diversity estimates were similar across regions and populations, and comparable to other long-lived perennial species. Within-population genetic variation accounted for 92% of the total genetic variation found in the species. To maintain the relative genetic distinctiveness of the Georgia populations and the variability identified in the sampled North Carolina populations, they also recommended that *in situ* conservation efforts should limit exchange of genetic material across the three identified genetic clusters (Georgia, north Fort Bragg, and south Fort Bragg). Populations not examined by Wall et al. (2014), such as those on the Savannah River Site, should probably be considered distinct until shown otherwise.

Propagule Availability – Fruit set is commonly quite low relative to the number of flowers. Fruit production varies as a function of plant size and fire exposure (Wall et al. 2012). No fruits are produced the same year as an early growing season burn, regardless of plant size. Large plants will produce a mean of approximately 20 fruits one and two years post-fire. In contrast, large plants will produce a mean of roughly 30 fruits the same year as a dormant-season burn. Seeds can be collected from June to October either by stripping mature pods from racemes (Figure 7) or shaking seed from dehisced pods into paper or mesh bags. Collections from wild individuals should not exceed 10% of the year's total seed set, and occur no more frequently than every 10 years to ensure protection of natural populations (Guerrant et al. 2004).

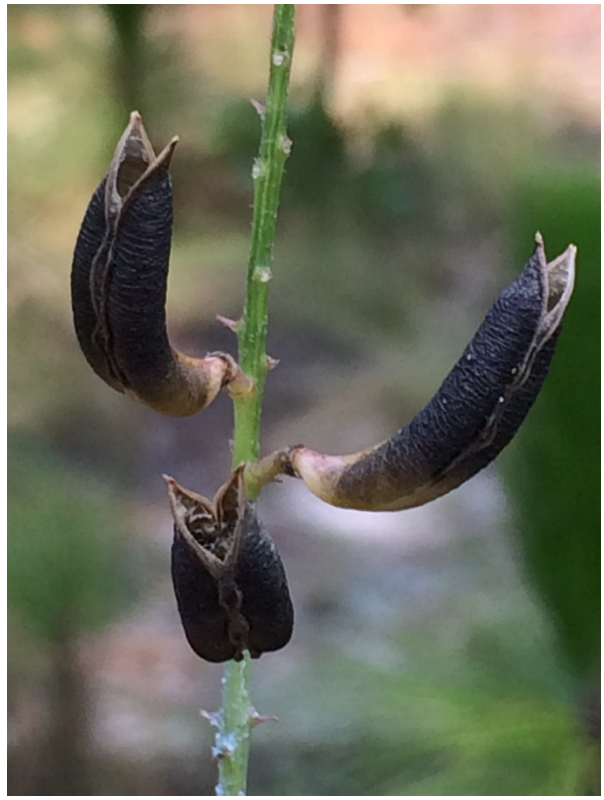

Figure 7. Raceme with mature *A. michauxii* pods.

Processing for Storage – Viable seed should be separated from inviable seed by either hand-sorting or use of a commercial seed blower. Viable seeds withstand light pressure, appear slightly glossy, are approximately 2 mm in length, and are tan colored with variable degrees of mottling or speckling (Figure 8). Heavily mottled and dark seeds may exhibit abnormal development and low survivorship (Kunz et al. 2016). After cleaning, seeds should be stored in a cool, dark, low-humidity environment (65 ^{o}F and 25% relative humidity). Although studies of the effects of moderate to long-term storage on *A. michauxii* seed viability have not been conducted, other species of *Astragalus* have been tested and show little decline in viability during long-term storage (e.g., Molnár et al. 2015). If properly stored, declines in *A. michauxii* seed viability should not be expected for at least several years.

Propagation

Germination - Astragalus michauxii seeds exhibit physical dormancy (Kunz et al. 2016). To prepare seeds for propagation they should first be scarified by nicking both inner and outer seed coats opposite the hilum with a razor blade, or using another method of mechanical scarification, such as sand paper or automated devices (e.g., Townsend and McGinnies 1972).

Figure 8. Variability in *A. michauxii* seed coloration.

