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Evaluating Restoration Potential of an Endangered Legume, Baptisia Arachnifera: Shade & Litter Effects on Early Life Stages

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EVALUATING RESTORATION POTENTIAL OF AN ENDANGERED LEGUME,
BAPTISIA ARACHNIFERA: SHADE & LITTER EFFECTS ON EARLY LIFE STAGES

by

TIMOTHY JOHN ESTEP

(Under the Direction of Lissa M. Leege)

ABSTRACT

Baptisia arachnifera, hairy rattleweed, is a federally endangered, herbaceous, legume endemic to Wayne and Brantley Counties in Georgia. The species has declined by 89% in the past 20 years. Therefore I examined the early life stages of the species for weevil predation, fungus infection, and germination; information used to help prevent the species extinction. Seed pods of *Baptisia arachnifera* from six sites were examined for weevil predation and fungal infestation. Germination was examined under greenhouse conditions. One site had intense weevil predation, fungal infection, and reduced germination compared to other sites. Over 62% of seeds germinated within the greenhouse.

To determine the effects of light and litter on *Baptisia arachnifera,* I planted 320 seedlings into a 2x2 factorial shade and litter experiment within the natural range of the species. Another 480 seedlings were planted across 12 sites within three habitat types: four replicates for each of two types of pine plantations and power-line cuts. Both experiments were compared for germination and seedling growth. Of seeds planted in the field <8% germinated in the shade and litter experiment; while <1% germinated within the forest and power-line cut habitats. Shade and litter increased seed germination within treatments. All germinated seeds died for both field experiments. Transplanted seedling

survival dwindled down to 40%. Four percent of seedlings across forested habitats survived initial planting, and plant numbers dwindled down to <1% by the end of the study. Neither experiment showed an effect of shade or litter on seedling growth.

This study showed seeds that escaped predispersal mortality collected from the natural range of *Baptisia arachnifera* can be used to obtain numerous seedlings within a greenhouse, and additional factors other than light and litter determine germination and seedling survival within the species natural range. Reintroduction of greenhouse grown seedlings showed potential use for restoration projects, field sown seeds did not. Future research should focus on increasing reintroduced seedling survival within the species range for use within restoration projects.

INDEX WORDS: *Baptisia arachnifera*, Hairy Rattleweed, Restoration, Transplanting, Shade, Litter, Germination, Endangered Species, Georgia

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CHAPTER 1

NATURAL HISTORY OF AN ENDANGERED LEGUME: *BAPTISIA ARACHNIFERA*

Baptisia arachnifera (Duncan 1944), hairy rattleweed, is a federally endangered, perennial, herbaceous legume, 4-8dm tall, covered with cobwebby hairs. The remaining natural populations of *Baptisia arachnifera* are limited to 16 square kilometers in sandy pine/palmetto woodlands of Wayne and Brantley counties in Georgia, USA (Isely 1998, United States Fish & Wildlife Service [USFWS] 1984; Figure 1.1). Populations are most abundant on high, dry sandhill pine communities that have a history of natural fires (USFWS 1984).

Little is known about the biology of the species. *Baptisia arachnifera* seedlings are rarely seen in the field, but are thought to germinate in early spring. Seedlings grow until the winter, when the plant enters a dormant phase. The above-ground portion of the plant dies back, and the roots overwinter to re-emerge in early spring. Adult plants flower in the summer, with seeds developed in the early fall (USFWS 1984). Some individuals are estimated to be at least 20 years old (Personal communication Lissa Leege, GA). Predators of *Baptisia arachnifera* include caterpillars of the moth species *Uresiphita reversalis* that eats above ground portions of the plant (Durden et al. 2011) and Say's weevil (*Apion rostrum*) that eats seeds within the fruit (USFWS 1984, Young et al. 2007, Leege 2007 & 2009). Newly germinated plants have rarely been seen within the natural range of the species (Leege 2009), which may indicate recruitment as a limiting factor.

Low numbers of individuals and endemic habit has placed and maintained *Baptisia arachnifera* on the endangered species list since 1978 (USFWS 1978). A monitoring report showed the population of the species declined 89% over the past 20 years in sites managed for timber (Leege 2007). Timber stands make up the majority of the species natural range, though one site has been protected by The Nature Conservancy. The continued decline has been linked to the pine tree seedling bedding practices of the timber industry and fire suppression (USFWS 1978). Population monitoring has shown that without help, the species could go extinct (USFWS 1984). The recovery plan developed for delisting the species calls for: 1) eight self-sustaining populations, 2) an optimum frequency and percent cover in the populations, 3) adequate biological knowledge, 4) and continued protection (USFWS 1984). The plan objectives include: 1) protecting existing population of the species, 2) monitoring the population, 3) conducting surveys of the species, 4) storing germ plasm, 5) and conducting autecological research. In this study I hope to provide information on the predispersal status of seeds, examine the potential for germination, and determine the effects of light and litter on *Baptisia arachnifera* germination and growth of seedlings to determine management strategies to this endangered species.

CHAPTER 2

SEED – FROM POD TO PLANT: WEEVILS, FUNGUS, & GERMINATION

Introduction

Of the 307,674 plant species described in the International Union for

Conservation of Nature database (IUCN), 9,098 are listed as globally threatened, nearly

twice the 5,328 listed in 1998 (IUCN 2011). This is an alarming increase of species

headed toward extinction, and the number is expected to accelerate (Naeem et al. 1994,

Pimm & Russell 1995, Thomas et al. 2004). Plants provide genes to improve domestic

crops and chemicals and products for medical and industrial use (Wilson 1988,

Hoisington et al. 1999, Johnson 2008). The main threats to at-risk plants can be linked to

human actions including habitat destruction, introduction of non-native species, and

pollution (Wilcove et al. 1988, Silva et al. 2007). To protect these plant species from

extinction, active management will be required.

Due to concerns about species extinction in the United States (U.S.), The

Endangered Species Act was passed in 1971. The Endangered Species Act determines if

a species is endangered or threated by: 1) destruction of its habitat or range, 2)

overutilization, 3) disease or predation, 4) inadequacy of existing regulatory mechanisms,

5) and other factors affecting its continued existence ("Endangered Species Act" 1973).

Preventing extinction would require a reversal of the factors responsible for habitat loss

and species decline. This becomes problematic as there is often inadequate biological

information on what is causing the species decline (Schemske et al. 1994, Campbell et al.

2002, Kozlowski 2008).

To help in the recovery of endangered species, factors that limit seedling recruitment are investigated (Aparicio & Guisande 1997, Wenhui et al. 2006, Burgos et al. 2008, Weekley et al. 2008). Each stage in the life cycle of a plant acts as a "sieve" that limits a species population size (Harper 1977). If not by asexual/vegetative means, plants must recruit new individuals into the population as seeds. Seeds are removed by predispersal predation and disease (Harper 1977, Louda 1982, Kaye 1999). Seeds must then break dormancy in order to germinate (Harper 1977). Germination cues vary depending on the species. After germination, growth and survival will determine whether a seedling will reach reproductive maturity.

Predispersal seed predators can reduce seed output by 40-75% in a population (Green & Palmbald 1975, Louda 1982, Kaye 1999, Young et al. 2007). Many predators specialize on seeds as a food source (Steven 1983, Szentesi & Jermy 1995, Hemborg & Despres 1999, Westerbergh 2004). Specialized seed predators can infiltrate fruit structures before they develop (Szentesi & Jermy 1995, Hemborg & Despres 1999, Westerbergh 2004).

 Fungal infections can also limit seed production by killing all seeds produced by an individual and reducing 10-90% of seed produced in a population (Green & Palmbald 1975, Drake 1981, Tewksbury et al. 2008b). Moist conditions can cause fungal infections in plants (Green & Palmbald 1975). Insects may also act as vectors spreading fungus to other seeds within an individual plant or throughout a plant population (Tewksbury et al. 2008b). As with seed predation, fungus removes seeds from the pool of potential recruits.

When seeds survive predispersal factors, germination becomes the next obstacle. Germination cues vary among species and include light (Keeley 1987), litter (Falkner et

al. 1997, Fowler 1988), moisture (Baskin et al. 2003, Garwood 1983), chemical (Baskin et al. 2003), temperature, including fire or heat shock (Keeley 1987) as well as a cold period (Caplenor 1967), passage through the gut of animals (Cosyns et al. 2005, Tweksbury et al. 2008a, Lieberman et al. 1979), scarification (Boyle & Hladun 2005), and others. When germination cues are identified, individual plants can be obtained when seeds are available.

Obtaining new plants is a vital step in the recovery of endangered species. Seedlings can be grown under natural conditions (Pemadas & Lovell 1975, Laman 1995, Falkner et al. 1997, Qulichini & Debussche 2000) as well as within controlled environments such as greenhouses (Dutra et al. 2008, Cervera et al. 2006, Kadis et al. 2010, Carasso et al. 2011, Keeley 1987, Falkner et al. 1997, Noe 2002). Once numerous plants are obtained, endangered species recovery projects can proceed (Kaye 2008).

There are no published studies on obtaining *Baptisia arachnifera* seedlings. Viable seeds must be collected and identified before being planted. Previous examinations have suggested weevil seed predation may reduce seed production of *Baptisia arachnifera* (Young et al. 2007, Leege 2007). An efficient germination method will be essential to obtaining numerous seedlings. Attempts to germinate the species using heat shock were found to reduce seed viability (Young et al. 2007), yet field observations showed germination in sites that were burned (Leege 2009). Little is known concerning *Baptisia arachnifera* seed production and germination. When this information is identified, reintroduction projects can be developed.

Say's weevil is known to eat seeds of *Baptisia arachnifera* (USFWS 1984). *Apion rostrum* Say, 1826, is a weevil that feeds on many *Baptisia* species (Blatchley & Leng

1916, Smith 1884, Haddock & Chaplin 1982, Horn & Hanula 2004, Petersen et al. 1998, Alstad 2008) from New Hampshire to Florida, west to Wisconsin and Texas. Females drill holes into the base of developing pods, lay, then push one or two eggs into the pods with their snout (Haddock & Chaplin 1982, Frost 1945). Larvae eat the seeds, damaging all seeds within the pod (Haddock & Chaplin 1982). Adults continue to feed on leaves and flowers of *Baptisia* species.

I addressed objectives outlined within the Hairy Rattleweed Recovery Plan that focused on the establishment of new individuals of the species (USFWS 1984). This study aimed to analyze factors that limit germination and recruitment of *Baptisia arachnifera*. The objectives of this study were to: 1) determine the rates of weevil predation and fungal infection within individuals of *Baptisia arachnifera*; 2) determine and compare greenhouse germination rates of seeds collected from multiple sites throughout the species remaining natural range.

