Assessing microsite and regeneration niche preferences when introducing endangered species

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ABSTRACT

KRISTIE SUSAN WENDELBERGER
ASSESSING MICROSITE AND REGENERATION NICHE PREFERENCES WHEN INTRODUCING AN ENDANGERED SPECIES
(Under the direction of Dr. Alan S. Weakley and Dr. Peter S. White)

As pressures from development and climate change grow, land managers are turning to introductions/assisted migrations to prevent rare species extinctions. When introducing a species, it is important that propagules survive long enough to reproduce and recruits establish and reproduce themselves. If the target specie's specific microsite and regeneration requirements are unknown, one can use experimental introductions to learn demographic information while attempting to create a new population. Planting into three distinct microsites within its native habitat, I used the introduction of the endangered plant, *Tephrosia angustissima* var. *corallicola,* as an opportunity to answer microsite-specific questions while attempting to establish a new population. Results showed the highest transplant and recruit growth, flowering, and survival occurred in shady, dry microsites; recruits germinated in shadier locations than where adults were planted. Lessons learned from introduction successes and failures are essential to building the scientific base needed for rare species conservation and policy decisions.

To my friends and family who supported and encouraged me to succeed.

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CHAPTER ONE: INTRODUCTION

1.1 The effects of climate change on plant species distributions

As habitat is developed and/or altered through climate change, biodiversity around the world is increasingly threatened (Hoekstra et al. 2005, Williams et al. 2007, Rosenzweig et al. 2007, IUCN 2008). The Intergovernmental Panel on Climate Change (IPCC 2007) determined, with high confidence, that biological systems within terrestrial, marine, and aquatic ecosystems are being affected by recent warming of the Earth's temperature. They found an increase in floral and faunal range shifts (poleward and upward in elevation), phenological changes, species abundance shifts with some local extinction, and increased water temperatures with changes in salinity, oxygen levels, and circulation (IPCC 2007, Rosenzweig et al. 2007). Models predicting the movement, creation, and extinction of current climate envelopes—areas with climatic conditions suitable for a given species— and a specie's ability to migrate within those envelopes show individual species responding to climate change differently, variously losing available habitat, gaining habitat, or not being effected at all (Berry et al. 2002, Williams et al. 2007). This variation in species response will likely change the natural community assemblages we see today with some novel assemblages forming while some current assemblages disappear (Berry et al. 2002). Tropical and subtropical regions are predicted to experience the most dramatic climate change (Williams et al. 2007). The circum-Arctic and tropical narrowly endemic species are thought to be the most threatened by these changes (Thomas et al. 2004, Hannah et al. 2005, Williams et al. 2007).

1.2 Development and climate change impacts on south Florida's plant species composition

South Florida vegetation is determined largely by elevation, drainage, hydroperiod, soil type, pH, disturbance, salinity, amount of yearly rainfall, and temperature (Kushlan 1990, Snyder et al. 1990). The Miami Rock Ridge (MRR) is a limestone outcrop 80 km long by 6 to 14 km wide (DERM 1993) running along the southeastern coast of south Florida, USA. Miami limestone is composed of small ovoid pellets of calcium carbonate originally developed during the Pleistocene (Perkins 1977) in a shallow marine environment (Hoffmeister et al. 1967, Hoffmeister 1974) about 130,000 years before present (Perkins 1977). The limestone dissolves readily in fresh water with rains resulting, overtime, in a karst surface with deep sinkholes. The MRR ranges from 7 m to 2 m above sea level declining in elevation from north to south and east to west (Snyder et al. 1990). Pineland (pine rockland) vegetation establishes on areas of higher elevation. All the pine rockland soils along the ridge have good drainage, only remaining saturated when inundated by high water tables (Snyder et al. 1990). Surrounding lowland vegetation ('transverse glades') can be as much as several meters to as little as a few centimeters below the pine rockland edge. These differences in elevation can lead to great variation in hydroperiods experienced by both pine rocklands and transverse glades, depending on location. Upper pine rocklands tend to flood only during extreme weather events (e.g., hurricanes) while those in areas of lower elevation can flood regularly during the wet season, remaining inundated for several months (Snyder et al. 1990).

Infrequent flooding and good drainage makes south Florida's pine rocklands at heightened risk to development. As of 1998, less than 2% of the natural pine rockland ecosystem remained, mostly as small parcel fragments (USFWS 1999) interlaced in a matrix of wildland-urban interface. Fragmentation and development both directly — through habitat loss and disconnectedness — and indirectly — through factors such as fire suppression, invasive species, and altered ecological processes — impact natural systems (DeAngelis and White 1994, Beckage and Stout 2000, Jackson and Sax 2010, Kong et al. 2010).

Pine rocklands are a fire dependent ecosystem (Robertson 1955, Snyder et al. 1990). Today, with fire suppression common and prescribed fire management difficult in an urban

system, the remaining south Florida pine rockland fragments are rarely, if ever, burned, resulting in hardwood invasion and accumulation of litter which drive the system toward a hardwood-dominated habitat (Robertson 1953, Snyder et al. 1990, Platt 1999, Beckage and Stout 2000).

Drainage throughout south Florida for agriculture and mosquito control has altered the hydrologic patterns of this immense wetland/upland matrix (Davis et al. 1994, McIvor et al. 1994). Additionally, Florida has 72 Category I and 73 Category II invasive plant species (64 in south Florida alone; Gann et al. 2002) infiltrating all habitat types, out competing native species and altering ecological processes (DiStefano and Fisher 1983, Volin et al. 2004, Possley and Maschinski 2006, FLEPPC 2009). Development and fragmentation has left little room for natural corridors to be created for species movement. Fairchild Tropical Botanic Garden is attempting to remedy this with their 'Connect to Protect' program (Fairchild 2010). However, as of yet, the land is far from connected, possibly leaving a number of threatened species with nowhere to go should climate change effects encourage species movement.

Predicted increasing storm occurrence and sea level from climate change (IPCC 2007) threatens south Florida pine rockland species. This system evolved under weather conditions that include hurricanes, rebounding quickly from extreme weather events (Duever et al. 1994). However, development, drainage, invasive species, fire suppression, and fragmentation have altered the system (Davis et al. 1994, McIvor et al. 1994, Beckage and Stout 2000, Volin et al. 2004, Possley and Maschinski 2006, FLEPPC 2009), possibly weakening its resiliency (DeAngelis and White 1994). An increase in storm activity may reduce the system's ability to rebound, altering microsite conditions essential to the regeneration of both common and rare native species, causing a shift in species composition and diversity. Further, though pine rocklands are in uplands, they can be found as low as 2 m above sea level (DERM 1993). Differences in only a few centimeters determine whether a natural area is a pine rockland or transverse glade (Snyder et al. 1990). Therefore, an increase in sea level that influence underground water salinity patterns may alter lower pine rockland areas causing a shift to a more wetland/halophitic plant composition (Williams et al. 1999, Williams et al. 2003, Desantis et al. 2007, Karim and Mimura 2008).

There are 238 Florida state endangered plant species in south Florida (Gann et al. 2002) under the influence of development and climate change. Many of these species are endemic to south Florida and within a restricted range. They exist in federal, state, county, and city parks that vary from a few to many thousand acres (Gann et al. 2002). A specie's ability to disperse (e.g. wind, water, animal), habitat patch size, and corridors connecting patches may determine its ability to migrate to new areas as climatic envelopes shift and individual species react. Disjunct habitat patches may be too far away for some species to migrate to leaving conservation biologists and land managers trying to determine the best conservation strategy for those species.

1.3 The use of introductions and/or assisted migration in rare species conservation

Rare species introductions and/or assisted migrations are being considered as possible solutions to help prevent extinction in an era of development and climate change (Maunder 1992, Barlow and Martin 2004, Guerrant and Kaye 2007, McLachlan et al. 2007, Richardson et al. 2009, Swartz & Dixon 2009, Vitt et al. 2009). I define the term 'introduction' as when one transplants a population of individuals into any habitat or habitat fragment that is historically known to be within the specie's range and supports that species regardless of whether or not the species was found on that exact patch. I define 'assisted migration' as transplanting a population of individuals for conservation purposes into any habitat or habitat fragment that is known to be outside of the species historic range but is, for some reason, deemed suitable to harbor the species. Many conservation biologists look at moving species — introductions and assisted migration alike — in lieu of protecting the habitat they are naturally found in as avoiding the fundamental issue of habitat loss attempting to treat the symptoms as opposed to addressing the true problem (Falk 1987, Howald 1996, Guerrant et al. 2004). Additionally, there are concerns of worsening the situation by introducing a threatened species into a new system, which in turn could create an invasive species issue (Mueller and Hellmann 2008, Ricciardi and Simberloff 2009). However, in cases where the habitat has been destroyed completely and, as climate change occurs, the original habitat no longer supports the species, introductions and/or assisted

migrations may be the only way to conserve rare species threatened with extinction. Additionally, past, long-term changes (e.g. glacial periodicity) have caused species to move long distances over time depending on climatic patterns (Jackson et al. 2000). If assisted migrations are kept to within a range that is likely to have occurred naturally based on historic global climatic pattern shifts, it may lesson chances of invasion risk.

As climatic envelopes shift and species become threatened, single species and community restoration via introductions or assisted migration may become our most important tools in the conservation of biodiversity. In order to make this effective, information from a range of conservation-related studies need to be assembled to better improve our chances of success including seed and/or germplasm storage (Li and Pritchard 2009, Vitt et al. 2009), demographic studies (Bottin et al. 2007, Baillie and Schaub 2009), species-species interactions (Gibbs et al. 2008), soil/microbial effects (Fahselt 2007), disturbance ecology (Duncan et al. 2008, Seastedt et al. 2008, Lyet et al. 2009), population viability analyses (Brook et al. 2000, Menges 2000, McCarthy et al. 2001), conservation genetics (Hufford and Mazer 2003, Frankham 2008, Kramer and Havens 2009), conservation horticulture (Benson et al. 2000, Maunder et al. 2001), corridors (Haddad 2008, Brudvig et al. 2009), and invasion ecology (Moody and Mac 1998, Mitchell et al. 2006, MacDougall et al. 2009, Ricciardi and Simberloff 2009). We need to learn from our successes and failures to understand the best ways to restore or establish specie's populations (Pavlik 1996).