Production – Weeks (2004) demonstrated that *A. michauxii* could be propagated, and Kunz et al. (2016) devised a simplified protocol suitable for efficient large-scale production. After scarification the seed should be treated with *Rhizobium* (Prairie Moon Nursery, Winona, Minnesota) and sown directly at an approximate depth of 3 mm into 38-cell deep seedling trays filled with a medium consisting of Starter size Gran-I-Grit (North Carolina Granite Corporation, Mount Airy, North Carolina), Black Gold Seedling Mix (Sun Gro Horticulture, Agawam, Massachusetts), vermiculite, and filtered pine bark compost in a 5:2:2:1 ratio and inoculated with native soil. Plug trays should be placed in a greenhouse and watered from below as needed to keep the soil moist for two weeks. Germination will begin within 48 hours and be completed within seven days. Germination rates as high as 95% can be anticipated, but seedlings should be monitored for proper emergence from the soil to ensure maximum survival.

As the plants become established, they can be watered from above one day after the soil surface begins to dry, usually one to two times a week, and foliar fertilized biweekly at 50 ppm ($20N:20P_2O_5:20K_2O$ with micronutrients; Jack's Classic, JR Peters Inc., Allentown, Pennsylvania). After one month of growth, plants should receive 100-day slow-release fertilizer ($13N:13P_2O_5:13K_2O$ Nutricote Total, Type 100; Arystra Life-Science America Inc, New York, New York) at approximately 20 g/0.09 m^2. If fungal infections appear, the plants can be treated weekly with copper soap fungicide at a ratio of 15 g/l of water. After 8–9 months, plants will reach approximately 10–12 cm in height and be large enough to outplant (Figure 9). Survivorship rates to this size should be greater than 60% during propagation and approximately 50% one year post-outplanting. Transplants >20 cm tall have survivorship of >90% one year post-outplanting.

Figure 9. *Astragulus michauxii* plants ready to be outplanted.

Reintroduction

Site Preparation – Site preparation prior to outplanting is important for reintroduction success. Sites should be prepped with a growing-season prescribed burn prior to fall outplanting. This will reduce aboveground competition and also give plants sufficient time (i.e., one or more growing seasons) to establish prior to being exposed to prescribed fire. Although *A. michauxii* habitats are fire-dependent, Wall et al. (2012) found that the species exhibits reduced reproduction following growing season burns.

Transplant hardening – If grown in a greenhouse, transplants should be hardened off for several weeks outside prior to outplanting in the fall. Plugs should be maintained completely weed free and any weeds must be removed prior to moving plants to the reintroduction sites. When ready to outplant, transport the plug trays to reintroduction sites using an enclosed cargo space.

Outplanting – Plugs can be planted in transects or in a grid pattern to facilitate future monitoring. Excavate a hole with a trowel the size of the plug, but not deeper. If available, a power auger will aid outplanting. Drill a hole to the depth of the plugs, using an auger bit about the same diameter as the plugs. Place the plugs in holes so the crown remains at the soil surface (not above or below), gently pack in soil to ensure soil contact with the root system, and water in. After watering, check to ensure the plant is still properly seated.

Not all outplants will survive. Mean first year survival within four reintroduction sites on Fort Bragg over one year was approximately 50%. To establish a population having 100 individuals five years post-outplanting (Pavlik 1996), it will be necessary to propagate and outplant approximately 1,000 plants. Outplanting over successive years can help minimize impacts of annual climatic variability and introduce some variation to the population size structure.

Post-reintroduction Maintenance

Astragalus michauxii reintroduction sites should be subjected to regular prescribed fires and/or other treatments to maintain an open canopy and understory. Although growing season burns are often recommended for hardwood management, herbaceous *A. michauxii* may also benefit from dormant-season burns, which will not impact annual growth or reproduction. Additionally, with greater awareness of potential for fire-related mortality of resprouting perennials (e.g., Thaxton and Platt 2006, Gagnon et al. 2012), there may be some benefit in removing woody debris and pine cones away from established outplants prior to scheduled burns.

Supplemental, regular irrigation did not have a significant impact on either survivorship ($p > 0.05$) or growth ($p > 0.05$) of *A. michauxii* (Wall et al. in prep). If the first growing season post-outplanting happens to coincide with drought (Stahle et al. 1988), providing occasional supplemental water may increase survival.