Methods

Seed Collection & Examination

On August 21, 2009, I collected seed pods of *Baptisia arachnifera* from six sites within the remaining population of the species. Sites selection was based on locations known to contain high numbers of reproductive plants (Figure 1.1). Due to plant and pod availability, I collected pods from 85 individual mother plants (24 from Wire Road, 24 from Long Branch, 15 from 110W, 14 from E3, 6 from E2, and 2 from GA Power). Within a week after collection, weevils, seeds infected with fungus, and clean seeds (without fungus) were separated from the collected pods and stored in small paper envelopes at room temperature until October 25, 2009. I calculated the percent of weevil

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infection at the plant level for each site. Fungus was visually identified by white hairs, presumed hyphae, protruding from seeds. The presence of fungus was evaluated by applying pressure to the seeds with forceps; infected seeds would easily crumble, whereas uninfected seeds would stay intact. I calculated the percent of seed fungal infection by dividing fungal infested seeds by the total number of seeds at the plant for each site. The average number of seeds per pod was calculated by dividing the total number of seeds, both infected and uninfected, by the total number of collected pods for each mother plant. To examine seed weight I took the weight of all uninfected seeds collected for each plant and divided by the total number of seeds of that plant using a Denver Instrument Company XE Series Model 400 precision/scientific scale.

Greenhouse 2009 Planting

From the seed collection, 2655 seeds were planted in 26 trays filled with Miracle Grow™ Moisture Control Potting Soil Mix and monitored for germination in the greenhouse at Georgia Southern University, Statesboro, GA, from October 25, 2009 to February 28, 2010. To determine differences in germination within containers of various soil amounts and depth, three different types of trays were used: 11 plastic Cone-tainer™ trays with 98 [3.8cm diameter opening x 14cm depth] cells in rows of 7 x 14; 9 small styrofoam trays with 128 [2cm x 2cm opening x 6.25cm depth] cells in rows of 8 x 16; 6 large styrofoam trays with 72 [2.5cm x 2.5cm opening x 7cm depth] cells in 6 x 12 rows.

Seeds were planted haphazardly. At random, an individual plant was chosen with 21 seeds from that plant planted across the three tray types (Figure 2.1). Individuals were not reselected until all individual plants had been chosen, then the process repeated. Seeds were planted in this way until there were no longer seeds available to fill a row of

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the tray or there were no longer trays of the tray type. I continued to plant seeds in until all available trays were filled. A single seed was planted just below the soil surface in each cell within the trays. Planted seed trays were haphazardly placed within the greenhouse and watered every other day. Germination was monitored weekly for 18 weeks.

Greenhouse 2010 Planting

On September 7, 2010 I planted the remaining 1274 seeds from the 2009 seed collection. These seeds had been stored at room temperature, within envelopes containing seed from a single mother plant, for one year. Seeds remained from four sites: Wire Road, Long Branch, E3, and E2. With few seeds remaining for most mother plants, seeds were combined by site. Cone-tainers™ were used for ease of planting after determining tray type did not have an effect on germination in the 2009 greenhouse planting. Seeds were planted into 13 Cone-tainers™, exhausting all seeds. Trays were haphazardly placed within the greenhouse with monitoring and watering methods as above. Germination was monitored through November 16, 2010, as described above.

Statistics

Statistical analyses were conducted with JMP® 8.0 (2009). Data were tested for assumptions of normality using a goodness of fit test and for equal variance using the Levene test. To determine differences among tray types in 2009, cumulative germination percentages after 18 weeks were compared (assumptions met, ANOVA test; N = 26). To determine differences among sites (N = 6), average seeds per pod (assumptions met, ANOVA test), seed mass (assumptions met, ANOVA test), averages in weevil predation percentage (assumptions not met, Kruskal-Wallis test), and percent of fungal infection

was compared (assumptions not met, Kruskal-Wallis test). To determine differences in germination among sites in 2009, cumulative germination percentage at the end of 18 weeks were compared (assumptions not met, Kruskal-Wallis test; N = 78, as seeds of seven mother plants were all infected with fungus and not planted in the study). To determine differences in germination rates among sites and between years, the germination percentage of sites replicated across trays from weeks 1-10 for 2009 and 2010 were compared among the four sites from which seeds had been planted for both the 2009 and 2010 planting (assumptions not met, Scheirer-Ray-Hare test; N = 112). Differences between two means were compared using the student's t-test, and among means were compared using the Tukey HSD test.

Results

Predispersal Effects

Comparison of seeds from different sites revealed differences in weevil predation, fungus infection, and seeds per pod among sites. Weevils were present in the pods of two sites, eating all seeds within the pod. The 110W site had significantly higher weevil predation compared with other sites (H = 46.3235, DF = 5, P = <0.0001, Figure 2.2). Fungal infection was present at each site. Fungal infection rate was 11% $\pm$ 0.02 Standard Error for the entire 2009 seed collection. The rate of fungal infection was significantly different among sites, most intense at the 110W site (H = 16.3717, DF = 5, P = 0.0059, Figure 2.2). Pods collected in 2009 had 1.8 $\pm$ 0.1 seeds per pod. Seeds per pod differed among sites with Wire Road and Long Branch having double the number of seeds per pod than 110W (H = 13.2601, DF = 5, P = 0.02, Figure 2.3). Across all sites the seeds

from the 2009 seed collection average seed weight was 10.1 ± 0.2 mg per seed (Table

2.1). Seed weight did not differ among sites ($H = 4.6713$, $DF = 5$, $P = 0.4573$).

Greenhouse Germination

Over half of the seeds successfully germinated in the greenhouse. The type of tray

used had a negligible effect on germination ($F = 1.7619$, $DF = 2,23$, $P = 0.1941$, Figure

2.4). The 2009 germination rates continued to highlight problems with the 110W site.

Cumulative percent germination differed with 110W having half the cumulative

germination of the Wire Road and Long Branch sites ($H = 17.2790$, $DF = 5,72$, $P =$

0.0040, Figure 2.5). A pattern could be seen when comparing the germination rates

between years. Germination rates for the 2009 and 2010 planting peaked from weeks 3-5

(Figure 2.6). Seeds took at least 10 days to germinate; no plants germinated in the first

week. Germination slowed to less than 1% at week 17 for the 2009 planting and at week

8 for the 2010 planting at which monitoring discontinued (Figure 2.6). A comparison of

germination rates indicated no germination differences between years (Table 2.2).

Discussion

Comparative Studies

Weevil predation impacted two sites in this study. The weevil infection

percentage was similar to preliminary reports and other studies on *Baptisia arachnifera*

and related species. My findings of weevil predation ranged from 0-27% and coincided

with a noted 6-57% in 1979 among three sites (USFWS 1984) 0-37% in 2005 and 0-17%

in 2006 (Leege 2007), but are lower than a report of 70% weevil predation from a survey

in 1982 (USFWS 1984). The large difference between this study and the 1982 study may

be due to differences in seed collection time. Seeds collected later in the year may have

allowed more time for weevil predation. My study coincided with other *Baptisia* species studies that showed 26.7% on *B. lactea* (Alstad 2008) and 32% *B. lanceolata* (Horn & Hanula 2004) weevil infection, but was different from a study of 5.4% *B. leucantha* and 64.7% *B. leucophaea* (Haddock & Chaplin 1982).

It is unclear why 110W had the highest weevil predation and fungal infection, but an examination of the area's vegetation and land history may provide answers. The 110W site was unusual compared to other sites as it is next to an open field, paved road mowed for vegetation and within a young planted pine plantation. Other sites are located beside dirt roads with denser vegetation and older pine plantations. The GA Power site may be most similarly mowed and open as the 110W site, yet this study had too few mother plants from that site to indicate any differences.

Seeds per pod and seed mass were similar to other studies on *Baptisia arachnifera*. Wire Road seeds per pod of 2.2 $\pm$ 0.2 and seed weight of 10.1 $\pm$ 0.0mg (N = 24) coincided with findings at a site on the same road reported by Young et al. in 2007 with 2.4 $\pm$ 0.3 seeds per pod and 10.4 $\pm$ 0.5mg seed mass. Uninfected seeds of 110W had similar mass compared to other sites and sites of other studies. The high intensity of weevil predation and fungal infection may explain why the 110W site had a reduced number of seeds per pod. Weevils eating seeds may cause the reduction in seeds per pod for 110W. Young et al. (2007) noted that seed predation by weevils appeared intense on *Baptisia arachnifera* potentially impacting the species. In a related Midwest species, *Baptisia leucophaea* seed predation by *Apion rostrum* promoted pod abortion (Petersen et al. 1998). As a further seed reduction, fungus has been found to kill all seeds within the seed pods of some legumes (Green & Palmbald 1975). Fungal infections can also cause

seed abortion (Drake 1981). The combination of both weevil predation and fungal infection are removing seed from the 110W site.

Observing both weevil and fungus presence bring up the question if there is an association between the weevil and the fungus. Weevils (De Nooij 1998) as well as other insects (Tewksbury et al. 2008b) have been found to act as vectors of fungus. In some systems the fungus outcompetes and starves weevils (Hinckley 1961). The relationship may be separate as predispersal seed predators were found to choose drier seed resources (Hudaib et al. 2010) rather than those under moist conditions that may promote fungal infections (Green & Palmbald 1975). Under moist, rainy conditions *Baptisia arachnifera* has been noted to show high rates of fungal infection even when weevil presence is low (Personal communication John Pascarella, GA). This would suggest weevils and fungus may not have a direct relationship with each other when infesting *Baptisia arachnifera*.

Obtaining Plants for Restoration

This study shows that large numbers of *Baptisia arachnifera* seedlings can be successfully grown in the greenhouse from seed, whereas seedlings are rarely seen in the field. I estimated that without any stratification or other germination stimulation, over half the seeds collected will germinate. The difference in environment may explain why observed germination was high in the greenhouse compared to monitoring the species in the field. Placement into the soil, a regular water regiment, and absence of competition are conditions seeds experienced in the greenhouse that they would not have in nature. USFWS personnel working on germ plasm storage of *Baptisia arachnifera* have reported 90% germination when seeds are cold stratified (Personal communication Peter

Pattavina, GA). A study involving cold stratification on the seeds of *Baptisia arachnifera* may provide a strategy to obtain higher germination rates.

Implications for Management

For initial seed collection, managers may want to collect pods and separate seeds soon after they mature to limit time for weevil predation or fungal infection. *Baptisia arachnifera* seeds taken directly from the field can be expected to have over 50% germination up to a year following collection. Because tray type did not influence germination, maximizing the number of cells can provide the greatest number of seedlings for space provided. Ideal trays may be those that allow easy removal of seedlings from cells for planting. Based on my study, managers can expect the first seeds to germinate within two weeks of planting, with germination peaking in weeks 3-5. After 8 weeks it can be assumed the majority of expected seed germination has occurred. This time scale can be used in preparing seedlings for transplanting of future reintroduction and augmentation projects. Also seeds stored for a year showed no significant loss in viability indicating the seed storage potential.

This study has direct implications for the Hairy Rattleweed Recovery Plan (USFWS 1984). This study proposes a method for obtaining new individuals of *Baptisia arachnifera,* which may be used to restore or augment current population of the species in order to satisfy requirements for species delisting (Recovery Objective 1). This study also provides information on the early life stages of the species as requested by the plan (Recovery Objective 3, Sec. 5). The methods described here can aid managers in the cultivation and storage of the species *ex situ* (Recovery Objective Sec. 4). Germination

(Sec. 516) and effects of weevils and fungus (Sec. 524) are also emphasized within this study (USFWS 1984).

