Factors affecting safe-guarding of the federally endangered *Schwalbea americana* L. (Orobanchaceae)

by

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Abstract

Schwalbea americana L. (American chaffseed) is a federally endangered hemi-parasitic plant (USFWS 1992). Its tendency to persist with limited recruitment and threats to its habitat make it vulnerable to extirpation. Additionally, any attempt to safe-guard a population of *S. americana* is made difficult by the fact that no clearly defined protocol exists for propagating and out-planting, with published studies reporting limited success. The goal of this research is to improve future conservation efforts of this rare species.

The chapters in this work describe studies with the following goals. Chapter 1 describes the historic abundance, species biology and current status of *S. americana*. Chapter 2 uses the size class and reproductive status of plants at the one known natural site in Alabama from 2010 to 2016 to describe current trends and present short-term projections of site viability. Chapter 3 uses shade huts to examine the effect of three shade levels and the size and presence of host plants on *S. americana* size in an attempt to improve propagation protocols and out-planting site choice. Chapter 4 describes three preliminary studies aimed at improving future out-planting efforts. Preliminary Study 1 compares five host species by number, size and number of leaves of attached *S. americana*. Preliminary Study 2 examines the effect of fertilizer on *S. americana* size with and without a host present. Preliminary Study 3 assesses the ability of *S. americana* to regrow from cuttings. It also assesses the effect of smoke on regrowth of cuttings and on regrowth of the original seedlings following removal of aboveground biomass.

An assessment of demographic trends of the one known *S. americana* site in Alabama is described in Chapter 2. If the observed trends persist there will be a minor reduction in number of active individuals of *S. americana* at the site in 2017 but a slight increase in number of reproductive individuals. However no definite conclusions can be drawn as to the long-term viability of the site. An apparent

dormancy or extirpation of *S. americana* from much of the site occurred between 2008 and 2010. If *S. americana* has been extirpated from numerous locations at the one known Alabama site it is at increased risk of local extinction, which is of conservation concern. Long-term monitoring is needed to shed light on the viability of the known *S. americana* site in Alabama.

The effect of shade on *S. americana* size was examined in Chapter 3. The first study in this chapter included a host presence/absence treatment with *Pityopsis graminifolia* (Michx.) Nutt. as the host and two shade levels. No effect of shade or host presence/absence was detected. When only *S. americana* individuals with a host present were included, *P. graminifolia* was correlated with increased *S. americana* height and leaf size. This suggests that a host's increased ability to provide nutrients improves parasite health. The second study did not include a host treatment and examined three shade levels: no shade, 33% shade and 61% shade. The 33% shade treatment yielded *S. americana* with significantly greater height than those grown without shade and stems with more nodes than those grown in 61% shade. Among the three shade levels 33% shade is recommended for propagation and selection of out-planting sites over the other two.

Three preliminary studies are described in Chapter 4. Study 1 examined *S. americana*'s response to five host species. Attached clusters of greenhouse-grown *S. americana* and its hosts were unearthed and measured. *Eupatorium capillifolium* (Lam.) Small was found to have more attached *S. americana* than three other host species. It was also found to have attached *S. americana* with significantly greater height than two other species. *E. capillifolium* is recommended as a potential host for growing *S. americana* for out-planting. Study 2 examined the effects of fertilizer on *S. americana* size. The first portion of the study did not include a host and included seedlings grown from seeds collected in Alabama and South Carolina, analyzed separately. South Carolina seedlings were significantly taller and had significantly larger leaves when fertilized. Alabama seedlings were significantly taller when fertilized but did not have significantly larger leaves. The second portion of the study examined *S. americana* grown from seeds collected in South Carolina only, with a host present. The fertilizer treatment was correlated with significantly taller *S. americana* but had no detectable effect on leaf size. Fertilizer is therefore

recommended when growing *S. americana* for out-planting in order to increase seedling size. Study 3 used the seedlings from the second portion of Study 2 to determine the potential of cuttings as a method of *S. americana* propagation and the effects of smoke on regrowth. Half of the flats were applied with smoke then all aboveground biomass was clipped and stems were replanted. 42 out of 48 cuttings exhibited new growth following replanting. Cuttings are therefore recommended when growing *S. americana* for out-planting in order to increase number of stems. Smoked plants did not regrow at a greater percentage than unsmoked plants either in the original flats or as cuttings.