Population Monitoring

Monitoring outplants for survival, growth and reproduction can help document reintroduction success and inform adaptive management. Monitoring should continue for at least five years. Outplants can be annually relocated and monitored if marked at time of outplanting with unique identifiers. One common approach is to affix aluminum tree tags (Ben Meadows or Forestry Suppliers) to pin flags positioned next to the base of plants.

Lilium pyrophilum (Sandhills lily)

Site selection

Lilium pyrophilum has a narrow geographic range within the Carolina Sandhills, requires specific habitats, and maintains only small local populations. It is an edaphic endemic, restricted to sandhill seeps, streamhead pocosin ecotones (Figure 10), and more rarely small stream swamps (Skinner and Sorrie 2002, Schafale and Weakley 1990). Frequent fire is essential for maintaining the integrity of these communities and assurances about the ability to implement regular prescribed burns over the long-term is an important criterion for site selection. The dominance of shrubs over herbaceous vegetation within these communities is spatially and temporally dynamic (Schafale and Weakley 1990), depending on fire frequency and intensity. Monitoring data from natural populations suggests that individuals and populations do not persist where woody shrub cover becomes too great (Wall, unpublished data), indicating that the ground layer of reintroduction sites should be predominately herbaceous and maintained as such. Consultations with species experts or quantifying natural communities as a baseline will aid in choosing the most appropriate sites. Site-specific insight from fire managers about anticipated fire behavior may also help guide site selection.

Figure 10. Herbaceous ecotone between upland savanna (left) and streamhead pocosin (right).

Propagule collection

Sourcing Considerations – Examining chloroplast and nuclear genes, Douglas et al. (2011) showed that *L. pyrophilum* possesses substantial genetic diversity despite its current rarity. They also found that as a recent (late Pleistocene or early Holocene) peripheral isolate of *L. superbum*, much of the original genetic diversity of *L. pyrophilum* has been retained and could be conserved. A more in-depth study of *L pyrophilum* population genetic structure and gene flow based on microsatellite markers additionally found that most of the genetic variation occurs within, rather than between populations (Douglas et al., in prep). Consequently, all populations warrant conservation efforts. They also recommend that if *L. pyrophilum* seeds are collected for *ex situ* or *in situ* conservation, they should be maintained separately by each distinct population. *In situ* conservation efforts should utilize seed from the nearest geographic population.

Figure 11. *Lilium pyrophilum* capsule and seeds.

Propagule Availability – The capsules of *L. pyrophilum* mature in late October (Skinner and Sorrie 2002). The number of seeds in capsules varies from few to many (x = 62.8, sd = 70.9, range = 5-283, n = 28) (Kunz, unpublished data), with the former likely indicating pollination failure. Seeds can simply be shaken out of the capsule and do not require further cleaning prior to storage (Figure 11).

Processing for Storage – Although no specific data for *L. pyrophilum* seed viability under storage are available, the seeds of other *Lilium* spp. are known to maintain viability after drying and exposure to freezing temperatures (Kew Seed Information Database).

Propagation

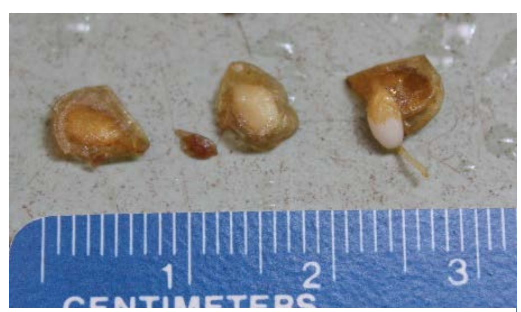

Figure 12. Developing *L. pyrophilum* seeds.

Germination – With patience (4–5 years) bare root bulbs can be propagated from seed for population augmentation or reintroduction. Seeds of *L. pyrophilum* exhibit double dormancy, requiring two years before a single leaf emerges the second growing season. This process can be expedited by artificially stratifying seeds. Place seeds in moistened sphagnum and seal in a plastic bag or other air-tight container. Seeds should be kept at 22 ^{o}C for 120 days and then at 4 ^{o}C for another 60 days (Baskin and Baskin 1998). Seeds may form a bulblet during this time (Figure 12). This sequence of warm/cold stratification should be timed to allow transfer to growing media in May.