As reports show the population in decline, managing the remaining plants of *Baptisia arachnifera* will be essential in preventing the species extinction. Methods that may alter weevil predation or fungus infection across sites may increase seed yield. Opportunities to reintroduce greenhouse grown plants should be taken advantage of in order to buffer the species numbers until a successful management program for the species is devised and secured. Future investigations may include identifying ways to increase seed germination. This study provides a method for obtaining numerous seedlings, the next step can involve finding an efficient way to transplant the greenhouse grown seedlings back into the species native range.

CHAPTER 3

SHADE & LITTER EFFECTS ON EARLY STAGES OF *BAPTISIA ARACHNIFERA*

Introduction

Humans dominate landscapes to the detriment of native plants. Part of the problem can come from how the landscape is managed. Through forest management, humans have caused the decline of understory species (Watkins et al. 2003, Deal 2001, Paillet et al. 2009). In the United States 203.9 million hectares (67%) of forests are used for timber and 21.7 million hectares (7%) of forests have been planted (United States Department of Agriculture Forestry Service 2001). Forests managed for timber are different from old-growth forests. Examples of these differences include: species compositions such as the number of exotic or weedy species compared to native or slow-growing species (Watkins et al. 2003, Sullivan et al. 2009, Halpern & Spies 1995, Thomas et al. 1999, Paillet et al. 2009), litter load amount and type (Kirby et al. 1998, Vanderwel et al. 2008), and resource cycling where unmanaged forest systems store more carbon than managed forests (Chatterjee et al. 2009). Often, replanted forests are composed of even-aged monocultures. These forests have higher tree density, denser canopy cover, and more leaf litter than natural forests (Lugo 1992). Forest management practices can cause the decline of understory plant species through increased canopy density and increased leaf litter.

Canopy closure within managed forests may be causing the decline of understory species. The forest canopy determines the amount of light reaching understory species (Felix et al. 1983, Espelta et al. 1995, Halpern & Spies 1995, Valverde & Silvertown

1997, Thomas et al. 1999, Franklin et al. 2002, Gillespie et al. 2006). Light affects understory species based on their tolerance to reduced or increased light levels (Gillespie et al. 2006, Small & McCarthy 2002, Lindh 2005). Compared to plants in an open canopy, shade-intolerant plants under shaded conditions have reduced growth, biomass (Small & McCarthy 2002, Galloway & Etterson 2009), and survival rates (Gillespie et al. 2006). In relation to reproduction, shade-intolerant species have reduced flower production under shaded conditions (Lindh 2005, Galloway & Etterson 2009). The opposite can be found for shade-tolerant species. Shade-tolerant plants under full sunlight can have reduced growth, biomass, and survival (Halpern & Spies 1999, Small & McCarthy 2002). After forests have been clear cut, increased light penetration can reduce the amount of water available in the soil, which can hinder shade-intolerant species (Ellensworth & Reich 1992).

Leaf litter load can also influence understory species. Litter can change light, temperature, and moisture in a habitat (Molofsky & Augspurger 1992). As with the amount of light, the effect litter has on understory plants varies based on the species' litter tolerance (Molofsky & Augspurger 1992, Facelli & Ladd 1996, Fowler 1988; Vellend et al. 2000). Leaf litter can decrease seed germination (Cavieres et al. 2007, Facelli 1994, Molofsky et al. 2000, Molofsky & Augspurger 1992, Xiong & Nilsson 1999, Bartuszevige et al. 2007, Xiong et al. 2003) by acting as a physical barrier that prevents seedling emergence and blocks light (Vellend et al. 2000, Foster & Gross 1997). Litter can aid pathogen establishment (Facelli & Ladd 1996) and leach-germination inhibiting chemicals in the soil (Cavieres et al. 2007). Litter can stunt growth or kill plants (Molofsky & Augspurger 1992, Fowler 1988, Foster & Gross 1997) and can

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decrease a plant's chance of flowering (Bloom et al. 2003). Litter has been found to affect plant community competition (Facelli 1994, Xiong & Nilsson 1999) by limiting the density of species (Facelli 1994, Molofsky et al. 2000, Carson & Peterson 1990). In some cases it promotes species diversity by preventing a single species from dominating (Molofsky & Augspurger 1992, Xiong & Nilsson 1999).

No studies have directly indicated that a change in the amount of light and litter has led to the decline of a species that resulted in its listing as an endangered species. Fire suppression has been directly linked to species decline; however, fire removes canopy (Barden & Woods 1976, Bergeron & Brisson 1990, Odion et al. 2004, Veblen 2003) and litter (Crane & Fischer 1986, Emlen 1970, Lemon 1949, Stephens & Moghaddas 2005). A review of United States plant species recovery plans indicated fire suppression as the primary cause of threatened or endangered listing for 4 out of 98 species (Schemske et al. 1994). Another analysis of 723 U.S plant species listed as threatened, endangered, or proposed for listing showed that 20% of the species were harmed due to fire suppression (Wilcove et al. 1998). The return of fire to the prairie habitat of the endangered *Lomatium bradshawii* has helped stabilize populations of the plant (Kaye et al. 2001). The absence of fire may allow reduced light and increased litter to persist causing continued population declines for endangered plant species adapted to fire disturbed habitats.

Endangered plant reintroduction projects may be required when remaining populations are under immediate threat of extinction or additional populations are needed for recovery plan objectives (Kaye 2008). The Endangered Species Act mandates recovery plans (1973), and 72% of 181 endangered plant recovery plans call for some type of reintroduction (Hoekstra et al. 2002, Kaye 2008). Recovery plans often specify a

number of stable populations before the species can be removed from the endangered

species list. Reintroduction projects reestablish populations lost in areas of their previous

species range (Kaye 2008). Defining clear objectives, obtaining numerous individuals of

the species, and effectively reintroducing the species are vital toward recovery success

(Kaye 2008).

The Nature Conservancy bought a property containing "the best population" of

Baptisia arachnifera in December 2008 and placed a conservation easement on the

property in December 2009 to help protect the species (Georgia Department of Natural

Resources 2010). This is the only protected land containing *Baptisia arachnifera* within

its remaining range. Much of the property is made up of timber plantations. Devising a

strategy to reintroduce plants into this habitat will help the restoration of new sustainable

populations of the species that can lead to the species' delisting. Preliminary studies are

required to develop a successful and efficient method for *Baptisia arachnifera*

reintroduction and augmentation into this preserve and within its natural range.

There are no published results on the effects of light and litter on *Baptisia*

arachnifera, yet plants persist along edges of timber plantations, roadsides, and power-

line cuts. In this study I investigate the potential for reintroduction of *Baptisia*

arachnifera into managed timber forests by determining the effects of shade and litter on

seeds and seedlings of the species. The objectives of this study were to: 1) analyze seed

germination and seedling growth of *Baptisia arachnifera* under shade cloth and litter

addition treatments to mimic managed forest conditions in a 2x2 shade and litter factorial

design; and 2) examine and compare the growth of seeds and seedlings planted into two

managed forest plantation types and a power-line cut to observe the effects of light and litter under habitat conditions and the potential for reintroduction.

Methods

Shade & Litter Experiment

A 15m x 19m site was selected within the natural range of *Baptisia arachnifera* in a power-line cut of property owned by The Nature Conservancy in Brantley County, GA (31.33N,81.89W, Figure 1.1) for the placement of 80, 1m x 1m plots, separated by 1m (Figure 3.1). The site was selected within the natural range of the species to follow the home-site advantage hypothesis, a site where the plant has adapted best (Montalvo & Ellstrand 2000), and within the area where reintroduction efforts would occur. Plots were divided into four quadrants, a randomly selected *Baptisia arachnifera* individual, grown in the Georgia Southern University greenhouse for seven months (see Chapter 1: 2009 greenhouse planting), was planted into the center of each quadrant on May 2010 (Figure 3.2). Plants were watered every other day for two weeks, with at least two plants in every plot remaining before the treatments were initiated. On June 10, 2010 seed baskets and treatments were added to each plot. I buried two seed baskets in the center of each plot (Figure 3.2) that measured 12cm x 12cm x 3cm (length x width x height), open at the top and constructed out of fiberglass mesh (Figure 3.3). For each seed basket I dug a hole, placed the seed basket into the hole, and replaced the removed soil within the seed basket. I buried seed baskets below the surface with sides of the mesh basket slightly protruding from the soil. Into one basket of each plot I planted 20 seeds. The other seed basket acted as a seedless control to determine if seeds were present within the soil. Seeds

were planted just below the surface of the soil as a 5 x 4 seed grid (Figure 3.2) within the basket.

The shade and litter treatments for the plots followed a 2x2 factorial design: 1) control with no shading, no litter (S^-L^-); 2) shading, no litter (S^+L^-); 3) shading, litter (S^+L^+); 4) no shading, litter (S^-L^+). Shade cloth addition was designed similar to those used within another study, which used painted glass houses to test the shade tolerance of plant species (Portsmuth & Ninemets 2007). Instead of painted glass, shade cloth was used to test shade tolerance (Vandenberghe et al. 2008). Also shade cloth allows better air and moisture flow than solid glass. Shade cloth structures were constructed using a PVC framed cubed, 1m x 1m x 0.8m tall apparatus covered with green, 70% Easy Gardener Inc. shade cloth for shaded plots (Figure 3.4). The shade level was selected based on previous literature on longleaf pine ecosystems that indicated light reduction under full canopy closure ranged from 57%-80% (Brockway & Outcalt 1998, Palik et al. 1997, Battaglia et al. 2003). At the base of each shade cloth apparatus was a 0.2m gap to allow for airflow (Portsmuth & Ninemets 2007). To determine the amount of litter to be used for the litter treatment, ten 1m x 1m plots were sampled from within a 15+ year old, closed canopy, pine plantation in Wayne County, GA using a Soehnle 5kg scale. Average litter load per square meter was 2.66 ± 0.1kg SE. For litter treatments 2.5kg of pine tree leaf litter, collected from a local managed pine forest, was scattered across the plot. Treatments were randomly assigned to plots, with 20 replicates plots per treatment (Figure 3.1). Survivorship was examined to determine differences among treatments. To examine seedling growth, I measured total length of all stems and branches and summed them for a total length (cm) and I counted the number of leaves for each plant. I recorded

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the number of seedlings present in seed baskets monthly. This variable was used rather than germination as there was uncertainty whether seedlings observed had survived from the previous month, or those observed in the previous month had died and new seedlings had germinated.