1.4 A case study for experimentally introducing rare plant species

When introducing a species inside or outside of its native range, it is important for introduced individuals to survive long enough to reproduce and recruits to establish and reproduce themselves (Sakai et al. 2001). A failure of either step may result in the introduction failing. Further, in cases where the native habitat is destroyed or the species is being moved to a new habitat via assisted migration, the best microsite to support the species may not be known, or the microsites in the new location may be slightly different to those of the native location. Because introduced plants are often nursery grown, it is likely that the conditions which support

transplant growth and survival are different than those that support seedling germination and establishment (Guerrant and Kaye 2007). Therefore, each introduction can be looked at as two different studies asking two different questions: what are the factors driving the survival, growth, and reproduction of introduced individuals and what are the factors driving the recruitment, establishment, growth, and reproduction of the F_1 generation? In this thesis, I present a rare plant introduction designed to address both of these questions (Chapters 2 and 3, respectively).

Using an experimental introduction including six years of demographic monitoring of the Florida state rare plant, *Tephrosia angustissima* var. *corallicola* (Coral hoary pea), I assessed which of three microsites found in the species' native habitat best supports the survival, growth, and reproduction of introduced individuals (Chapter 2) and which three microsites best supported germination, growth, and reproduction of F_1 individuals (recruits; Chapter 3). There is only one remaining population of *T. angustissima* var. *corallicola* in the United States. This population is located in an agricultural area where the substrate has been plowed and the nutrient and water regimes changed. There is little information in the literature on this specie's microsite preference, and Herbarium labels do not give details on the species specific microsite requirements, only that it was found in pine rocklands. Therefore, I knew little about the species microsite requirements prior to introduction. The introduction was performed in a 10.22 acre urban area protected by Miami Dade County's Environmentally Endangered Lands program.

Lessons learned from this experimental introduction can be used in the future for both introductions and assisted migrations (Pavlik 1996). It is important to note this study was not an assisted migration. It was a rare plant introduction performed within the historic range of the species, *Tephrosia angustissima* var. *corallicola*. However, information learned from both within native range introductions and outside of native range assisted migrations can be used to further both fields simultaneously. We need to learn from the successes and failures of within range introductions to build the scientific base needed to make conservation and policy decisions for out-of-range assisted migrations and rare species conservation in general (Guerrant and Kaye 2007).

CHAPTER TWO: EXPERIMENTALLY ASSESSING BEST MICROSITE CHARACTERISTICS FOR INTRODUCING PROPAGULES OF THE RARE PLANT *TEPHROSIA ANGUSTISSIMA* VAR. *CORALLICOLA*

2.1 Introduction

As fragmentation, development, and climate change increase in their impacts on natural populations, land managers are turning to introductions as a means of preventing rare species extinctions (Guerrant and Kaye 2007, McLachlan et al. 2007, IUCN 2008). Although past rare species introductions typically have had low success rates (e.g. Hall 1987), with improved techniques this is changing (Maschinski & Duquesnel 2006, Guerrant and Kaye 2007, Wendelberger et al. 2008). Studies from invasion ecology show introduced population success or failure is often case specific with factors relating to competition (Blumenthal 2006, Borer et al. 2007), mutualism (Richardson et al. 2000), niche/microsite characteristics (Thuiller et al. 2005, Broennimann et al. 2007), and/or a combination of these factors (Mitchell et al. 2006) influencing population survival. Unfortunately, because of the realities of rapidly changing environments, constrained funding, and, at times a sense of urgency possibly leading to a lack of careful observation and/or the assumption this information is too difficult to obtain, conservation biologists and land managers often do not have information on microsite requirements prior to introduction. Commonly, propagules are introduced into one location deemed suitable by the introducer followed by brief monitoring, which can lead to little information that would give insight into reasons for success or failure (Sarrazin and Barbault 1996, Bottin et al. 2007, Dixon and Coates 2007).

Pavlik (1996) suggested defining specific introduction objectives and using hypothesis-driven experimental designs with long-term monitoring. This approach can lead to more information on a specie's biology while contributing information about introduction successes and

failures thereby providing baseline information for future introductions. Often, there is limited to no information about rare specie's biology or what microsite characteristics best support the target specie's propagule growth, reproduction, or long-term survival. Recently, some conservation biologists have used an experimental approach when introducing rare species, leading to new information on the specie's biology and appropriate introduction designs (e.g., Maschinski and Duquesnel, Guerrant and Kaye 2007). Wendelberger et al. (2008) experimentally introduced the federally endangered *Amorpha herbacea* var. *crenulata* using four propagule types: seeds, cuttings, 1-7 year-old nursery plants, and wild adult transplants. They found large plants, whether nursery grown or transplanted, tended to survive longer than seedlings or cuttings. With this information, further introductions of the species were conducted assessing the specie's microsite preference using large transplanted adults, knowing that survival was based on microsite preference not propagule type (Maschinski et al. 2007).

I used an experimental introduction approach and long-term monitoring to asses which microsite characteristics best supports the growth, survival, and reproduction of nursery grown cuttings and learn previously unknown demographic details of the Florida State endangered *T. angustissima* var. *corallicola*. Due to habitat destruction, only one population of *T. angustissima* var. *corallicola* remains in the United States (Gann et al. 2002). This population is located in an agricultural field where the substrate was plowed and there is regular mowing making it difficult to assess the specie's natural microsite requirements; on site crop production threatens the species with extinction from the United States. There is little information in the literature about this specie's specific microsite requirements or demographic patterns. Examination of Fairchild Tropical Botanic Garden (Fairchild) Herbarium labels indicated *T. angustissima* var. *corallicola* was historically collected from pine rockland habitat, but the labels provided no specific information about microsite or demographic requirements.

I monitored transplant demographic patterns in three different microsites within its native pine rockland habitat; each microsite shared some characteristics with the wild population site that could potentially be important to the specie's success. Insights gained about which microsite characteristics best support introduced *T. angustissima* var. *corallicola* propagules can now be

used to guide future introductions of the species with greater confidence in project success. Specifically, I asked several questions. Which of the three microsites supported optimal *T. angustissima* var. *corallicola* transplant survival? What light conditions and soil moisture characteristics best supported *T. angustissima* var. *corallicola* transplant survival? What light conditions and soil moisture best supported *T. angustissima* var. *corallicola* transplant growth? Which of the three microsites best supported flowering and fruiting of *T. angustissima* var. *corallicola* transplants?

2.2 Methods

2.2.1 Study species

Tephrosia angustissima var. *corallicola* (Fabaceae) is a prostrate, sprawling forb native to South Florida, USA, pine rockland habitats. It is listed as endangered by the Florida Department of Agriculture and Consumer Services (Coile and Garland 2003) and as critically imperiled by the Florida Natural Areas Inventory (FNAI 2007). Over the last 125 years, *T. angustissima* var. *corallicola* has suffered a north-south reduction of its original range running from Broward County south through the Everglade Keys (approximately 200,000 km^2; Gann et al. 2002), and is now confined to one population with a range of .027 km^2 (Maschinski et al. 2004) with little genetic diversity (Thornton and Maschinski 2004). Eight populations are known in Northwestern Cuba (Beyra Matos 1998). The one U.S. population is located in an agricultural area where the substrate has been plowed and the nutrient and water regimes changed. There is little information in the literature on this specie's microsite requirements, and Herbarium labels do not give details on the species specific microsite characteristics, only that it was found in pine rocklands. Therefore, I knew little about the species microsite requirements prior to introduction. However, after observing the wild population, I noted *T. angustissima* var. *corallicola* co-existed with regularly mown lawn grasses. The species spreads its branches out under the grass, sending shoots up to flower in full sun. Seeds drop near the parent, germinate beneath the grass, and grow in the protected area until maturity (Wendelberger *personal observations*).

2.2.2 Experimental introduction site

I performed the experimental introduction in a pine rockland fragment owned by the Miami-Dade County's Department of Environmental Resources Management, Florida, USA under their Environmentally Endangered Lands program. The site was situated a few blocks from the original population; there are no historical records of *T. angustissima* var. *corallicola* at this site.

South Florida pine rocklands are a diverse, fire dependent community (Snyder et al. 1990). The substrate is soft, karst, limestone layer containing many holes and depressions, due to erosion. A single species dominates the pine rockland canopy, *Pinus elliottii* var. *densa*. The pine rockland shrub stratum contains over 90 taxa mostly derived from the West Indies and highly influenced by proximity to other plant communities such as tropical hardwood hammocks or wetlands. The herb layer can contain more than 250 indigenous species from the tropics and temperate zone (Snyder et al. 1990).

I looked for three microsites within the pine rocklands with environmental characteristics similar to the wild location but varied in degree across the microsites, specifically: soil moisture during both wet and dry seasons and percent photosynthetic active radiation (PAR). The three microsites I chose (Open, Closed, Road) are commonly found within the pine rockland community. The Open microsite had few *P. elliottii* var. *densa,* direct sun, dense patches of *Serenoa repens*, scattered herbs, and deep pockets of bare sand between limestone outcrops. The Closed microsite had a dense *P. elliottii* var. *densa* canopy creating mostly shady areas with scattered light patches. There were a few *S. repens*, scattered hardwoods and herbs, and more exposed limestone than the Open microsite with shallower sandy pockets covered by a pine needle litter layer 1-2 cm deep. The Road microsite edged the pine rockland along a firebreak maintained by mowing but which had little car traffic. I chose these three microsites as opposed to others (e.g. varying nutrients, proximity to 'nurse plants', etc.) because they are common in this habitat, yet distinct enough to provide variation in light and soil moisture. Moreover, all three contained various environmental characteristics that mimicked those found at the wild site.