      S. americana is federally endangered and is at extreme risk of extirpation from Alabama. Its tendency to persist with limited recruitment indicates it may benefit from population augmentation. Efforts to out-plant *S. americana* are made difficult by a lack of clearly defined propagation and out-planting protocols. It is the goal of this research to improve future efforts through a greater understanding of the conservation concerns of *S. americana* in Alabama, effective propagation methods in the greenhouse and out-planting site choice for the species.

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Chapter 1

Introduction

The Endangered Species Act (ESA) was passed in 1973 with the goal of preventing extinction of listed plant and animal species. While preventing extinction is at the crux of any conservation initiative, the eventual goal is to improve the status of the species so that it no longer needs protection under the ESA. To accomplish this, existing populations must be managed appropriately. However, this is not always enough. Recovery may require establishment of new populations or augmentation of existing ones in order to prevent localized extinctions. Such efforts require reliable methods for *ex situ* conservation.

This study attempted to improve methods of protecting the federally endangered plant *Schwalbea americana* L. Work focused on the size and demographic trends of plants at a natural site, responses to levels of shade in a greenhouse experiment, and other observations of *S. americana*. Demographic trends from 2015 to 2016 were used to extrapolate future numbers at the one known *S. americana* site in Alabama. In a controlled setting, three levels of shade were applied to *S. americana* to identify which growing conditions yielded the largest seedlings. The current study may provide insights into better management, propagation, and safe-guarding of *S. americana* for future conservation efforts.

Species Description

S. americana is a generalist root hemi-parasite in the Orobanchaceae (USFWS 1992; Young et al. 1999). It was first described by Linnaeus (1753) and placed in Scrophulariaceae until Young et al. (1999) did a genetic analysis of plastid gene sequences and found it more closely related to other Orobanchaceae. Although it is now considered a monotypic genus, a second species, *S. australis,* was described by Pennell (1935). Fernald (1937) first unified the two species, observing that their supposedly

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distinguishing morphological features were inconsistent across herbarium specimens. According to the most recent analysis, *S. americana* is one of the basal lineages in the Orobanchaceae, but its exact placement is uncertain (Young et al. 1999). Therefore, there is no closely related species which might provide a useful comparison.

Leaves are alternate, sessile, entire, and elliptical to lanceolate, with the largest leaves occurring in the lower 1/3 of the stem. Leaves and stems are puberulent and yellow-green with purple undertones. Juvenile individuals are opposite-leaved, maturing to alternate-leaved (J. Glitzenstein, pers. comm. Feb. 7, 2016). Flowers are in a many-flowered, spikelike raceme produced from April to June in the Southeastern United States, the southern portion of its range (Kral 1983). They are pollinated by bumble bees but are not pollen limited and exhibit selfing (Norden and Kirkman 2004a). Kirkman et al. (1996) showed that bagging plants (thus preventing out-crossing) yielded seeds with slightly lower viability percentages than non-bagged plants. However, no analysis was done to determine statistical significance of those results and it is not clear if entire plants were bagged or just the flowering stalks. No publications exist regarding the germinability of seeds produced through selfing. Fruits are in persistent, dehiscent capsules that form in July and mature in September. Seeds are 2.5 to 3.0 mm long, numerous, greenish-brown, and linear-fusiform (Kral 1983).

Historic Abundance

Existing records conflict regarding how common *S. americana* was before European settlement. USFWS (1992) states that it was "always considered rare." However, a basis for this statement is not presented. The few existing historic records are separated into two species that would eventually be united as *Schwalbea americana* (*S. americana* and *S. australis*) so some confusion may be explained by that discrepancy. Pennell (1919) includes a description of *S. americana*'s coastal range, then states "inland apparently occasional." This suggests that, closer to the coast, it is not occasional but more common. Pennell (1935) states that *S. australis* is "occasional to locally frequent in the Coastal Plain from North Carolina to Florida and Louisiana." Pennell (1935) provides information regarding the other

physiographic regions in which it occurs but no descriptions of frequency in these regions. *S. americana* is also described as "occasional or locally frequent in the Coastal Plain from Delmarva to the Cape Cod Peninsula and Nantuket" (Pennell 1935). It was first described as rare in the late 20th century (Kral 1983).