Habitat Planting Experiment

Twelve sites were selected on timber company property within 4km of the remaining natural range of *Baptisia arachnifera* in Wayne and Brantley Counties for the seedling transplanting study (Figure 1.1). I used a three (habitats) x two (litter) factorial design. The sites were selected based on four replicates of three habitat types: 1) planted pine stand of trees over 15 years of age; 2) planted pine stand of trees 5-10 years of age; 3) power-line cut without trees. Sites were selected because: 1) they would not face disturbance for an extended time period (i.e. the timber or power company would not harvest, thin, or spray chemicals into stands); 2) the sites represent potential locations for *Baptisia arachnifera* plant reintroduction. These habitat types cover much of the remaining natural range of *Baptisia arachnifera*. From November 20-24, 2010, 40 ten-week old *Baptisia arachnifera* seedlings grown in the Georgia Southern University greenhouse (see Chapter 2: 2010 greenhouse planting) were planted into each of the 12 sites. The seedlings were "hardened off" by being placed outside for a week before planting. Seedlings were randomly selected for planting in 1m x 1m plots within a 6m x 8m grid at each site (Figure 3.5). Half of the plots at each site were randomly assigned the litter treatment: 2.5kg of pine litter scattered within the 1m x 1m plot. As with the shade and litter experiment, seedlings were measured monthly for summed stem and branch length and number of leaves.

In March 2011, I placed 10 seed baskets randomly into each of the 12 sites, as above (Figure 3.5). Five seed baskets acted as a seedless control, 20 seeds were placed within the other five baskets. I examined seed baskets for germination and number of seedlings present monthly from March to August 2011. Seedlings were mapped and marked with a colored pipe cleaner placed beside the plant to distinguish it from other and future emerging seedlings.

In each site I examined light levels on days of clear skies, August 11 & 13, 2011, between 11:00AM and 1:00PM. I measured light availability in photosynthetically active radiation (PAR) at each site with a Model PAR-80 AccuPAR ceptometer (Decagon Devices®, Inc., Pullman, WA). I took ten measurements for each site (Figure 3.5). The AccuPAR ceptometer has 80 sensors along a ~1m long bar to measure photosynthetically active radiation between the 400-700nm waveband of the spectrum of sunlight. I took 10 measurement using a convex densitometer at breast height to measure percent canopy cover within each site.

Statistics

Statistical tests were conducted with JMP® 8.0 (2009). Assumptions were tested using Goodness of Fit to determine normality, and Levene test to determine equal variance. To compare seedling survival in the shade and litter experiment, a χ^2 test was analyzed. The model analyzed treatment and survival status yes or no by frequency of survival yes or no. To compare seedling growth in the shade and litter experiment, the initial design was to use a repeated measures analysis across the months of the study. Instead the final month was examined as this indicated a period where plants were no longer dying due to being transplanted and were not dying back for the winter. Data were

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analyzed using a 2-way mixed model ANOVA. The model analyzed the X factors: shade, litter, shade*litter, and plots to remove plot variation by the Y factors: average summed stem and branch length for each plot, and leaf average number for each plot. To meet assumption, summed length data were log transformed and leaf number data were square root transformed.

My initial design called for repeated measures analysis, but with limited seed germination in many plots of the shade and litter experiment, and assumption of normality and equal variance violated, the Scheirer–Ray–Hare test (Sokal & Rohlf 1995, Dytham 2003) was used to compare cumulative number of seedlings within seed baskets of each treatment over the entire experiment.

The nonparametric Kruskal-Wallis test was used to determine habitat differences in light and canopy cover using the ceptometer and densitometer readings (N=120, 10 readings per site, for each instrument).

Tukey-Kramer HSD test was used to compare means between treatments.

Results

Shade & Litter Experiment

Overall transplanted seedling mortality was high and germination was low throughout the study. Overall transplanted seedlings in the shade and litter experiment showed 40% survival, with no difference among treatments (χ^2=2.435, DF=3, P=0.49, Figure 3.6). Examining growth using summed stem and branch length indicated no differences among treatments (Table 3.1). Examining growth using leaf count indicated no differences among treatments (Table 3.1). At the end of the study, 21% (67 plants) of the 320 planted seedlings remained alive after 16 months.

The germination and resulting seedlings present benefited when cover, shade and/or litter, was present. The more cover applied to seeds resulted in increased seedlings present. Shading had a significant effect on germination (H=21.62717, DF=1, P=<0.00001), litter had a significant effect on germination (H=6.146637, DF=1, P=0.013), and the interaction was not significant (H=0.633004, DF=1, P=0.43, Table 3.2, Figure 3.7). The S^+,L^+ treatment had the most seedlings present; followed by S^+,L^-; then the S^-,L^+; finally S^-,L^- with the lowest. No seedlings were observed within control baskets suggesting that seeds in the treatments were not from a seed bank within the soil of the study site. All germinated seedlings died by the end of the study.

Habitat Planting Experiment

In forested plots, photosynthetically active radiation was found to be reduced by 65% from that found within open canopy power-line cuts. The 5-10 year old plots and the 15+ year plots were similar with restricted light, but were different from the canopy-absent power-line cut sites (H = 75.44, DF = 2, P = <0.0001; Figure 3.8). A 65% reduction validates our use of 70% shade cloth for the shade and litter experiment.

The 5-10 year old plots and the 15+ year plots were found to have a similar percent of canopy closure, but differed from the canopy-absent power-line cut sites (H = 82.5016, DF = 2, P = <0.0001, Figure 3.9).

Seedlings planted into the 12 sites were dormant over the winter, reemerging in March 2011. Mortality was high among the transplanted seedlings. As the ceptometer and densitometer measurements indicated no difference between the 5-10 year and 15+ year aged tree stands (Figure 3.8 & 3.9), results were combined for planted pine stands. No transplanted seedlings reemerged within the power-line habitat throughout the study, and

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few seedlings reemerged in the planted pine stands. The maximum observed seedlings across all sites was 16 (3% of the 480 planted) in March 2011. By August 2011 seedling numbers were down to 4, <1%. Plant survival was too low to compare among habitats using statistical methods.

Seeds in seed baskets fared poorly in this experiment. Twelve seeds germinated (1%) of 1200 planted. Ten seeds germinated from within the power-line cut sites, two seeds germinated from the 15+ year pine tree stand sites, and none were observed within the 5-10 years pine tree stand sties. Seedling germination was too low to compare among habitats using statistical methods. All planted seeds within the twelve sites died by the end of the experiment.

Discussion

Manipulated Shade & Litter Effect on Growth & Germination

Shade and litter did not influence *Baptisia arachnifera* seedling growth in this study. Other studies indicate shade and litter may cause plant mortality or reduced growth (Galloway & Etterson 2009, Gillespie et al. 2006). Used as a measurement of growth in my study, the number of leaves can represent a convergence response, plants acclimate to the environment (Givnish 1988). As sunlight is a resource essential to plant growth, a reduction of this resource was expected to reduce growth. Studies suggest that other plant parts such as roots should be taken into account to represent plant growth (Givnish 1988). In this study root measurements would not have been feasible as this would have led to plant mortality.

Germination was affected by the amount of cover, shade and/or litter. Although germination was observed, none of the seedlings remained alive until the end of the

study. This may be the result of the intense summer conditions that dried the soil and caused seedling mortality. Litter maintains more moisture in the soil to aid plant germination and survival (Facelli & Pickett 1991); therefore moisture may explain why seedling presence within baskets was greater in covered treatments. The lack of rain probably caused seedling mortality. Determining there was no difference in the survival of plants used in the light and litter experiment suggests that when seedlings become established, shade and litter does not show an effect on seedling survival, although mortality was seen within all treatments. A study with an increased watering regime to aid plant survival would be the next step toward a successful reintroduction strategy.

Shade & Litter Effect on Growth & Germination of Transplanted Seedlings

The 5-10 year aged stands had the same light and percent canopy cover measurements as stands of 15+ year age. This indicates canopy closure occurs early within these managed forests. Canopy closure of other tree species can vary as early as 15 (Felix et al. 1983, Espelta et al. 1995) to 20-25 (Cattelino et al. 1979, Franklin et al. 2002) to 28 (Halpern & Spies 1995) years. At a smaller scale, gaps that develop in the forest have been found to close in nine years (Valverde & Silvertown 1997). When present within the ecosystem, pine often dominates the canopy in forest succession (Pessin 1933, Cattelino et al. 1979, Felix et al. 1983). The light measurements of the pine plantation in this study were lower than those of natural longleaf pines forests (Brockway & Outcalt 1998, Palik et al. 1997, Battaglia et al. 2003). This was expected as pine plantations have more closed canopies due to higher tree density than that of natural forests (Lugo 1992). I found that mortality is high when individuals are introduced into

either completely open or closed canopies. A look at an intermediate light level would be the next step to determine if light has an effect on *Baptisia arachnifera*.

Intermediate light levels can occur within natural pine communities. Lightning strikes, wind, and suppression are the natural killers of longleaf pines (Palik & Pederson 1996). As trees die, gap openings develop in the canopy (Palik & Pederson 1996). Fires burn unevenly through forests causing irregular canopy openings (Palik & Pederson 1996, Ford 2010). Irregular openings allow for patches of canopy that are not completely open or closed (Palik & Pederson 1996, Ford 2010). This type of habitat may result in increased *Baptisia arachnifera* survival. Although this study used both extremes of canopy cover, canopy closure was not expected to occur as early as measured in the 5-10 year tree plots. The return of fire to the area may benefit *Baptisia arachnifera* by providing forests gaps with intermediate light levels.

Comparison of Techniques

Transplanted seedling survival and seed germination was poor in all habitats. The habitat-planted seedlings had lower survival rates than those of the shade and litter experiment. This is probably due to a difference in watering regime or timing of planting between the studies. The shade and litter seedlings received more water and more survived. Habitat introduced seedlings were planted as winter began. Seedlings may not have had enough stored resources or become established before dying back to survive the winter. Seed germination was probably influenced by water as there were few rain events during our summer study.

Responses of Reintroduced Endangered Species

The reintroduction of *Baptisia arachnifera* in this study shares similarities with other reintroduction projects. Germination and survival of other endangered species have benefitted from the presence of litter with increased germination and survival (Jõgar & Morora 2008). Similar *to Baptisia arachnifera*, other endangered species such as *Abronia macrocarpa* have exhibited germination with high seedling mortality (Goodson & Williamson 2011). Although germination occurred within some treatments of the study, additional factors must be responsible for *Baptisia arachnifera's* decline. Where introduction of *Baptisia arachnifera* showed low seedling survival, other reintroductions have had great success (McLoughlin & Vajda 2005, Yadav et al. 2009). Although all sown seeds of *Baptisia arachnifera* died by the end of the study, seeds of other endangered species within their native range have successfully reached maturity (Davis et al. 1999). In my study one-fifth of seedlings planted within the shade and litter experiment survived. The survival rates of transplanted seedlings have varied in other reintroductions. Examples include 60% survival for *Lilaeopsis schaffneriana* (Titus & Titus 2008) and 90% survival for *Argyroxiphium kauense* (Moriyasu & Robichaux 2003).

Low recruitment is not always responsible for the decline of endangered species. When in a stable environment, endangered species can have stable populations with low recruitment when mortality is low such as with *Liatris ohlingerae* (Weekley et al. 2008). In the case of *Baptisia arachnifera*, poor recruitment within an area of high mechanical disturbance from development may lead to high plant mortality (USFWS 1984). Protecting *Baptisia arachnifera* from such a disturbance may allow the species to recover. Until then, reintroduction projects can help maintain the species persistence.