2.2.3 Propagation protocol

To balance genetic diversity among the three microsites, I propagated cuttings for clonal replicates. As I did not know the population's genetic variability at the time of introduction, I collected several cuttings per wild plant from as many wild plants as possible (800 cuttings; 1-8 cuttings/wild individual) to create three clonal replicates per wild plant, and processed them in the Fairchild nurseries according to the Maschinski et al. (2006) propagation protocol. Once established, I moved them to quart pots containing three soil strata; a top and bottom layer of 1:1 limestone to standard potting soil mix and a central layer of native soil where the plant's root ball was planted. Because mycorrhizae have been known to benefit plant growth in nurseries, leading to better introduction successes (e.g. Giri et al. 2005), soil containing native mycorrhizae was added to the planting soil to potentially enhance growth and survival.

2.2.4 Study design

To assess which microsite best supports *T. angustissima* var. *corallicola* introduced propagules, in June 2003, I placed 57 plants in the Open microsite, 57 plants along the Road, and 27 plants into the Closed microsite for a total of 141 plants. Due to propagation limitations, there were 27 wild individuals represented by three clones each (81 transplants) and 30 wild individuals represented by two clones each (60 transplants). Therefore, all three microsites received one clone each of the 27 wild individuals and the Open and Road microsites received an additional one clone each of 30 different wild individuals. I chose the Open and Road microsites to receive 30 more individuals than the Closed because prior observations led me to believe one of those microsites might support the introduced populations more effectively. Later, it was determined that there was little genetic diversity in the wild *T. angustissima* var. *corallicola* population (Thornton and Maschinski 2004) leading me to analyze the data with all individuals in each microsite rather than distinguishing between clones across the microsites.

Because pine rocklands have shallow (0-<5 cm) limestone bedrock, I selected planting locations for individual plants by prodding the ground with a piece of rebar to approximately a 10 cm depth (the depth of the pint pot). To ensure complete saturation, I watered planting holes pre-

and post-planting with ¼ gallon of water per plant. After transplanting, I watered every three days until the rains began in August, at which time I shifted to watering only when the soil around the plants was dry 5-8 cm below the surface.

To characterize soil moisture in each of the three microsites, I collected soil moisture data during the wet and dry seasons. I collected soil from each planting hole at the time of transplanting, 23 June 2003, and at the end of the first dry season after transplanting, 23 April 2004, using a soil corer to 10 cm deep as close to the transplant as possible without damaging roots. I sealed collected soil into Ziploc bags to prevent moisture loss, brought them back to the Fairchild lab, and began processing immediately. I sifted samples removing particles > .25 cm^2 and weighed each processed sample obtaining a wet mass. After drying samples at 100 C^o for 8 hours, I obtained dry mass. I calculated gravimetric percent moisture for each microsite in the 2003 wet (June) and 2004 dry (April) season and used analysis of variance to determine differences between microsites.

To characterize light levels for each planting site and each of the three microsites, I measured photosynthetically active radiation (PAR) at each introduced adult using a Li-Cor LI-191SA Line Quantum Sensor. I determined PAR in the full sun and at each plant, calculated percent PAR reaching each individual, and then used analysis of variance to test for differences between microsites.

For each introduced individual, I recorded geocoordinates using a Trimble Pro XRS GPS Unit and quantified plant size and survival. Using three nested hoops with diameters of 10, 50, and 100 cm centered on the rootstock, I recorded the number of stems crossing the hoop edge. Some stems grew out from the rootstock, doubled back, and grew toward the rootstock; I counted each stem only once, even if it crossed the hoop twice. To test the effects of microsite on transplant growth, I used repeated measures analysis of variance.

To measure reproduction, I counted the total number of flowers and fruits. I collected these data quarterly for one year. To test the effects of microsite on transplant growth and reproduction, I used repeated measures analysis of variance. Due to the large number of zeros in

the reproduction data and to test the effects of microsite on transplant reproduction, I used a log count plus 0.5 transformation prior to analysis of variance.

I measured propagule survival every week for the first two months after planting and quarterly thereafter. Data were analyzed using survival analysis (Muenchow 1986). The Kaplan-Meier estimates of the survivorship were obtained and differences in transplant survival across microsite were assessed using a log-rank test. Because I planted pint-size transplants from a protected nursery environment into an exposed natural area where they experienced hot temperatures and minimal watering, transplant effects such as drying or other planting stresses may have influenced transplant survival. This influence would be seen as a faster rate of death in the beginning of the study as opposed to the end. Therefore, to test if early introduction effects influenced survival more than long-term effects, I used the Gehan-Wilcoxon test, a variation of the log-rank test, which weights differences by the number of individuals still at risk, thus giving less importance to later survival time, when there are fewer individuals. To test the effects of PAR and wet season/dry season soil moisture on transplant survival across microsites, I used a Cox proportional hazards regression model. Statistical significance of predictors was assessed using a likelihood ratio test (LR statistic). Both a formal test and a graphical analysis suggest that the proportional hazards assumption was violated for PAR. Spline smoothers added to the plot suggested the presence of two distinct PAR regimes; PAR had a negative effect on survival early in the study, but no effect later. To account for this, an extended Cox model that allows for time dependent covariates was fit dichotomizing the effects of the exposure variable PAR (Kleinbaum and Klein 2005). A systematic search yielded 380 days as the optimal break point.

In response to significant recruitment, I began monitoring recruits in February 2004. I located and tagged each recruit within one meter radius of the parent plant. To help relocate the recruits I measured the distance and compass bearing from the parent plant to each recruit. I monitored a total of 3,000 *T. angustissima* var. *corallicola* recruits within a 1 m radius of adults introduced in 2003. More recruits with cotyledons were noted more than a meter away; however, I excluded them from the study.

Analyses were performed using the survival package (Therneau 2009) and the nlme package (Pinheiro et al. 2009) of R 2.10.1 (R Development Core Team 2009).

2.3 Results

2.3.1 Characterizing the three microsites

The three pine rockland microsites significantly differed in soil moisture in both the dry (April 2004) ($F_{2,127}$ = 31.15, P < 0.001, Fig. 2.1a) and the wet (June 2003) season ($F_{2,127}$ = 5.75, P = 0.004, Fig. 2.1b). In the dry season, the Road had significantly higher percent soil moisture (5%) than the Closed (3%, P < 0.001) or the Open (2%, P < 0.001) sites; Closed and Open soil moisture was not significantly different (P = 0.066, Fig. 2.1a). Similarly, in the wet season the Road had significantly higher soil moisture (21%) than the Open (12%, P = 0.001), but not Closed (18%, P = 0.32, Fig. 2.1b), and the Open and Closed did not differ as much or significantly (P = 0.07).

Photosynthetically active radiation (PAR) was significantly different across the microsites ($F_{2,134}$ = 8.91, P = 0.0002, Fig. 2.1c); the Open had highest percent PAR (83%), the Road had intermediate (75%) and the Closed had lowest percent PAR (48%, Fig. 2.1c).

2.3.2 Introduced propagule growth across the three microsites

Ten months post introduction, propagules grew and died back seasonally with amount of growth varying across microsites. Propagule branch growth was significantly different across time ($F_{3,316}$ = 59.19, P < 0.0001, Fig. 2.2); introduced adults grew during the wet season dying back during the dry season. This corroborates nursery observations that some aboveground plant material dies back during the dry season. Rank order of growth changed across monitoring dates indicating an interaction between microsite and season ($F_{6,316}$ = 5.0, P < 0.0001, Fig. 2.2); Open plants had the most growth by January 2004, but had the least amount of growth by April 2004 ($F_{6,316}$ = 5.0, P < 0.0001, Fig. 2.2).

2.3.3 Introduced propagule flower and fruit production across the three microsites

Introduced proagules had significantly different flower production across time ($F_{3,322}$ = 66.62, P < 0.0001, Fig. 2.3a); more flowering occurred in the wet season than the dry season. When controlling for date, there was a significant microsite effect ($F_{2,137}$ = 4.96, P = 0.008, Fig. 2.3a); Open plants produced significantly more flowers than the Road, while Closed plants produced intermediate levels. Similarly, fruiting was significantly different across time ($F_{3,316}$ = 216.9, P < 0.0001, Fig. 2.3b) with more fruiting occurring in the wet than the dry season. There was an interaction between habitat and time ($F_{6,316}$ = 3.7, P = 0.0014); in the dry season those individuals in the Open microsite produced more fruits than expected from the no interaction model (t = 3.06, P = 0.002).

2.3.4 Introduced propagule survival across the three microsites

Survival of introduced *T. angustissima* var. *corallicola* propagules varied over time across microsites. Survival across the three microsites was significantly different when early time points were weighted more heavily (X^2 = 19.2, P < 0.001; Table 1, Fig. 2.4), but not when survival was weighted evenly across the study period (X^2 = 5.5, P = 0.064; Table 1, Fig. 2.4). The microsite that supported the most transplant propagule survival changed over time with the Closed showing the most survival early on, switching to the Open having the best survival, and, finally, no significant difference between microsites. Six years after introduction, two (0.01%) of all transplants survived (one each in the Open and Road) — four died from human disturbance, but the remaining died from microsite suitability, transplant stress, or it reached the end of its lifespan.

PAR negatively influenced propagule survival (LR statistic = 8.56, 1df, P = 0.003). A plot of the Shoenfeld residuals showed that the effect of PAR varied with time (X^2 = 4.6, P = 0.032); higher percent PAR had a negative impact on survival before 380 days after planting (coef = 0.044), while > 380 days after planting PAR had a negligible positive effect on propagule survival (coef = -0.0005, LR statistic = 136.2, 2df, P < 0.0001). Given the introduced plants were pint-size, nursery-grown plants accustomed to adequate water and nutrient supplies, it is not

unexpected that those individuals planted into the drier, sunnier microsite (Open) would show greater die off within the first year. This is congruent with survival data showing the Open microsite, with overall higher percent PAR, had the least amount of transplant survival early on and the shadier Closed microsite maintained the most early transplant survival (Fig. 2.4).