Production – Sow stratified seeds and bulblets into #606 deep inserts (TO Plastics, Clearwater MN) containing a soil mix of 1:1 sand and peat and placed into a 11 x 21.5 in. flat. Plants may produce a single leaf the first growing season and in subsequent seasons may continue to produce single leaves (Figure 13) or possibly a short stem with one to several whorls of leaves. Regardless of leaf type, the bulb will continue to grow. Some years, bulbs may not produce any leaves, so do not discard pots without checking the soil for a bulb. *Ex situ,* the minimum time to achieve flowering is 5 years. Place trays in sub-irrigation and maintain wet at all times. Foliar fertilize individuals monthly during the growing season at 50 ppm ($20N:20P_2O_5:20K_2O$ with micronutrients; Jack's Classic, JR Peters Inc., Allentown, Pennsylvania). Plants should be of suitable size for outplanting (bulbs >1 cm diameter) by the fourth or fifth year (Figure 14).

Figure 13. *Lilium pyrophilum* seedlings.

Figure 14. *Lilium pyrophilum* bulb ready for outplanting at a reintroduction site.

Although vegetative propagation of *Lilium* spp. from bulb scales is common (e.g., Heus 2003), and known for *L. pyrophilum* (Tony Avent, Plant Delights Nursery, pers. comm.), we had poor success using this method *in situ* (Kunz and Randall, unpublished data). Moreover, this approach produces clones with a potentially limited representation of the source population's genetic diversity. It is not recommended for reintroduction.

Reintroduction

Site Preparation – To prepare sites for outplanting, they should be subjected to a prescribed fire during the growing season before the scheduled fall planting. This will not only reduce aboveground vegetative competition, but also extend the period of outplant establishment prior to first exposure to fire (e.g., two years assuming a three-year return interval). Demographic data from natural populations indicate that *L. pyrophilum* plants exposed to growing season fires do not resprout that season (Wall et al., unpublished data) and a small percentage do not resprout even the following growing season.

Transplant Hardening – Bulbs should be outplanted in the fall after plants senesce. To prepare the bulbs for outplanting, empty the growing container and gently loosen the soil to expose the bulb and wash them in clean water to remove any weed seeds and spores. Carefully wrap bulbs in moist paper towel and seal in plastic bags or other air-tight container. Bulbs should be kept moist and cool until time of outplanting.

Outplanting – Bulbs can be planted in transects or in a grid pattern to facilitate future monitoring. A >1 m spacing will facilitate movement between plants during monitoring and provide room for recruitment of second-generation seedlings. Small (<2.5 cm diameter) bulbs should be planted closer to the surface (~3 cm) than large (>3 cm) bulbs (~6 cm) and oriented with the scale pointing upward. Given the small size and precise depth needed, holes should be dug with a hand trowel. After being positioned at the proper depth and covered with loose soil, bulbs should be watered-in and checked to ensure they remain properly covered with soil.

Our demonstration on Fort Bragg and Weymouth Woods Sandhills Nature Preserve (SNP) showed that herbivore exclosures did not statistically increase outplant survivorship (67% cage vs 48% no cage; $p > 0.05$) or growth ($p > 0.05$). However, deer are known to browse *Lilium* spp., halting growth and reproduction during that growing season (Fletcher et al. 2001). We documented browsing on 12.5% of *L. pyrophilum* plants (n = 706) over eight years across monitored natural populations (Wall et al. unpublished data), and 1% of outplants within four reintroduction sites over four years. Rodents (e.g., mice, squirrels, and chipmunks) can potentially have greater negative impacts on reintroduction success, as they are known to dig up and consume *Lilium* spp. bulbs (Fletcher et al. 2001). Some success in protecting outplants from these herbivores can be achieved by erecting exclosures (approximately 1 m high x 0.5 m wide) made of hardware cloth (Figure 15). Exclosures should be monitored annually to confirm they do not interfere with plant growth. Invasive feral swine, which are an emerging threat in the Fall-line Sandhills, are known to cause extensive damage to the vegetation communities occupied by *L. pyrophilum* (Engeman et al. 2007, Felix et al. 2014) and consume *Lilium* spp. bulbs (Howe et al. 1976, Bratton 1974). To prevent impacts to *L. pyrophilum* reintroduction sites from feral swine, a more robust exclosure would be required.