Recovery Plan

The study addresses objectives outlined in The Hairy Rattleweed Recovery Plan (USFWS 1984). Seed and seedling methods of reintroduction and its application to various habitats were examined (Sec 2.1, Sec 3.2). The study does provide new information on the early life stages of the species (Sec 5.1). Conclusion of this study has added 50+ *Baptisia arachnifera* plants within The Nature Conservancy protected land, an initial test of reintroduction (Sec 5.1.2). This study also addressed the species germination within the field (Sec. 5.16). This study examined light in association with the species (Sec 5.2.2) and litter (Sec 5.2.4). I expect that these findings will be useful to future managers of the species if a restoration project developed for *Baptisia arachnifera* (Sec 5.3.4).

Management Implications

This study provides managers with information they may utilize for *Baptisia arachnifera* restoration projects. Many seedlings planted into habitats may initially die. Although mortality was observed throughout the study, some established plants have remained and look quite well. Continued monitoring of the planted seedlings in the following years will indicate how successful the reintroduced plants are performing and if plants will obtain reproductive maturity. Plants can be successfully replanted within their natural site, but currently not with high rates of survival. A study increasing seedling survival can provide support for large scale restoration projects. Sites with full canopy closure or full sunlight, within pine stands of 5+ years or in power-line cuts should not be considered extensive reintroduction habitats for the species. This study placed plants in the center of each habitat type; perhaps a study in reintroducing plants to habitat edges

may produce more favorable results. If a supply of seedlings can be maintained for use

for future restoration studies, a successful method for maintaining the species existence

can be developed.

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Table 2.1 Summary of *Baptisia arachnifera* seed collection and germination data. Values are mean ± SE of data values at the plant level. Numbers in parentheses are range of measured values at the plant level. Seeds were pooled for the 2010 Greenhouse Planting, SE could not be calculated at the plant level.

Sites	Mean Seed Weight (g)	N (Without Fungus)	2009 Greenhouse Planting Germination (%)	2010 Greenhouse Planting Germination (%)
2009				
Wire Road	0.011 ± 0.0 (0.0071 – 0.015)	23	66.4 ± 3.2 (18.2 – 88.7)	45.9
Long Branch	0.010 ± 0.0 (0.0068 – 0.015)	23	61.1 ± 4.2 (6.67 – 93.2)	50.4
E3 Tom Dan Harper	0.009 ± 0.0 (0.0058 – 0.012)	14	52.4 ± 4.1 (28.6 – 77.1)	49.2
E2 Oil Well Road	0.010 ± 0.0 (0.0082 –0 .010)	6	50.2 ± 5.5 (30.8 – 69.2)	17.9
GA Power	0.007 ± 0.0 (0.0054 – 0.0095)	2	34.3 ± 20 (14.3 – 54.3)	- -
Hwy 110 W	.010 + 0.0 (0.0072- 0.013)	10	37.9 ± 8.1 (0 – 71.43)	- -
Total seed collection	**0.010 ± 0.0** **(0.0054 – .015)**	**78**	**56.6 ± 2.3** **(0 – 93.2)**	**47.6** **(17.9 – 50.4)**

Table 2.2 Statistics table for Kruskal‑Wallis comparison of *Baptisia arachnifera* greenhouse germination between 2009 and 2010 from seed collected from four sites: Wire Road, Long Branch, E2, and E3.

Source of Variation	SS	df	MS	H	P Value
year	2048.192	1	2048.192	1.909887	0.166976
site	4986.067	3	1662.022	1.549794	0.670827
Y*S	11489.22	3	3829.739	3.571134	0.311653
Within	99442.85	105	947.0748		
Total	120110.5	112	1072.415		

SS=sum of squares, df=degrees of freedom, MS=means squared, H=test statistic, P Value=the statistic representing the probability as extreme as the observed assuming the null hypothesis is true with 0.05 representing a statistically significant difference.

Table 3.1 Statistics table using ANOVA for seedlings growth using summed stem length and leaf count within the shade & litter experiment. F=test statistic.

Shade and Litter Experiment			
Sum Length Square Root Transformed			
Source	F	df	P Value
Shade	0.0488	1	0.82
Litter	1.2618	1	0.27
Shade*Litter	1.1316	1	0.29
Leaf Count Log Transformed			
Source	F	df	P Value
Shade	2.773	1	0.1
Litter	0.2964	1	0.59
Shade*Litter	1.08	1	0.3

Table 3.2 Statistics table for seed germination within the shade & litter experiment.
* represents a statistically significant difference.

Source of Variation	SS	df	MS	H	P Value	
S(+,-)	10260.45	1	10260.45	21.62717	<0.0001	*
L(+,-)	2916.113	1	2916.113	6.146637	0.013	*
S*L	300.3125	1	300.3125	0.633004	0.43	
Within (Error)	24002.63	76	315.824			
Total	37479.5	79	474.4241			
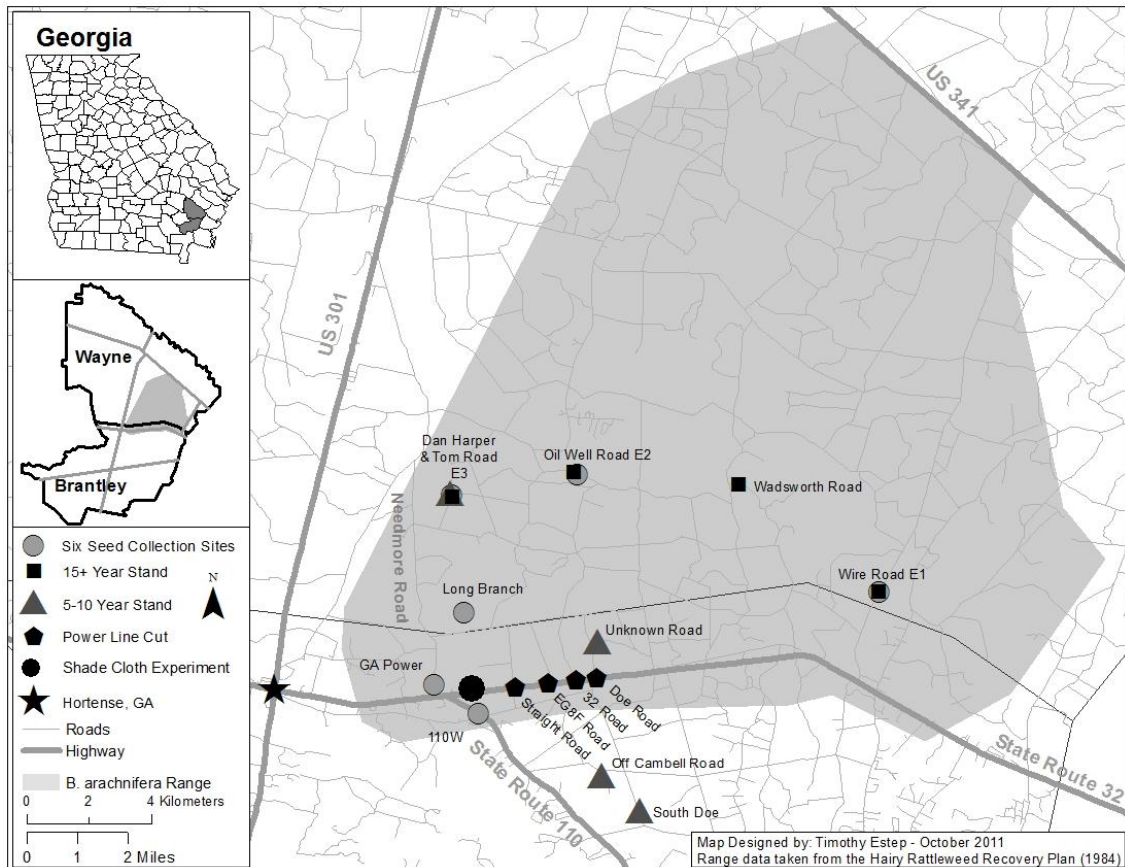
Figure 1.1 Map indicating 2009 seed collection, shade and litter experiment, habitat experiment study sites, and *Baptisia arachnifera* range. Range as described in the 1984 Hairy Rattleweed Recovery Plan (USFWS). Range on map represents an area larger than that reported by Isely (1998).