2.4 Discussion

Because I performed the experimental introduction of *T. angustissima* var. *corallicola* with adequate replication in multiple microsites and maintained long-term demographic monitoring, insights into microsite characteristics supporting propagule growth, survival, and reproduction were obtained. Adults showed the best survival in the Closed microsite until October 2006, when survival was no longer significant across the microsites. Had I monitored for only one or two years, I would have reported the Closed microsite as the best for propagule survival not realizing that long-term monitoring showed poor survival in all microsites. The Open microsite is the sunniest and driest part of the pine rockland habitat. Though the original population grows in an agricultural field that is regularly mowed, the species tends to stretch its branches under grasses providing some shade for stems. Additionally, *T. angustissima* var. *corallicola* recruits germinate prolifically in the nursery near irrigation spigots and are often considered weedy indicating this species would require a wet, shady environment for a successful introduction. Had I picked only the shadiest microsite (Closed) or the microsite with the highest percent soil moisture for introduction (Road), I would have seen introduced individuals decline until nearly all died and not learned that transplants flowered, fruited, and grew more in the Open microsite even though initial survival tended to occur in shadier locations. Further, as of September 2009, results of seedling establishment in Chapter 3 show a population of 162 *T. angustissima* var. *corallicola* individuals in all stages of development at the site. It is possible that a seed bank has been established and the population may become sustainable. However, monitoring long into the future will be needed to assert this.

Introducing propagules into microsites containing environmental characteristics needed to support propagule survival, growth, and reproduction is essential for introduction success

(Sakai et al. 2001). Transplanted propagules need to survive long enough to reproduce and contribute to the seed bank while recruits need to establish and grow to maturity. It is common for introductions to be performed with little knowledge of the target specie's specific microsite requirements (Maunder 1992). Land managers decide where to introduce individuals in the broader habitat/community often under critical time constraints, with limited resources, not determining microsite preferences prior to introduction (Sarrazin and Barbault 1996, Bottin et al. 2007, Dixon and Coates 2007). Selecting a few to several possible microsite types deemed by the introducer as sharing similar characteristics with microsites currently supporting the species elsewhere (e.g. light, soil moisture, aspect, gradient, altitude, nutrient, etc.) can increase the chances of transplant success while providing an opportunity to learn about the species biology and microsite preference through seedling establishment (Guerrant and Kaye 2007).

Experimental introductions have been performed in a number of different ways with varying degrees of long-term monitoring and success using birds, mammals, plants, insects, and reptiles (Valutis and Marzluff 1999, Belousova et al. 2005, Shute et al. 2005, Bottin et al. 2007, Guerrant and Kaye 2007, Ye et al. 2007, Jogar and Moora 2008, Watts et al. 2008, Hill et al. 2009). Guerrant and Kaye (2007) detail lessons learned from seven introduction projects involving 10 plant species, all of which involved a variety of introduction techniques unique to the project. They found propagule type (e.g. seeds, cuttings, transplants), microsite characteristics and preparation, source population number and location, propagation protocols, and post planting care to be the common factors influencing introduction survival and important to discuss prior to any introduction effort. They assessed that transplants tended to establish better than seeds. Interestingly, though the *T. angustissima* var. *corallicola* introduction was performed with transplants, the seeds that were generated from the transplants established more successfully leading to the hypothesis that an introduction of seeds, rather than transplants, may be a more effective means of restoration for this species.

Microsite requirements, in addition to other aspects of the species biology, can be learned using an experimental introduction design. Number of microsites and each microsite chosen would be decided by the researcher based on what they know of the specie's native

microsite and habitat within its historic range attempting to mimic the dominant environmental variables that may be driving the specie's presence (e.g. light availability, substrate, nutrients, moisture, etc.). Further, it is necessary to monitor the study yearly until introduction goals are met (e.g. a certain population size, a better understanding of microsite preference or biology of the species, etc.). Should the land manager not have resources to study the introduction as intensely as I did, he or she could still make yearly qualitative observations of which habitat had the biggest plants, most recruits, and where recruits were germinating compared to where adults were planted. By performing introductions with an experimental component, one can contribute to the understanding of the target specie's demography and microsite preference while increasing the likelihood of creating a viable population in the face of habitat fragmentation and climate change.

Tables

Table 2.1. Introduced adult *Tephrosia angustissima* var. *corallicola* survival comparing survivor functions across microsites. The log-rank test weights all survival times equally while the Gehan-Wilcoxon test weights early survival times more heavily. Asterisks indicate significant differences.

Microsites	Gehan-Wilcoxon Test		Log-rank Test	
Road vs. Closed	$X^2 = 1.6$	- $P = 0.2$	$X^2 = 0.1$	- $P = 0.741$
Road vs. Open	$X^2 = 13.4$	* $P = 0.0003$	$X^2 = 4.6$	* $P = 0.031$
Closed vs. Open	$X^2 = 9.9$	* $P = 0.002$	$X^2 = 2.5$	- $P = 0.11$
All combined	$X^2 = 19.2$	* $P < 0.001$	$X^2 = 5.5$	- $P = 0.064$

Figures

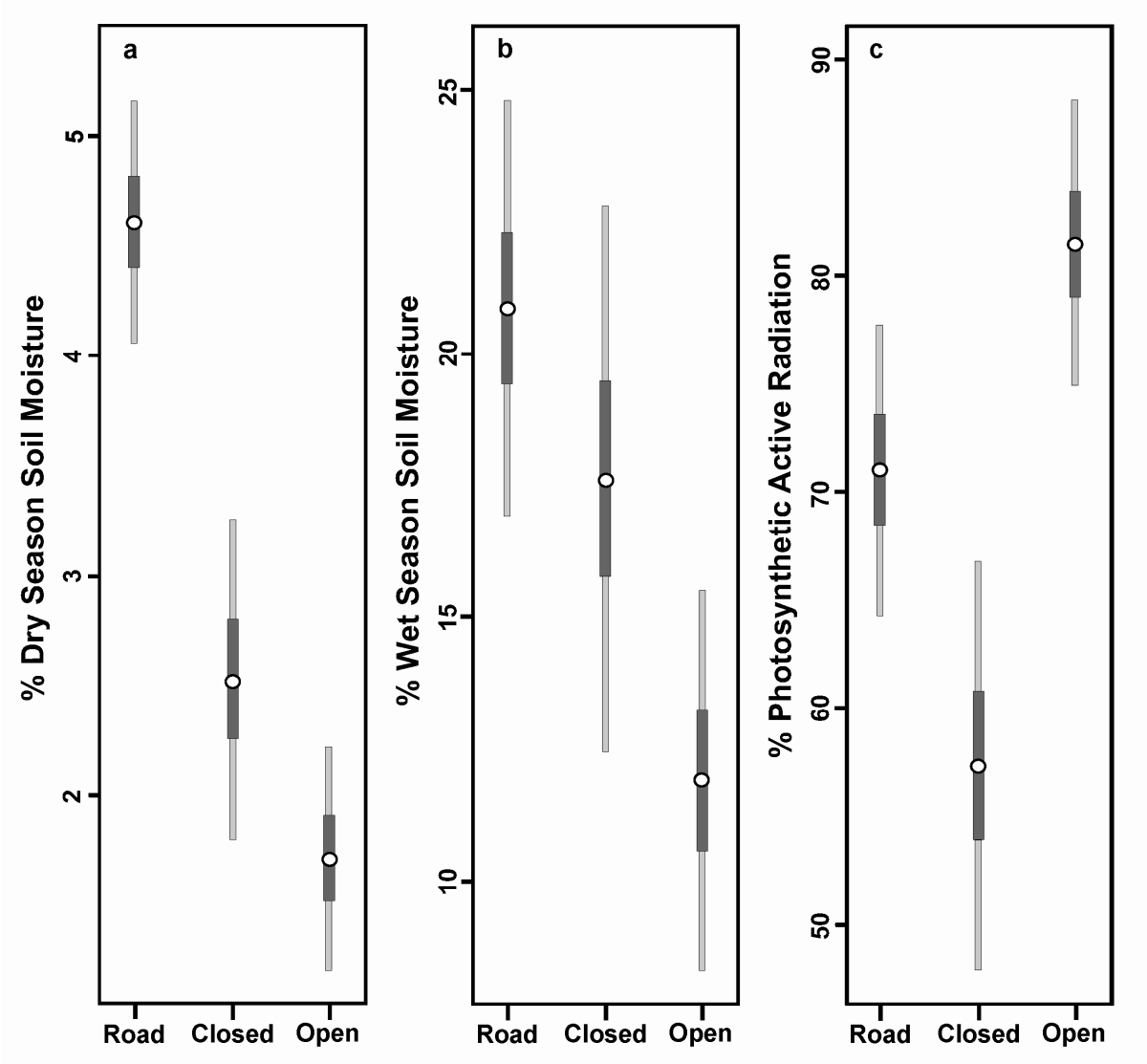

Figure 2.1. Dry and wet season soil moisture and percent photosynthetic active radiation (PAR) of the Road, Closed, and Open microsites used in the introduction of *Tephrosia angustissima* var. *corallicola* propagules. The figures shows ninety-five percent confidence intervals (light grey), 50% confidence intervals (dark grey), and a point estimate for the mean (white circle) obtained from analysis of variance.