Our demonstration on Fort Bragg and Weymouth Woods SNP also showed that vegetative competition reduction increased survivorship (p = 1.23 e-08) of four- and five-year-old *L. pyrophilum* plants at reintroduction sites, but did not affect growth (F = 0.245, p = 0.63) (Figure 16). We cleared woody vegetation within reintroduction sites just prior to outplanting in the fall, which likely had an effect comparable to prescribed fire. This approach could be used as a fire-surrogate, site-maintenance method when and where application of prescribed fire is constrained. It is possible vegetation clearing and prescribed fire both affect *L. pyrophilum* survivorship indirectly, by influencing rodent abundance and foraging behavior (Krall et al. 2014).

Although most natural populations of *L. pyrophilum* contain few individuals, reintroduction efforts should strive to establish population sizes that have prospects for long-term viability. Common recommendations for a target minimum population size of 50 individuals should be sought (Pavlik 1996), recognizing that roughly 5% of individuals could be dormant in a given year. Mean three-year survivorship of outplanted bulbs was 24% in four reintroduction sites at Fort Bragg and Weymouth Woods SNP. Therefore, it is probably prudent to make this target number a long-term goal that is achieved by collecting seeds from within reintroduction sites to rear additional outplants instead of from natural populations. Interestingly, Fletcher et al. (2001) also found that large patches (3–30 plants/0.04 ha) of outplanted *L. superbum* plants suffered lower herbivory than small (1–2 plants/0.04 ha) patches.

Figure 15. Hardware cloth exclosures erected to protect *L. pyrophilum* bulbs at a reintroduction site.

Post-reintroduction Maintenance

Lilium pyrophilum reintroduction sites should be subjected to regular prescribed fires and/or other treatments to maintain an open canopy and understory. Growing-season burns are recommended for hardwood and shrub management. Given that several of the largest known natural populations occur within power line cuts (Skinner and Sorrie 2002), mowing also appears to be a suitable maintenance approach.

Population Monitoring

Like other *Lilium* spp., *L. pyrophilum* is presumably long-lived and slow to mature. Age at first reproduction is >5 years in cultivation (Kunz, pers. obs.) and has been estimated to be approximately 20 years in natural populations (Douglas et al. 2011). These life-history traits have important ramifications for establishment of reintroduced populations. First, observations of recruitment and population growth are not likely to be observed for a decade or more. Second, the size structure of reintroduced populations will not be similar to that observed in natural populations, but will instead consist of mostly one size class. To more quickly achieve a stage structure representative of natural populations, which would include individuals from multiple size classes, it will be necessary to plan successive outplantings every few years. Demographic monitoring data will inform when a demographically diverse population is attained and outplanting can be stopped (assuming a minimum viable target number is also achieved).

The prolonged vegetative dormancy exhibited by *L. pyrophilum* (Wall et al., unpublished data) will complicate interpretation of monitoring data, as there will be uncertainty about whether unobserved individuals are dead, or are merely dormant. This uncertainty, along with the species' other life history traits will limit the ability of land managers to adopt adaptive management without a long-term commitment to monitoring.