Figure 2.1 Photo of three tray types with *Baptisia arachnifera* seedlings planted from the 2009 seed collection (Left: Cone-Tainers™; Middle: large styrofoam tray; Right: small styrofoam tray). Counting cells from left to right along one row of the trays totals 21 cells.
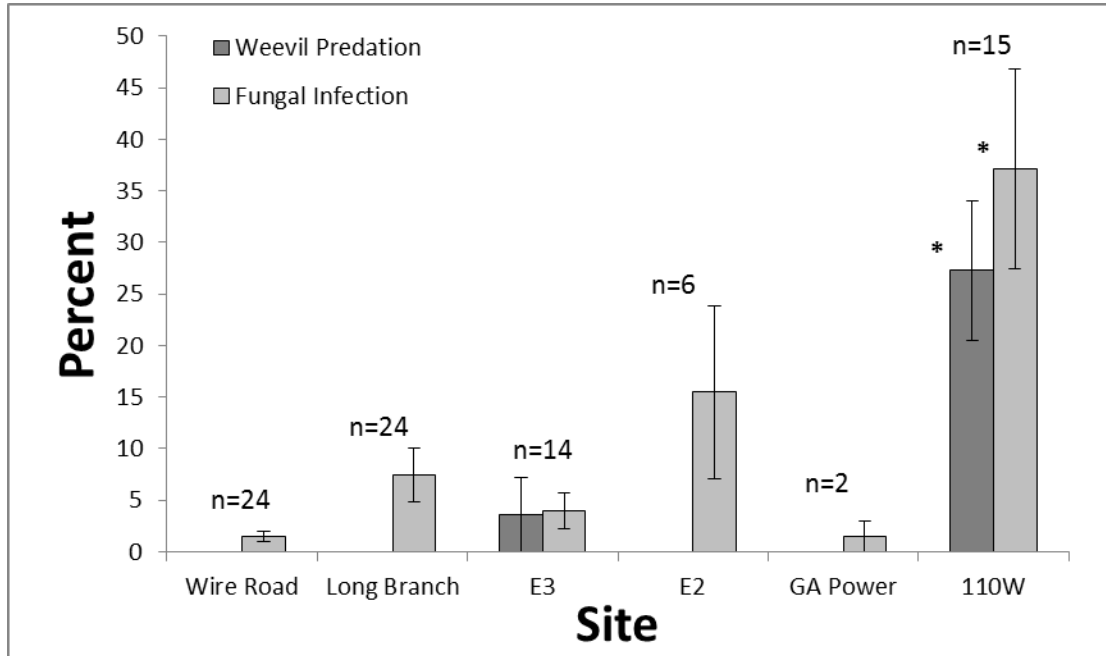
Figure 2.2 Percentage of pods with weevil predation and seeds with fungus infection in six collection sites ($\pm$ SE) n = mothers per site. The 110W site had statistically significant differences among other sites for both weevil predation and fungal infection.
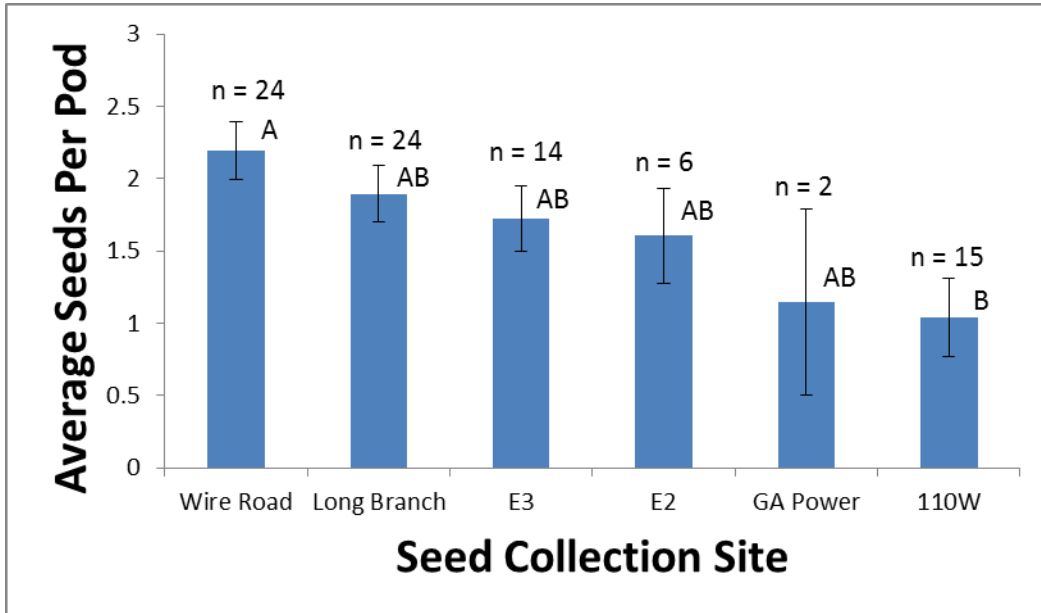
Figure 2.3 Average seeds/pod in six collection sites ($\pm$ SE) n = mothers per site. Different letters indicate differences among sites.
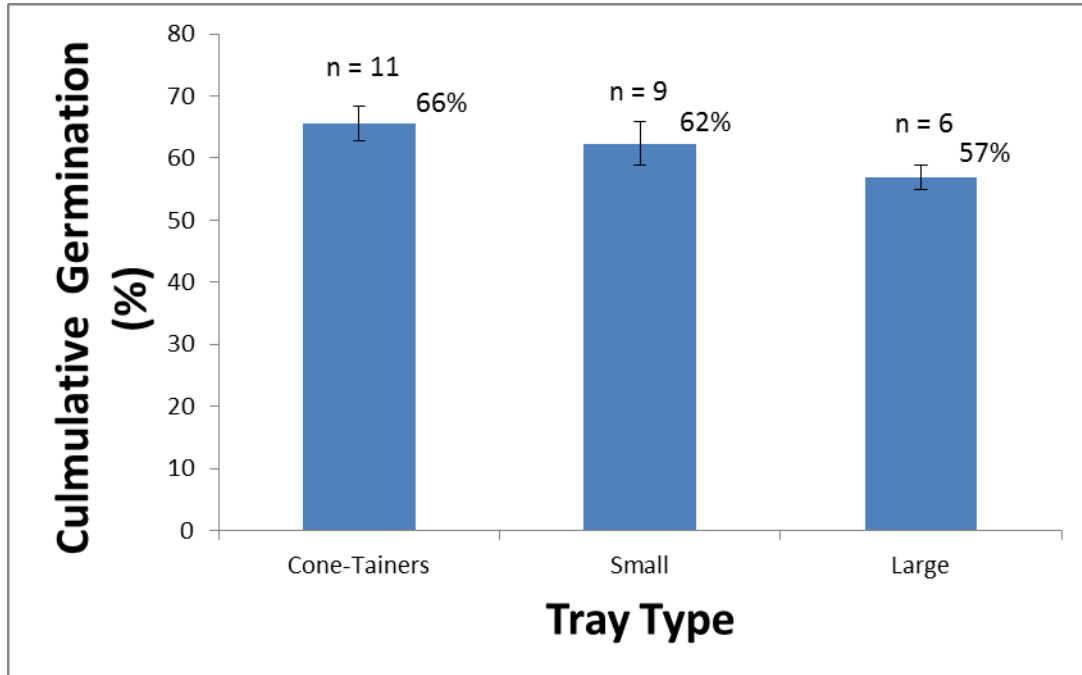
Figure 2.4 Mean percentage of seed germination within three tray types ($\pm$ SE) n = number of trays. Germination did not differ among tray types.
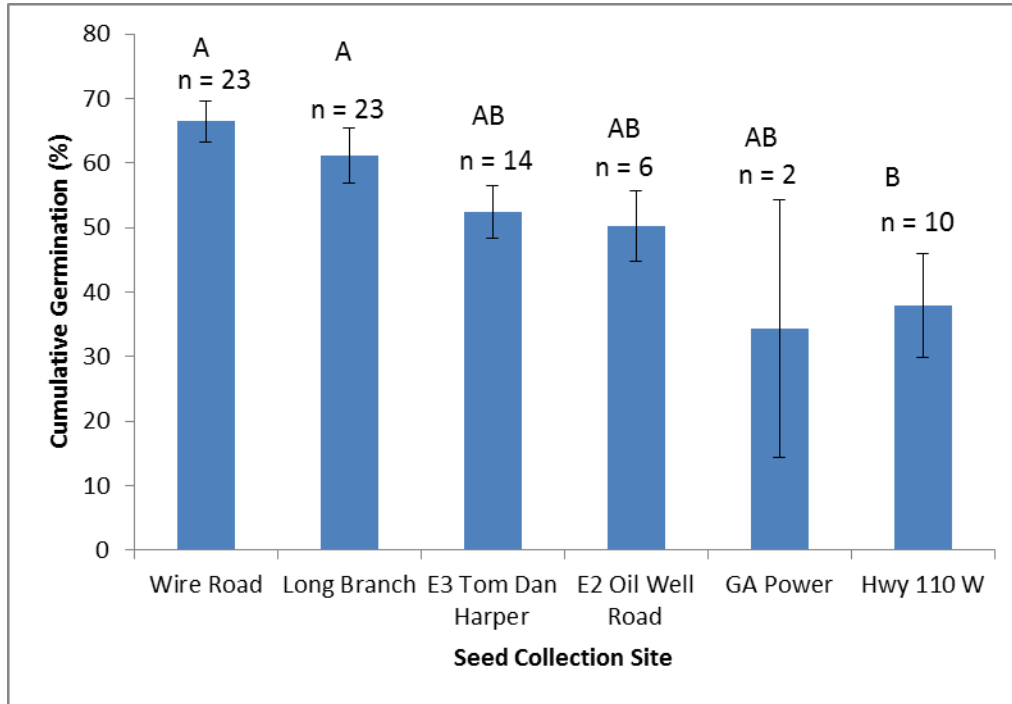
Figure 2.5 Comparison of germination of the 2009 greenhouse planting among sites (Table 2.1) (± SE) n = mothers per site. Different letters indicate differences among sites.
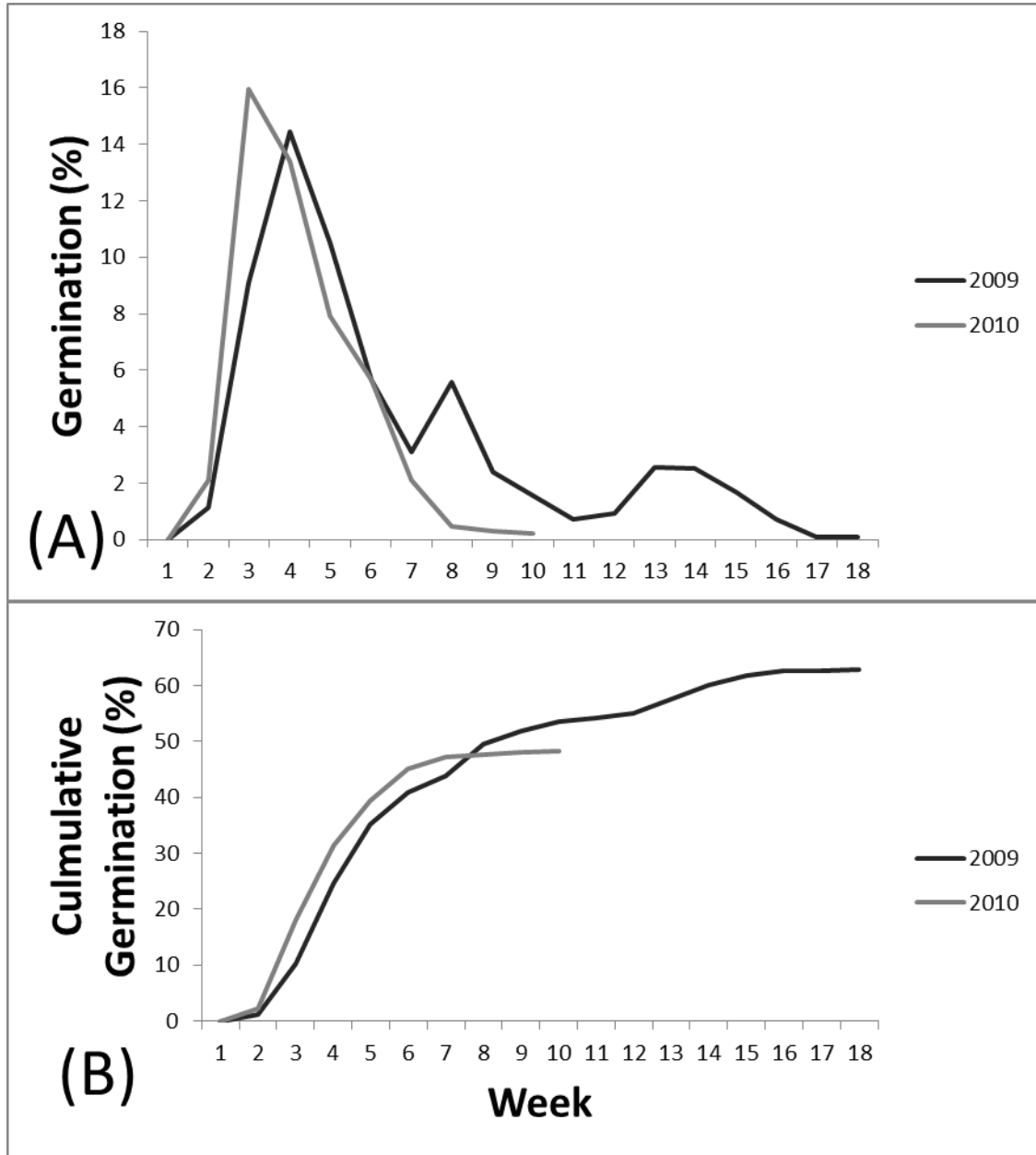
Figure 2.6 Comparison of greenhouse planting between years 2009 & 2010. **(A) Top:** weekly germination over the 18 week period. **(B) Bottom:** cumulative percent germination over the 18 week period.