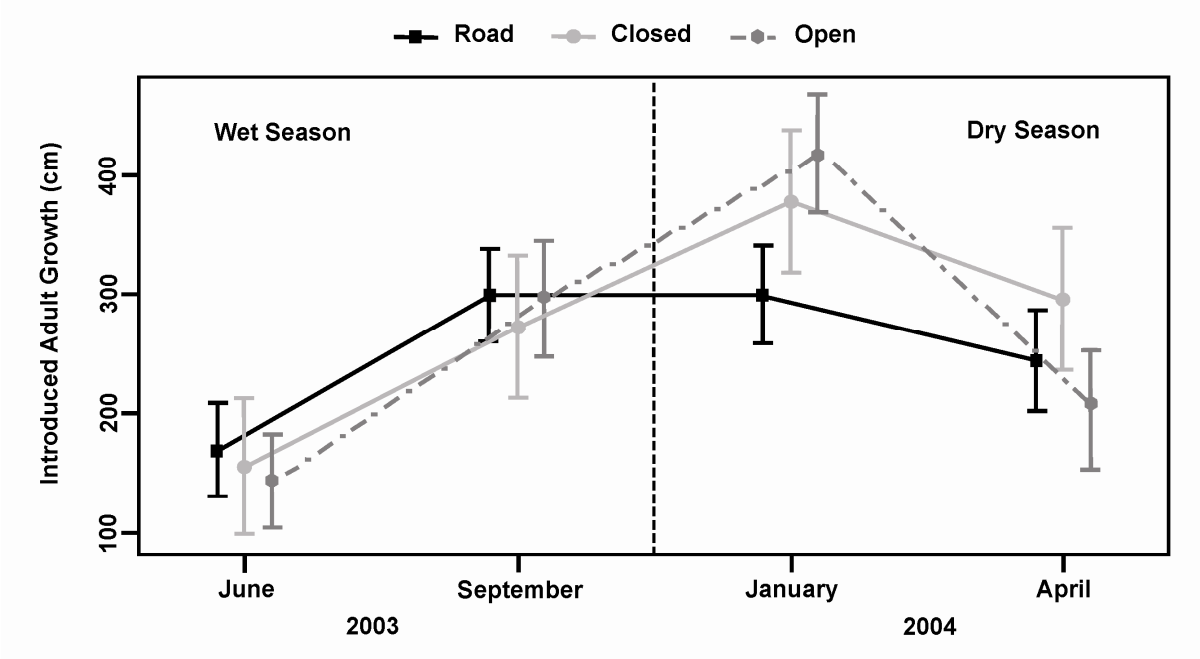

Figure 2.2. Introduced *Tephrosia angustissima* var. *corallicola* propagule growth across the Road, Closed, and Open microsites and time from June 2003 – April 2004.

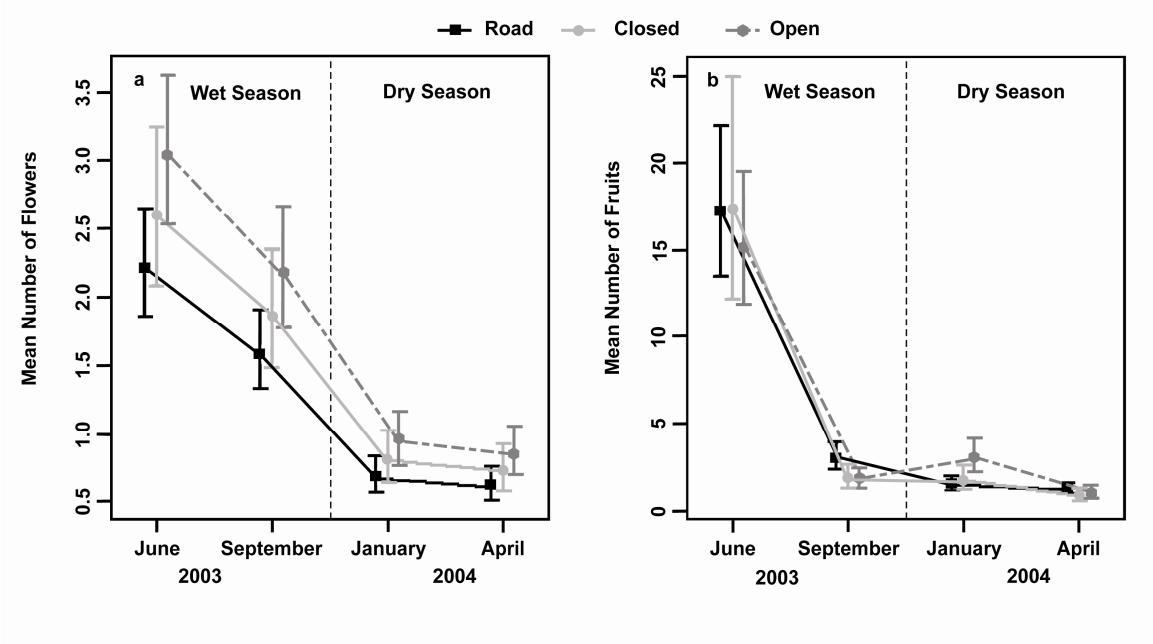

Figure 2.3. Introduced *Tephrosia angustissima* var. *corallicola* propagule flower and fruit production across the Road, Closed, and Open microsites. Error bars for both graphs are 95% confidence intervals of the mean obtained from analysis of variance. **a.** Mean number of Flowers found during the wet and dry season across microsites. **b.** Mean number of Fruits found during the wet and dry season across microsites.

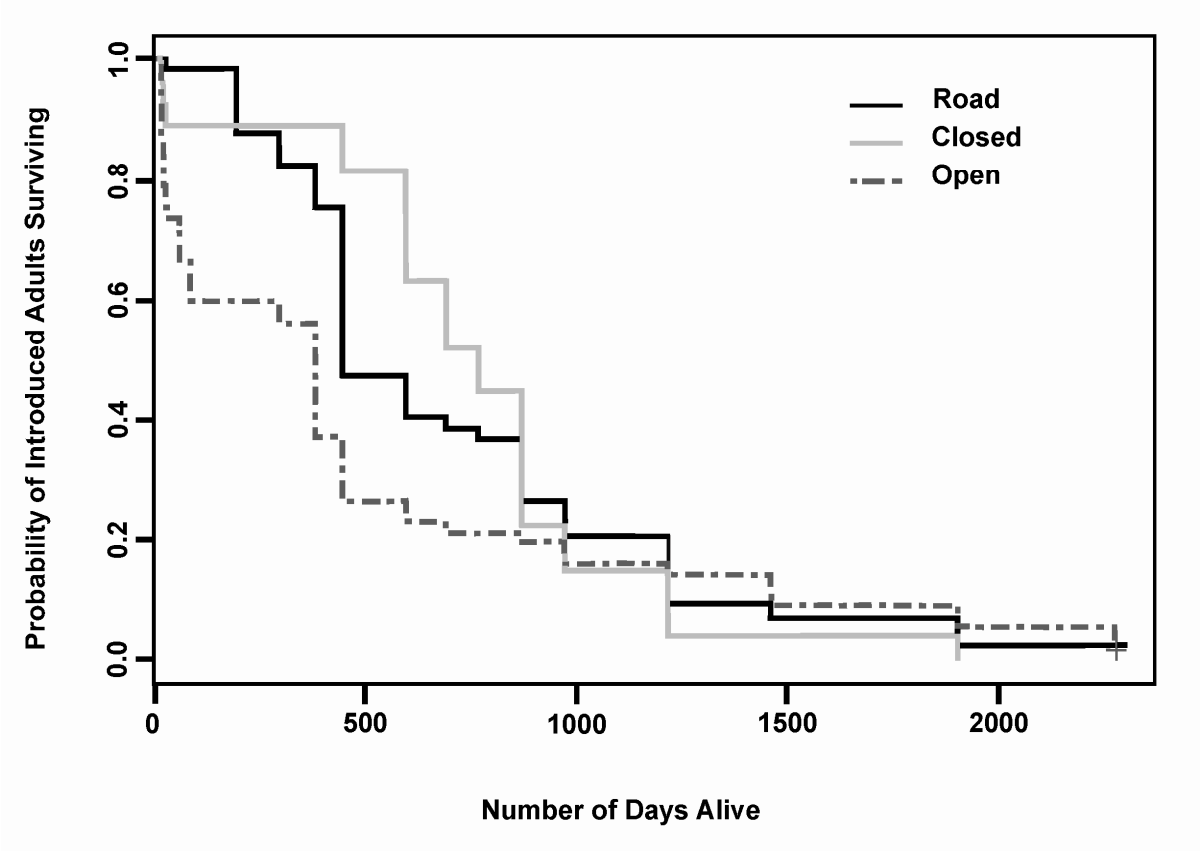

Figure 2.4. Kaplan-Meier estimates of survival for introduced *Tephrosia angustissima* var. *corallicola* propagules across the Road, Closed, and Open microsites over time. Dashed lines indicate 95% confidence intervals.

CHAPTER THREE: EXPERIMENTALLY ASSESSING *TEPHROSIA ANGUSTISSIMA* VAR. *CORALLICOLA* RECRUITED SEEDLING MICROSITE PREFERENCES

3.1 Introduction

To successfully introduce a rare plant species, one needs to select sites with appropriate microsites which support propagule survival and recruit germination, growth, and reproduction (Sakai et al. 2001). Studies from invasion ecology show that an introduced population's success or failure is often species specific with factors relating to competition (Blumenthal 2006, Borer et al. 2007), mutualism (Richardson et al. 2000), niche/microsite characteristics (Thuiller et al. 2005, Broennimann et al. 2007), and/or a combination of these factors (Mitchell et al. 2006) influencing population survival. Therefore, an *a priori* understanding of which ecological processes support the specie's regeneration may be helpful for a successful introduction. Unfortunately, realities of rapidly changing environments, constrained funding, and, at times, a sense of urgency may lead to a lack of careful observation and/or the assumption that this information is too difficult to obtain; land managers often do not know a specie's microsite requirements prior to introduction. Furthermore, contemporary and future human induced pressures (e.g. fragmentation, development, and/or climate change) may alter ecological processes changing microsite conditions to a point where the species can no longer regenerate in its historic location (Bottin et al. 2007, Rannap et al. 2009). When microsite and regeneration niche requirements are not known or ecological processes have been altered, performing an introduction experimentally with enough replication and long-term monitoring such that demographic, microsite, and regeneration niche insights can be obtained may help both present and future introduction success increasing the likelihood of conservation effectiveness (Bottin et al. 2007).

In chapter two, I discussed the results of an experimental introduction of the Florida state rare plant, *Tephrosia angustissima* var. *corallicola,* I performed in June 2003 and monitored thru

August 2009. Some of the propagules flowered and dispersed seed. This chapter reports on the subsequent fate of the recruited population.