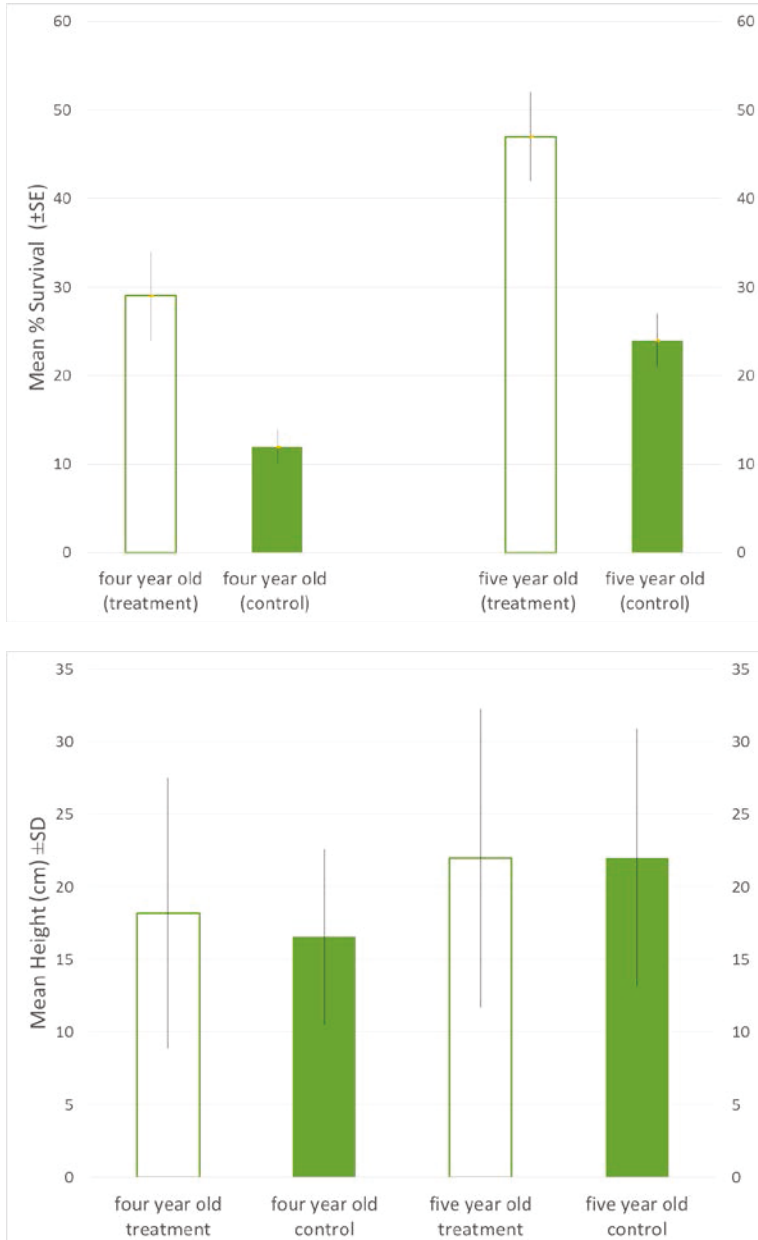

Figure 16. Mean percent survival (upper) and mean height (lower) of four and five year *L. pyrophilum* old plants after removing (treatment) or leaving (control) groundlayer vegetation during outplanting at four reintroduced populations.

Lysimachia asperulifolia (rough-leaved loosestrife)

Site selection

Rough-leaved loosestrife has been found in six natural communities: wet pine flatwoods, pine savanna, streamhead pocosin, sandhill seep, low pocosin, and high pocosin (Schafale and Weakley 1990, US Fish and Wildlife Service 1995). The species generally occurs in ecotones between wetland areas and drier uplands that have historically been maintained by fire (Schafale and Weakley 1990). If population reintroduction is pursued, care should be taken to target sites that have suitable vegetation communities, are protected and subjected to regular prescribed fires, and are within the species' historic range (Fiedler and Laven 1996). It would be beneficial to evaluate soil moisture conditions across different seasons or years within selected sites, as success may be partially determined by soils maintaining adequate moisture.

Propagule Collection

Sourcing Considerations – Many *L. asperulifolia* populations do not produce viable seed, especially smaller, more-isolated populations. If a large, seed-producing population is not within close geographic proximity to the reintroduction site, vegetative propagation may be considered. Rhizomes used to propagate *L. asperulifolia* can be sourced from impacted populations, production stock within *ex situ* populations, or healthy local populations. Extreme care should be taken to minimize impacts to natural populations.

Propagule Collection – For best success, rhizomes should be harvested in the fall after stem senescence. Using a shovel, cut out a roughly 0.5 m^2 portion of substrate to a depth of 15 cm and centered on one or more senescent stems. Viable rhizomes of *L. asperulifolia* will be white to pink in appearance. Then, using glove-protected hands, carefully remove the many roots of other community associates (e.g., *Toxicodendron vernix* [L.] Kuntze, *Arundinaria gigantea* [Walter] Muhl. ssp. *tecta* [Walter] McClure, *Ilex glabra* [L.] A. Gray) while being careful not to damage *L. asperulifolia* rhizomes. Rhizomes should be kept continuously moist and cool until being repotted within roughly 48 hours.