*Note: Differences in scale.

*Note: 2010 greenhouse germination was monitored for 10 weeks.
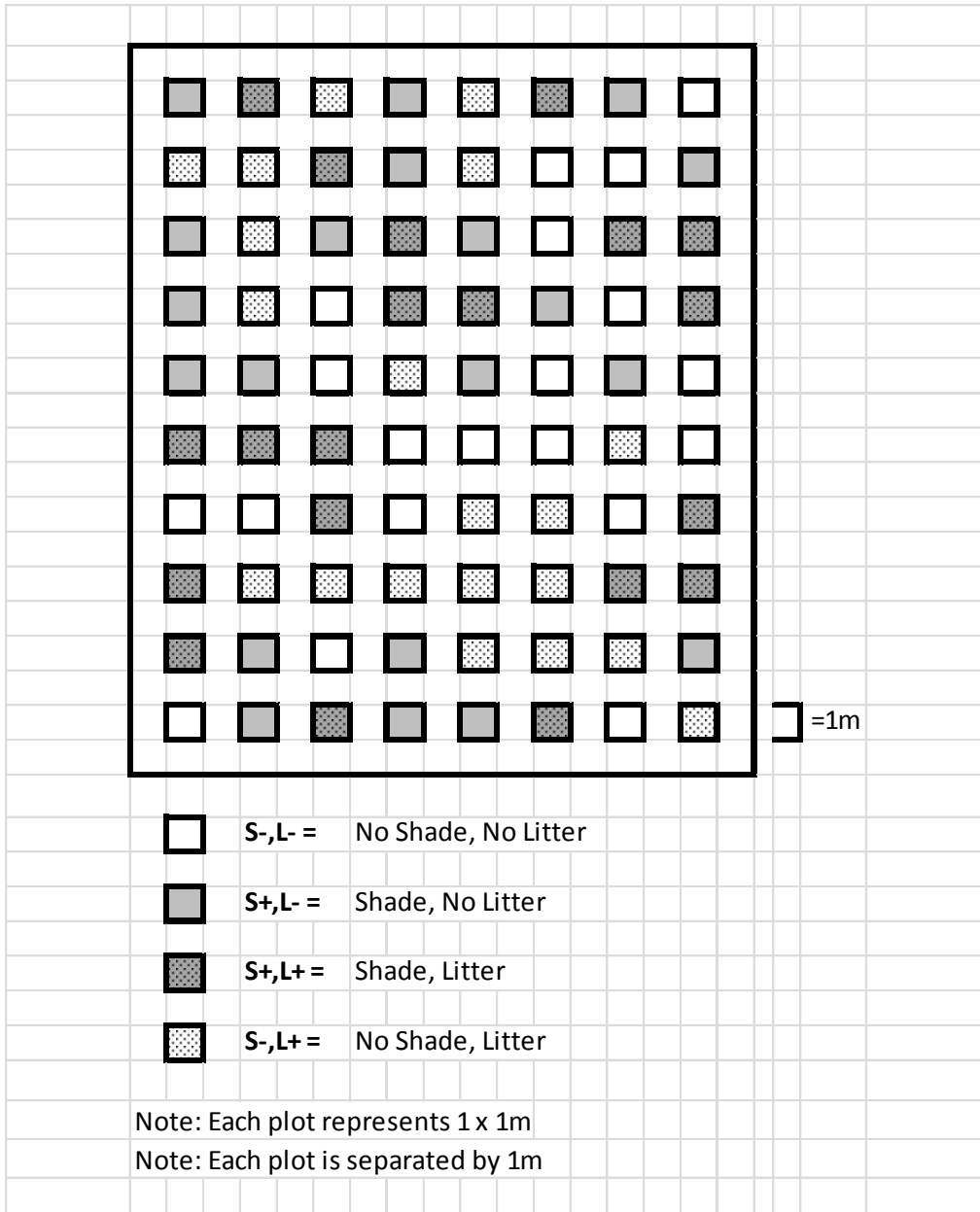
Figure 3.1 Distribution of 80 1x1m plots used in the factorial design for the study of shade and litter effects.
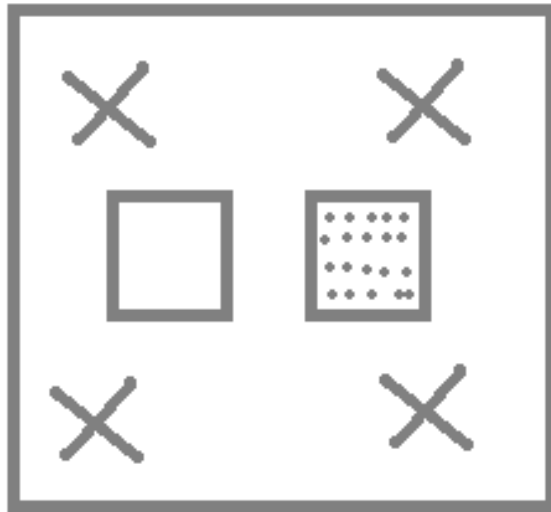
Figure 3.2 Shade and litter experiment plot design.
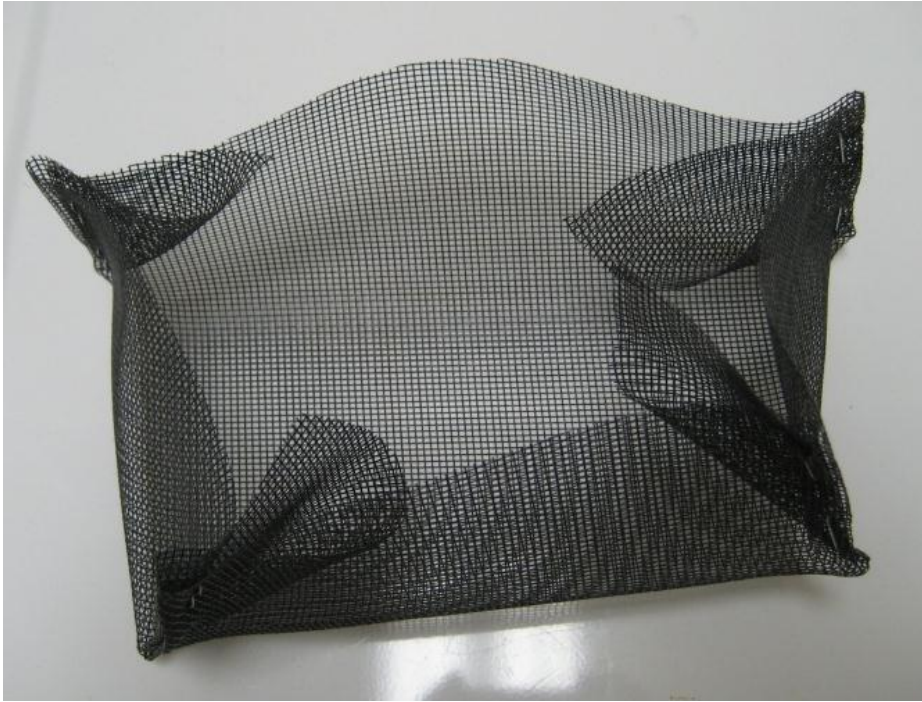
Figure 3.3 Photo of a seed basket used throughout the study. Seed basket dimensions were 12cm x 12cm x 3cm (length x width x height).