There are likely a number of reasons for the lack of historic records. For one, *S. americana* flowers are not brightly colored or especially showy and non-flowering individuals are small and easily overlooked. Additionally, sites at which *S. americana* populations occur can be converted to pine plantation or agriculture relatively easily, especially in the Southeast (Kral 1983; USFWS 1992). Historic sites were likely converted early on in European settlement before the occurrences of *S. americana* were recorded, as these sites had high profit potential. This is especially true where *Pinus palustris* Mill. (longleaf pine) occurred, due to its high timber value, leaving less than 3% of its original range with intact understory vegetation (Frost 1993; Noss et al. 2015). Although *S. americana* and longleaf pine frequently co-occur in the Southeastern United States, the former can occur outside longleaf pine's historic range and habitat. *S. americana* ranged from Texas to New York historically and can occur in prairies and mowed fields as well (Pennell 1935; Kral 1983; USFWS 1992). The earliest records report it growing only in moist, humid places (Pennell 1919) and Kirkman et al. (1996) describe it as occurring in ecotonal areas. However, it can apparently grow in a range of soil types and hydrologic conditions, as *S. americana* populations have also been found on xeric sites with either sandy soils or heavy clay soils (USFWS 1992).

Despite a conspicuous lack of abundance records, *S. americana*'s wide ecological niche, apparently expansive historic distribution, and tendency to parasitize a range of host species suggest it was once far less rare. Even supposing it was always rare, historic records indicate it has lost a great percentage of occurrences since European settlement (Pennell 1919; Pennell 1935; USFWS 1992).

Species Biology

S. americana has been observed parasitizing the following hosts *in situ*: *Aletris farinosa* L., *Panicum tenue* Muhl., *Pityopsis graminifolia* (Michx.) Nutt., *Carphephorus odoratissimus* (Gmel.) Herb.,

Gaylussacia dumosa (Andrz. T & G), *Aster adnatus* Nutt., *Ilex glabra* L. (Gray), and *Hypericum* sp. (Musselman and Mann 1977a; Kirkman et al. 1996). It has been observed parasitizing the following additional hosts in controlled settings: *Liriodendron tulipifera* L., *Pinus strobus* L., *P. palustris*, *Liquidambar styraciflua* L., *Nyssa aquatica* L., *N. sylvatica* Marsh., *Dyschoriste oblongifolia* (Michx.) Kuntze, *Aristida stricta* Michx., and *A. beyrichiana* Trin. and Rupr. (Musselman and Mann 1977b; Determann et al. 1997; Helton et al. 2000). In a controlled setting, Helton et al. (2000) showed that *I. glabra* and *Pityopsis graminifolia* exhibited the greatest parasitism rate by *S. americana* out of the following: *I. glabra* (95%), *Pityopsis graminifolia* (64%), *Panicum tenue* (27%), *A. beyrichiana* (21%), and *Pinus palustris* (13%).

Burning increases flowering of *S. americana* and timing of burns at least partially affects flowering phenology (Norden and Kirkman 2004b). The species' tendency to respond positively to fire and to persist in habitats with an open canopy led to the assumption that *S. americana* is "shade intolerant" (USFWS 1992). However, no published data exist regarding the effects of shade at ground level on growth of *S. americana*.

Occurrences of dormancy in some individuals, in which a plant does not appear aboveground one year and then re-emerges the following growing season, complicate efforts to monitor population dynamics (Norden and Kirkman 2004a). However, many researchers suggest that population recovery is slow for *S. americana* after disturbance. New recruits are rarely observed in the field (Kirkman et al. 1998; Norden and Kirkman 2004b), despite an abundance of seeds produced and germination rates at >90% in lab conditions (Kirkman et al. 1996; Obee and Cartica 1997). Possible explanations include 1) isolation and small population size leading to inbreeding depression, 2) microsite availability limiting seedling establishment, and 3) observer bias (Norden 2002; Kelly 2006). While the reasons for apparent low recruitment in *S. americana* populations are unknown, they present a challenge to population recovery. To minimize chances of extinction, this and other information must be addressed.

Cold stratification is often necessary for germinating seeds of various species. It does not appear to be necessary for germination of *S. americana* (Musselman and Mann 1977b; Kirkman et al. 1996;

Determann et al. 1997; Helton et al. 2000). However, this has been contested (J.S. Glitzenstein, personal communication, July 19, 2016). Obee and Cartica (1997) reported high rates of germination following wet-cold stratification but had almost no germination following dry-cold stratification. The reason for this is unknown, but it indicates that *S. americana* seeds may be intolerant of dry winter conditions.