Propagation

The color of rhizomes harvested in the fall varies from light pink to tan. White to pink rhizomes represent new growth and oftentimes will be associated with a new bud or hibernacle, while tan rhizomes are a year old, senescent, and no longer viable (Figure 17). Approximately 63% of pink rhizomes can be expected to survive harvesting and a round of propagation, while attempts to propagate intermediate colored rhizomes should expect lower (~15% survival) success (Kunz et al. 2014).

Harvested rhizomes can be divided into two or more sections each containing one or more nodes and a minimum length of 5 cm. Divisions are made by cutting the rhizomes between nodes with sterilized pruning shears or a sharp knife. Rhizome segments should then be placed in 25.4 x 50.8 cm (or comparably sized) flats containing a 1:1 sand:peat mixture, making sure to not overcrowd the rhizomes (~4–10 per flat). Depending on schedule, production capabilities, and goals, plants can be propagated on a natural annual cycle or an artificially extended growing season.

Flats containing the divided rhizomes should be placed in a cold frame or stratification refrigerator for 90 days, kept moist, and then moved to a warm greenhouse (>20 ^{o}C) and watered daily. Heat mats set to 70 ^{o}F may be useful in maintaining soil temperatures if extending the growing season. Once temperatures have stabilized above 20 ^{o}C, flats can be moved outdoors. Flats should be watered daily and individuals foliar fertilized biweekly during the growing season at 50 ppm (20N:20P_2O_5:20K_2O with micronutrients; Jack's Classic, JR Peters Inc., Allentown, Pennsylvania). After stem senescence, the rhizomes can be excavated and divided in preparation for reintroduction or another round of propagation.

Figure 17. *Lilium asperulifolia* rhizomes in a flat prepared for propagation. Pink viable rhizomes are on the left and tan senescent rhizomes are on the right.

Reintroduction

Site Preparation – Site preparation prior to outplanting rhizomes into reintroduction sites is important for success. Kunz et al. (2014) showed that the survivorship and subsequent reproduction of outplanted rhizomes (as assessed by stem counts) was greatly improved by reducing vegetative competition either through mowing or nonpersistent herbicide application during the growing season prior to outplanting. Prescribed fire will likely generate a similar benefit. In most cases, use of herbicide should be avoided, but selective application can be helpful for suppressing dominant woody species.

Outplanting – Like rhizome collection, outplanting should be conducted during the dormant season. Propagated rhizomes should be harvested from flats just prior to outplanting, and can either be left whole or divided to facilitate handling and outplanting. Protocols for rhizome division described above should be similarly followed for outplanting. After harvesting and during transport to reintroduction sites, rhizomes should be kept cool and moist by wrapping them in wet paper towel, sealing in plastic bags, and storing in a cooler.

Outplanting within a >1 m grid will facilitate relocation during monitoring and ensure heterogeneous microsites are selected within sites. To outplant the rhizomes, simply use a shovel or trowel to pierce a slot in the soil and place the rhizome at a depth of approximately 2–5 cm. After positioning the rhizome, pinch the gap at the surface to close. Individual planting sites should be watered in to facilitate contact between the rhizomes and soil. Approximately 36% of rhizome segments can be expected to survive through the first growing season. Kunz et al. (2014) showed an average 318 ± 145 SD % increase of stem counts within two translocation sites over a six year period.

Post-reintroduction Maintenance

Lysimachia asperulifolia reintroduction sites should be subjected to regular prescribed fires and/or other treatments to maintain an open canopy and understory. Growing season burns are recommended for hardwood and shrub management. Just et al. (2016) estimated that the fire return interval of herbaceous wetlands in the Sandhills is approximately 5.5 years, while it is approximately 3.5 years in savannas, pocosin ecotones, and seeps.

Population Monitoring

Monitoring of reintroduction success should be documented via annual counts of *L. asperulifolia* stems. The interannual movement of rhizomes and stems (Kunz et al., 2014) complicates attempts to track individuals, hence the recommended use of this simple approach. Stems can efficiently be counted using narrowly spaced transects and attempting a census. Simultaneous collection of data on vegetative competition within reintroduction sites may be useful in making adaptive management decisions. For example, a threshold cover of woody shrubs could be used to trigger implementation of local site maintenance such as selective cutting of woody stems, which reduce the likelihood that prescribed fires will move through the wetland ecotones occupied by *L. asperulifolia* (Just et al. 2016).

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