Figure 3.4 Left: Photo of the shade and litter experiment within The Nature Conservancy Property. Note the shade cloth apparatuses and the power-lines. **Right:** Photo of an opened shade apparatus revealing the litter treatment within.
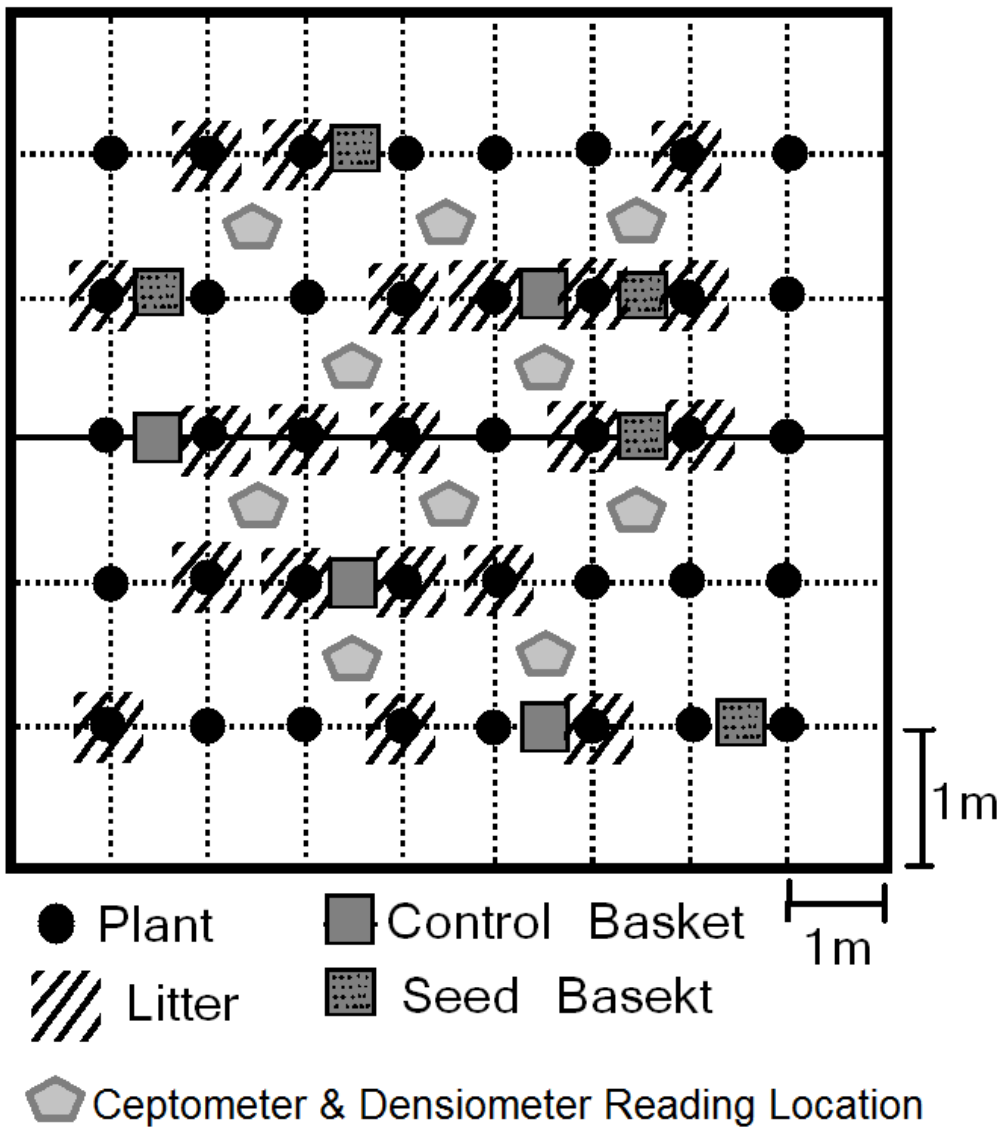
Figure 3.5 Diagram of seedlings and seed baskets placement, and ceptometer and densiometer reading locations within the 12 transplanting sites.
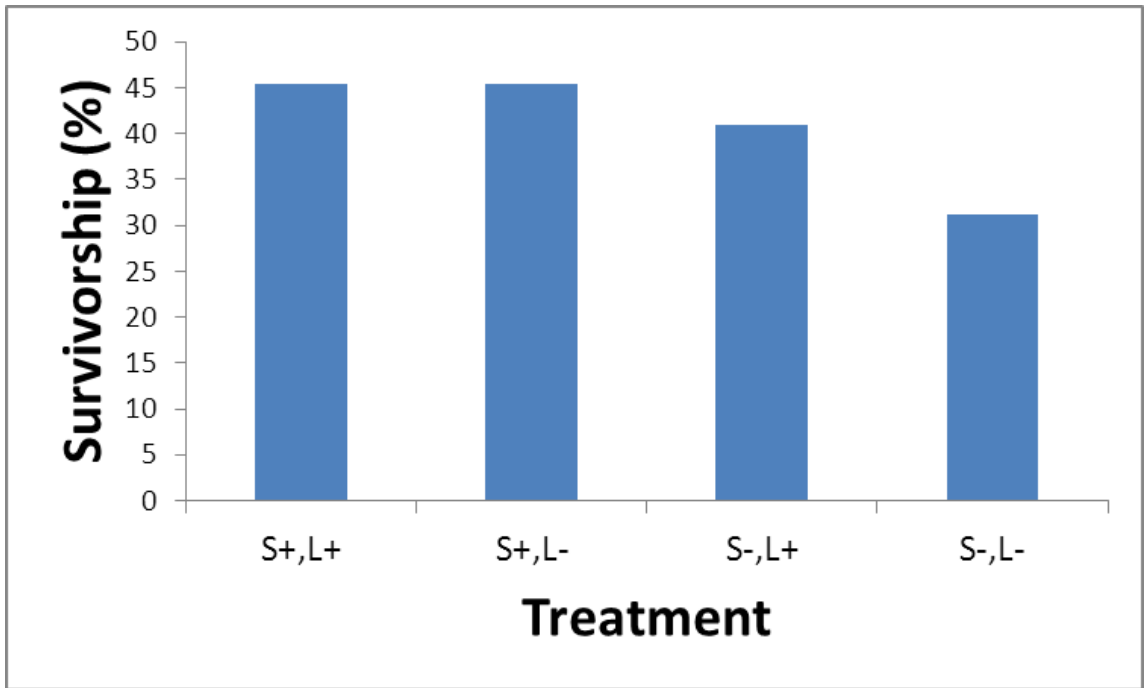
Figure 3.6 Survivorship of transplanted seedlings in the shade and litter experiment after treatment addition in June 2010 until the end of the study in August 2011.
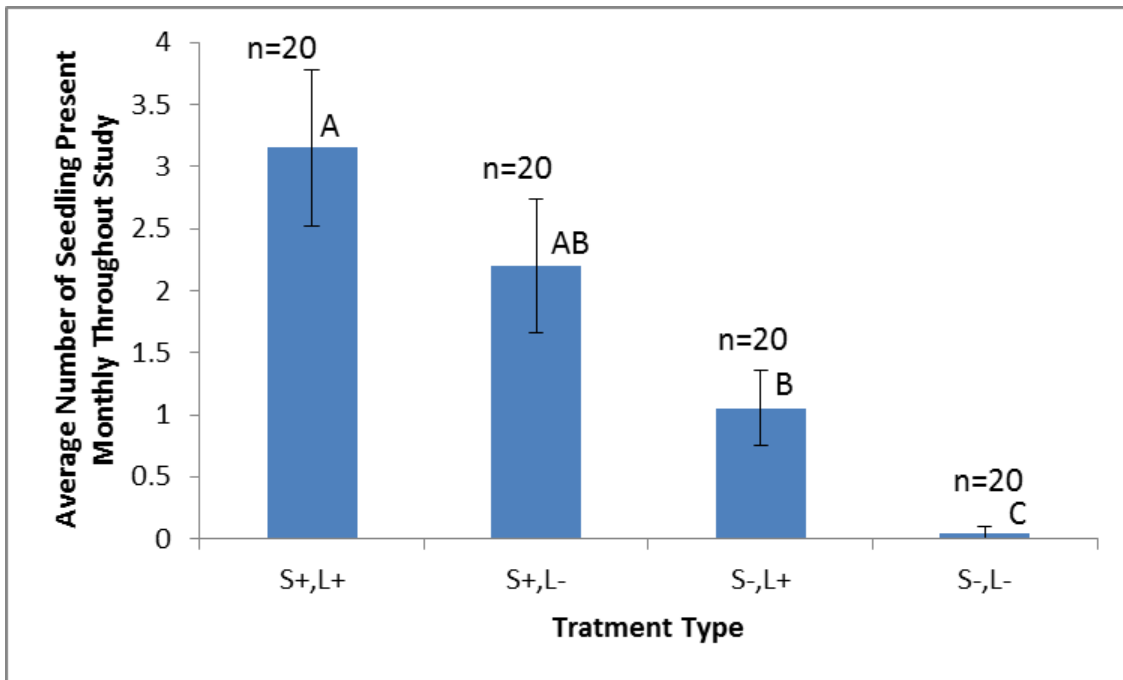
Figure 3.7 Average seedlings present within the shade cloth experimental plots ($\pm$ SE) n = number of plots. Statistics found in Table 2.2. Letters indicate differences among treatments.
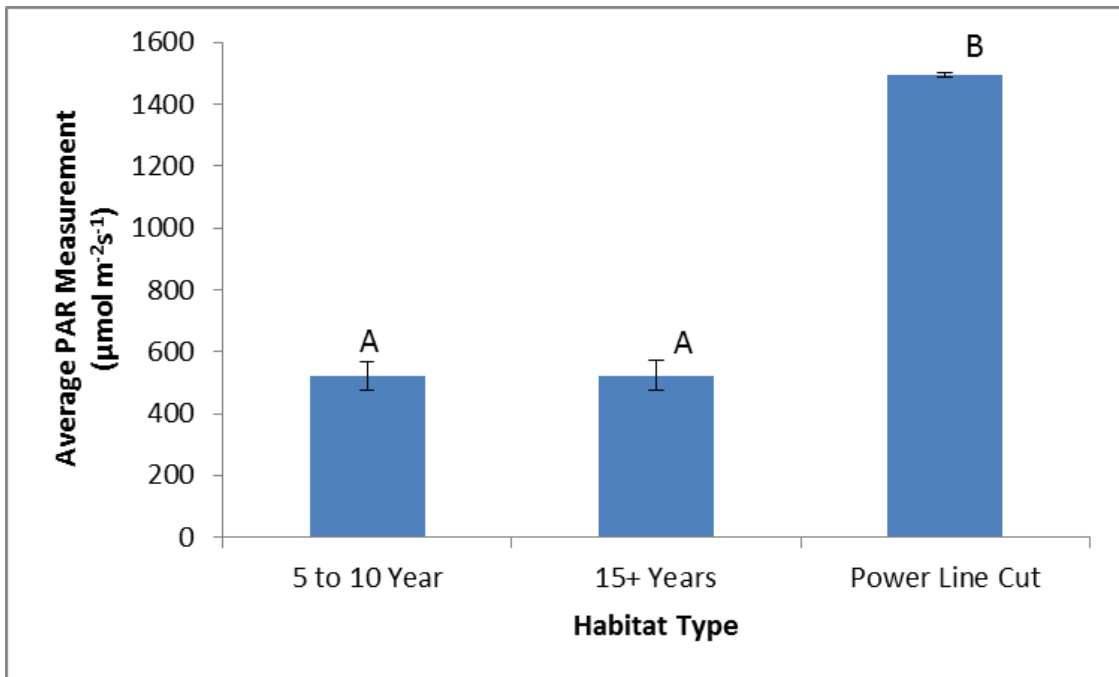
Figure 3.8 Mean PAR light measurements among different habitat types as measured with a ceptometer (± SE). Letters indicate differences among treatments.
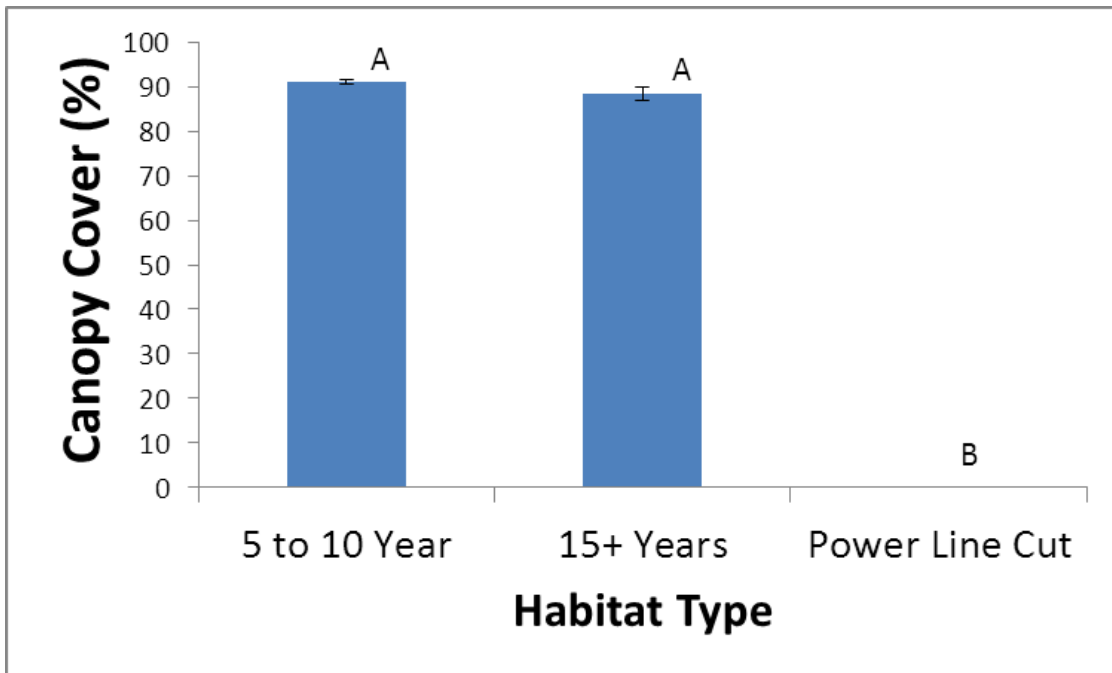
Figure 3.9 Mean percent canopy cover among different habitat types as measured with a densiometer ($\pm$ SE). Letters indicate differences among treatments.