To assess *T. angustissima* var. *corallicola's* microsite and regeneration requirements, I monitored demographic patterns of seedlings germinated from introduced transplants in three different microsites within its native pine rockland habitat. Insights gained from this introduction can now be used to introduce more populations of the species with greater confidence in its survival. Specifically, I asked which of the three microsites supported optimal *T. angustissima* var. *corallicola* seedling recruitment? Did recruits germinate under the same photosynthetic active radiation (PAR) regime as where the parents were planted? In which of the three microsites did *T. angustissima* var. *corallicola* recruits show the most growth? Which of the three microsites showed the highest incidence of flowering and fruiting of *T. angustissima* var. *corallicola* recruits? Which of the three microsites showed quickest median maturation time of *T. angustissima* var. *corallicola* recruits? Which of the three microsites had the highest survival of *T. angustissima* var. *corallicola* recruits?

3.2 Methods

3.2.1 Study design

In response to significant recruitment, I began monitoring recruits in February 2004. I located and tagged each recruit within one meter radius of the parent plant in an attempt to ensure seedlings came from that parent's seeds; seeds of *T. angustissima* var. *corallicola* disperse through explosive dehiscence tending to drop within a meter of the parent plant. To help relocate the recruits I measured the distance and compass bearing from the parent plant to each recruit. I measured recruit presence/absence, height (cm), and flowers and fruits. In all analyses, to account for potential spatial correlation, the seedling parent was included. I expected seedlings coming from the same parent and different parents to not be equivalent.

I collected PAR at a subset of randomly selected recruits within each microsite using a Li-Cor LI-190SA Quantum Sensor. I compared PAR at each recruit to a full sun PAR measurement

to calculate percent PAR. To test the percent PAR that recruits germinated into compared to the PAR where adults were planted (Chapter 2), I used a Wilcoxon signed-rank test.

Because this species grows during the wet season and dies back during the dry season, growth followed a quadratic pattern. To test in which microsite recruits showed the most growth, I used a quadratic linear mixed effects model in which all coefficients were treated as random on both parent and seedling level. The quadratic model was put into the standard form for a parabola to yield more interpretable parameters. The approximate variance of these parameters was obtained using a linearization called the delta method (Rice 1988).

Conditional on the number of recruits germinated at each time point, the number of recruits in reproductive state can be treated as an over-dispersed binomial random variable. Thus, to analyze which microsite showed the most recruit flowering, I used an additive quasi-binomial generalized linear model with date and microsite as the only predictors and obtained odds ratio estimates adjusted for date. To assess the mean recruit maturation time in each microsite and because of reproduction paucity in the Road and Closed microsites, I used a parametric survival analysis with a Weibull probability model. In the Weibull model I assume a common shape parameter, but a scale parameter that differs across habitats. Recruits that did not flower or those that died were treated as censored.

To analyze which microsite showed the most recruit survival, I used a log-rank test. To assess the median survival time of only those recruits that became reproductive, I used a Weibbull survival analysis. Conditional on the number of recruits germinated at each time point, the number of recruits in reproductive state can be treated as an over-dispersed binomial random variable. Thus, to analyze microsite effects on recruit reproduction, I used an additive quasi-binomial generalized linear model with date and microsite as the only predictors and obtained odds ratio estimates adjusted for date.

I monitored a total of 3,000 *T. angustissima* var. *corallicola* recruits within a 1 m radius of adults introduced in 2003. More recruits with cotyledons were noted more than a meter away; however, I excluded them from our study.

Analyses were performed using the survival package (Therneau 2009) and the nlme package (Pinheiro et al. 2009) of R 2.10.1 (R Development Core Team 2009).

3.3 Results

3.3.1 Recruit germination across the three microsites and comparing recruit PAR regime to that of the introduced parents

A total of 3,000 seedlings recruited within 1m radius of 131 parent plants. Additional recruits were found outside of a 1m radius of the adult plants. Total recruitment was highest along the Road (2,067 recruits), intermediate in the Closed (498 recruits), and lowest in the Open (435 recruits, Fig. 3.1). Recruits tended to germinate in shadier locations than adults were planted ($P < 0.001$, Fig.3.2).

3.3.2 Recruit growth across the three microsites

Recruit growth differed across the three microsites. Those recruits that survived in the Open tended to grow significantly bigger than the Road ($z = 3.5$, $P < 0.001$). Median maximum size in the Open microsite recruits was 10.0 cm, 95% CI = (7.41, 13.79), Road was 5.0 cm, 95% CI = (3.92, 6.46), and the Closed was 7.90 cm, 95% CI = (2.13, 29.30). Time to reach peak size did not differ across the three microsites. Mean time to maximum size in the Open microsite recruits was 580 days, 95% CI = (508.91, 652.56), Road was 498 days, 95% CI = (433.97, 561.54), and the Closed was 1,268 days, 95% CI = (468.93, 2,066.28; Fig. 3.3).

3.3.3 Recruit reproduction across the three microsites

Of the 141 introduced adults, 23 had recruits that produced flowers and/or fruits for a total of 63 reproductive recruits. Plants in the Open microsite had significantly greater odds of flowering than those in the other two microsites (Open vs. Closed; $t = 3.55$, $P = 0.003$, Open vs. Road; $t = 6.65$, $P < 0.001$; Table 3.1, Fig. 3.4). A total of 52 recruits flowered in the Open, 10 along the Road, and 1 in the Closed microsite.

Using all recruits, both reproductive and non-reproductive, median maturation time was significantly different across microsites using the Weibull survival analysis. It took a significantly shorter time to reproduce in the Open microsite than the other two microsites ($P < 0.001$). Median maturation time for Open microsite recruits was 1,212 days, 95% CI = (1048, 1490), Road was 4,264 days, 95% CI = (3134, 9181), and the Closed was 8,224 days, 95% CI = (3262, 36351).

3.3.4 Recruit survival across the three microsites

Recruit survival differed significantly across the three microsites ($X^2 = 179$, $P < 0.001$; Table 3.2, Fig. 3.5). The Open maintained the highest percent survival (2%; 8 out of 435 recruits), followed by the Closed (1%; 3 out of 498) and Road (0%; 0 of 2067; Fig. 3.5). Median recruit survival in the Road was 210 days, Closed 272, and Open 390 days. The median survival time of those that became reproductive was 1,324 days (3.5 years), 95% CI = (1207, 1452); at the study termination date, the maximum age of seedlings still alive was 2,033 days (5.6 years). By the end of the study, the Open had more recruits alive though it started out with the least number germinated. As of September 2009, 12 of 3,000 monitored recruits from six parents had survived—however, beginning in September 2008, the study was expanded to include recruits found outside of a 1m radius from the parent plant while still in the vicinity of the planting sites. As of September 2009, a total of 162 individuals were being monitored in all stages of development.

3.5 Discussion

Introductions can be used as a way to assess a specie's demographic and reproductive needs while creating more populations of that endangered species. Through the introduction of the Florida rare plant, *T. angustissima* var. *corallicola,* I was able to assess microsite preferences and, possibly, created a sustainable population. Results showed that, though recruits germinated more in the Road, they grew bigger, had a higher probability of becoming reproductive, and survived longer in the Open microsite. Ye et al. (2007) introduced micropropagated plants of the endangered *Symonanthus bancroftii,* a shrub known to have only one male and one female plant in the wild. This introduction brought to light the first insights on *S. bancroftii* pollination biology

while showing that the species could become reproductive through their introduction methods. Lessons learned by Ye et al. (2007) can now be used for future restoration efforts that may lead to a sustainable population of the endangered shrub. Similarly, the introduction of *T. angustissima* var. *corallicola* showed abundant recruitment across the three microsites providing an assessment of recruit germination, establishment, and microsite requirements that may best support this specie's long-term survival. Further, a turnover in the population was documented; new individuals are recruiting as older individuals are dying. The average reproductive recruit's lifespan was 3.5 years and the maximum was 5.6 years. I stopped tagging newly germinated recruits in 2006 using those that germinated prior to that as the study individuals. In 2008, new recruit monitoring was reinstated; as of September 2009,162 individuals were living in all stages of development; the study began with 141 planted individuals across the three microsites showing that turnover is occurring in the population. Additionally, species of Fabaceae are known to store well for long periods of time in the seed bank to then germinate later when the conditions are optimal (Baskin and Baskin 1989, Degreef et al. 2002). Seeds of *T. angustissima* var. *corallicola* have been found to survive at least a year in the seed bank (Wendelberger *unpublished data*), and if *T. angustissima* var. *corallicola* is like many other Fabaceae, possibly longer. Likely, adults as well as recruits were able to disseminate seed to the area stocking the seed bank. Long-term monitoring and analyses is needed to determine if this population is sustainable.

For a successful introduction, propagules need to survive long enough to reproduce and recruits need to establish, grow, and reproduce themselves (Sakai et al. 2001). Some rare species have been found to have specific microsite and regeneration niches (Grubb 1977) that are essential for population establishment (Wiser et al. 1998, Yates et al. 2007, Rannap et al. 2009). Wendelberger et al. (2008) found the federally endangered *Amorpha herbacea* var. *crenulata* requires one to two centimeters of litter for maximum seedling establishment; this suggests the litter's protection helps maintain microsite conditions that support seedling survival during the establishment phase. Introduced *T. angustissima* var. *corallicola* propagules and recruited seedlings tended to show better success in similar microsite conditions. Propagules grew and flowered more in the Open, sunny, dry microsite. However, higher light conditions

tended to negatively influence survival in the first year of planting. Interestingly, seedlings, though also more successful in the Open, tended to germinate in shadier areas than where propagules were planted. This suggests that future introductions of this species should focus on locations with similar soil characteristics as the Open, dry with deep sand, but with low light conditions, e.g. under shrubs. Contrastingly, Guerrant and Kaye (2007) found that seedling and transplant establishment of *Arabis koehleri* var. *koehleri* occurred in different microsite conditions; seeds tended to establish better on slopes with a southern aspect while transplants showed higher survival on south-western exposures. The contrasting results of these two studies highlight the variation in introduction response between two different species. Introducing rare species is a complex task that calls for varying techniques unique to each species and circumstance.

One of the struggles when introducing a species is deciding on introduction microsite. Whether one is introducing a species into its historic range or outside of its historic range via assisted migration, understanding the biology and microsite characteristics that will support regeneration is essential to introduction success (Sakai et al. 2001). Performing introduction experimentally with enough individuals, replication, and long-term monitoring can be a way to circumvent a lack of biological and demographic knowledge of the species; holes in this knowledge can be filled while, simultaneously, increasing the chances of creating a sustainable population.

Tables

Table 3.1. Odds ratio estimates for the effect of microsite on *Tephrosia angustissima* var. *corallicola* recruit flowering adjusted for date. Asterisks indicate a significant difference.

Microsites	Odds ratio	95% Wald confidence interval
Open vs. Road	12.96	* (6.09, 27.58)
Closed vs. Road	0.40	(0.05, 3.01)
Open vs. Closed	32.71	* (4.78, 223.67)

Table 3.2. *Tephrosia angustissima* var. *corallicola* recruit survival across the Road, Closed, and Open microsites. There was a significant difference in survival between all three microsites. Those recruits that germinated in the Open survived the longest, then the Closed, and the Road surviving the least number of days.

Microsites	**Log-rank Test**	
Road vs. Closed	$X^2 = 43.6$	$P < 0.001$
Road vs. Open	$X^2 = 153$	$P < 0.001$
Closed vs. Open	$X^2 = 29.8$	$P < 0.001$

Figures

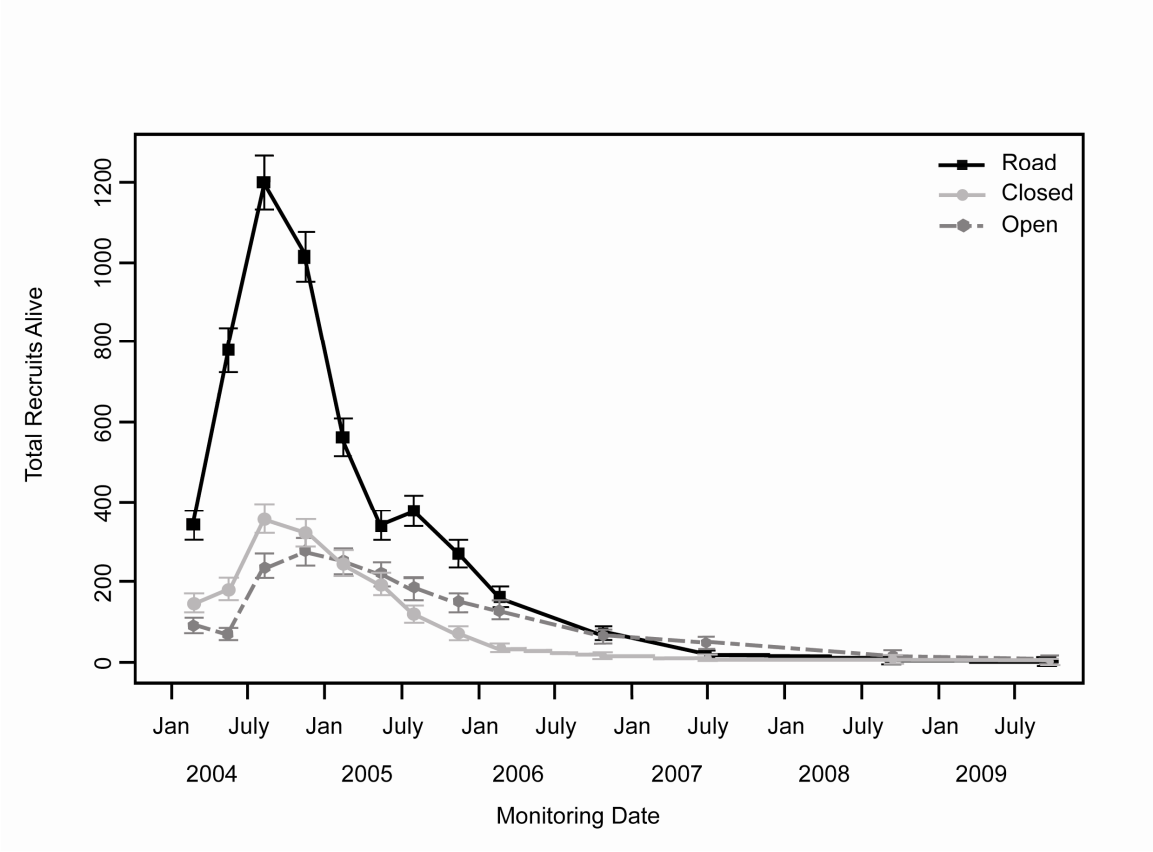

Figure 3.1. The total number of *Tephrosia angustissima* var. *corallicola* recruits alive at any one time in the Road, Closed, and Open microsites. Error bars are 95% confidence intervals.

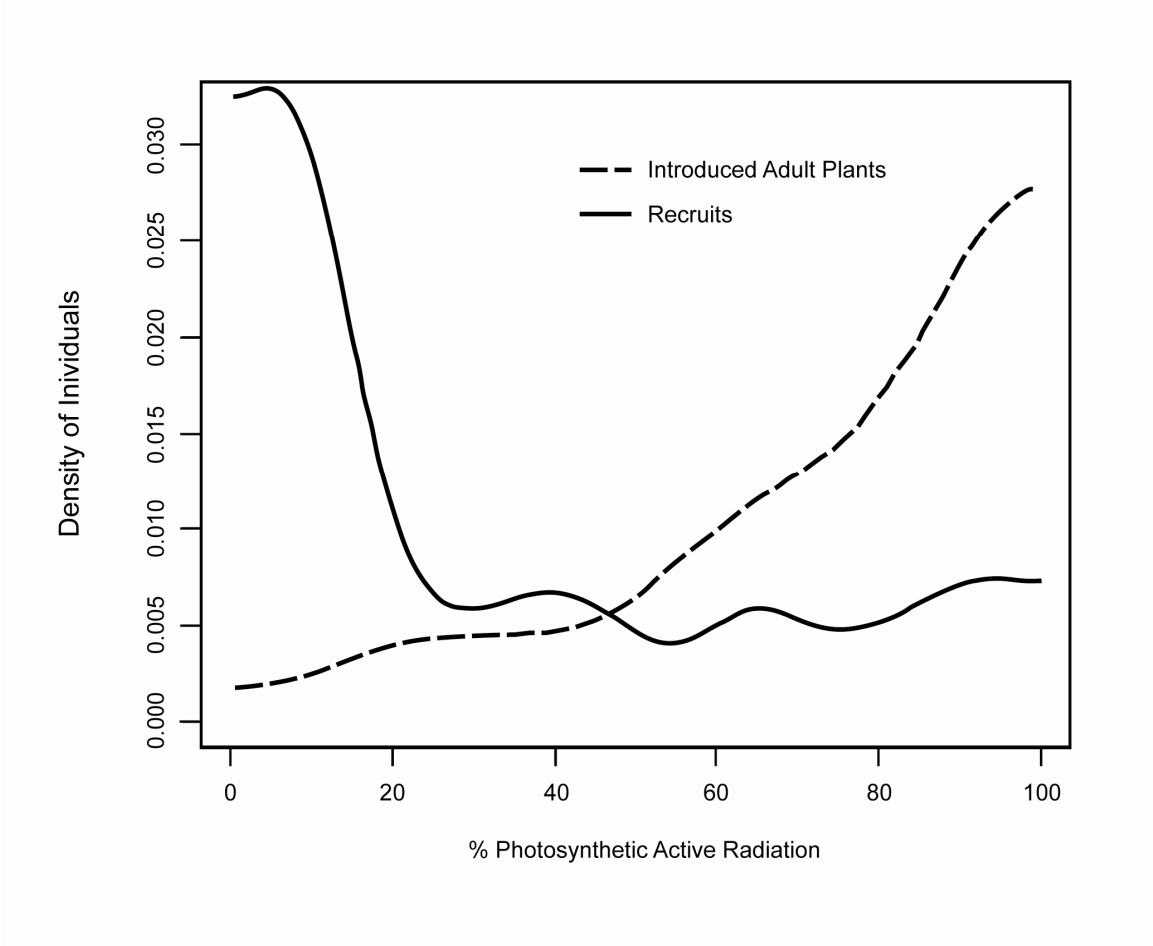

Figure 3.2. Percent photosynthetic active radiation *Tephrosia angustissima* var. *corallicola* recruited seedlings germinated into compared to where the adults were planted. Recruits tended to germinate in shadier locations (lower % photosynthetic active radiation) than those where adults were planted.

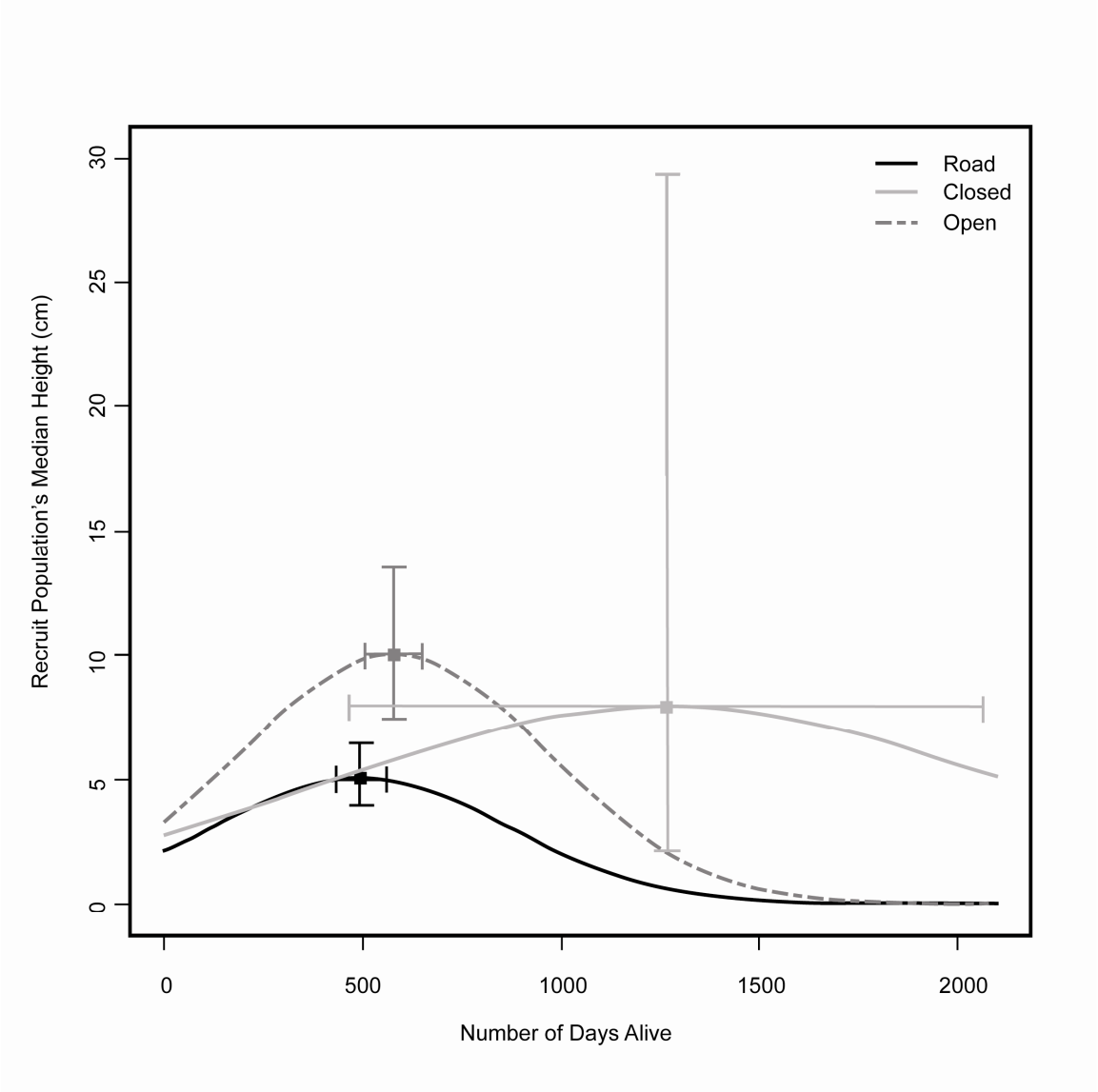

Figure 3.3. *Tephrosia angustissima* var. *corallicola* recruited seedling growth in the Road, Closed, and Open microsites. There is a significant difference in growth across the microsites. Those that established in the Open and Closed grew significantly bigger than those in the Road. However, those recruits that grew in the Open microsite tended to reach peak size at the same time as those that grew in the Road while the Closed took longer than both the Open and Road to reach peak size**.**

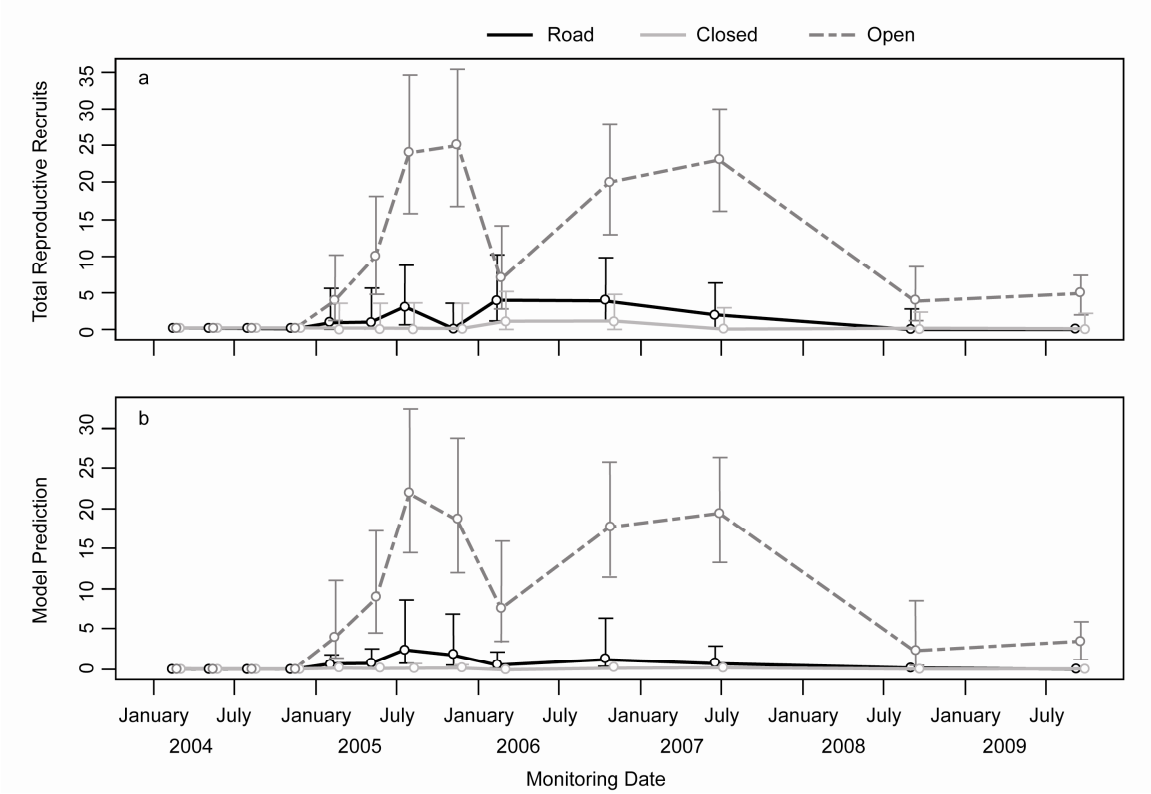

Figure 3.4. Number of *Tephrosia angustissima* var. *corallicola* recruits flowering across the Road, Closed, and Open microsites. **a**. Observed number of reproductive recruits per monitoring date. Error bars represent exact binomial 95% confidence intervals. **b**. Prediction recruit flowering from an additive quasi-binomial generalized linear model with date and microsite as the only predictors.

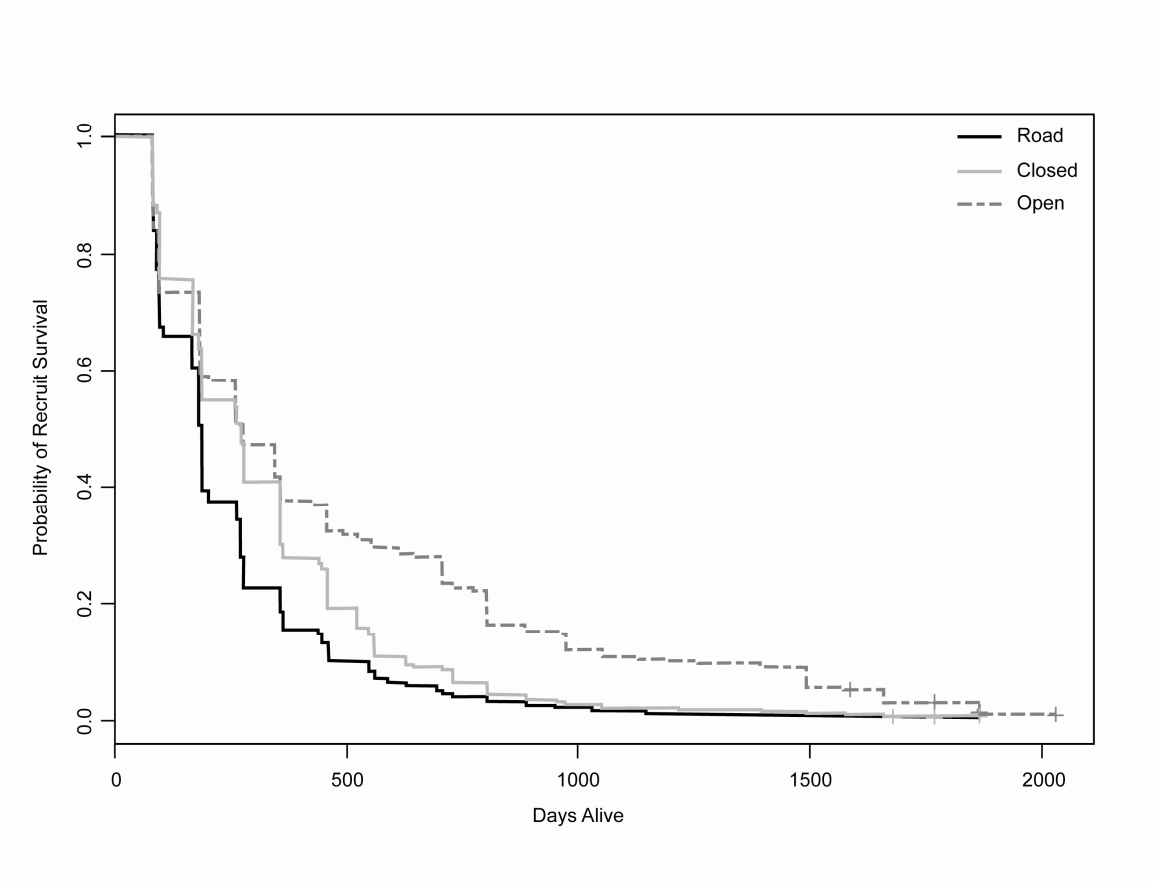

Figure 3.5. The probability of *Tephrosia angustissima* var. *corallicola* recruits surviving to 2,033 days within the Road, Closed, and Open microsites. Dashed lines indicate 95% confidence intervals.

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