Current Status

S. americana ranks as G2G3 globally, vulnerable to imperiled due to a restricted range, few populations, or widespread decline (ANHP 2014). In Alabama, it ranks as S1: critically imperiled due to extreme rarity or other special vulnerability (ANHP 2014). In South Carolina, it ranks as S3. Current threats to the species include habitat fragmentation and conversion due to agriculture and pine plantations, as well as housing developments and fire exclusion.

Soil disturbances from agriculture and intensive forestry have long been considered harmful to *S. americana* populations due to the plant's dependence on subsurface haustorial connections with a host (Kral 1983). Soils at these sites are level, deep, and suitable for building, and regional development pressures are severe and increasing in the Southeast (Rawinski and Cassin 1986; USFWS 1992; Napton et al. 2010). Even where suitable habitat still persists, fire exclusion and habitat fragmentation have led to considerable loss and reduction of historic populations, and isolation among those that remain (USFWS 1992). Fire exclusion alters community structure in fire-prone habitats by allowing hardwood stems to increase in density in the understory (Gilliam and Platt 1999). Because *S. americana* apparently requires relatively open habitat to thrive and because fire increases flowering of *S. americana*, fire exclusion is a likely contributor to population decline (USFWS 1992, Kirkman et al. 1998).

Habitat fragmentation and isolation have increased the risk of extinction of *S. americana* because a random event can eliminate an entire site relatively easily. In the case of *S. americana*, elimination of one site could mean elimination of a large portion of its range. Isolation can also result in a highly inbred population with low viability or fecundity (Chesser 1983). This is especially likely in *S. americana* populations, as the species exhibits low allozyme diversity (Godt and Hamrick 1998).

S. americana was thought to be extirpated from Alabama until it was discovered in 2008 in Bullock County (S. Hermann, pers. comm. May 15, 2014). A survey of the site in 2010 revealed 400+ stems of *S. americana* (S.M. Hermann and J.S. Glitzenstein, unpublished data). A census conducted in the summer of 2014 revealed only 150 stems aboveground, despite a fire regime of winter burns every two years. *S. americana* individuals are known to undergo periods of prolonged dormancy, casting doubt on the accuracy of population size estimates (Norden and Kirkman 2004a). However, dormancy during the growing season is thought to be a result of adverse conditions for either the host or the parasite, indicating poor conditions for a large portion of the site. Insect herbivory of *S. americana* stems is severe in some locations in Bullock County and may be a major contributor to this apparent decline. To date, the insect(s) has not been observed feeding on *S.americana*, so no identification has been possible.

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Chapter 2

Status and Demographic Trends of *Schwalbea americana* L. in Bullock County, Alabama

Introduction

Schwalbea americana L. was thought to be extirpated from Alabama until the species was discovered in 2008 on private property in northern Bullock County (Hermann, pers. comm. 2014). A survey of the site in 2010 revealed 400+ stems of *S. americana* in 15 patches (Hermann and Glitzenstein, unpublished data). Patches are defined as areas with *S. americana* present that are at least 100 m distant from the nearest neighboring one, and stem is defined as a major vertical shoot originating below the soil surface. Because we have no information on gene flow, we are not able to identify what might constitute a population on this property. In addition, *S. americana* may undergo periods of prolonged dormancy, perhaps the result of adverse conditions for either the host or the parasite, and this attribute may complicate attempts to assess populations and their viability (Norden and Kirkman 2004).

The current study 1) assesses plant size classes and reproductive states among patches over a three year period, 2) describes demographic trends among vegetative, reproductive, and absent (dead or dormant) stages, and 3) presents a preliminary projection of the status of *S. americana* in Bullock County in 2017. In this exercise a life stage-based approach to demography was examined, as opposed to age-based demography (e.g. Caswell 1989) because there is no way to determine age of *S. americana* stems once cotyledons disappear. This information will contribute to our understanding of the potential long-term viability of this rare species in Alabama.

Methods and Patch Descriptions

Estimates of *S. americana* stem numbers on the private property in Bullock County were made in 2010. There was a fire regime of winter burns every two years over much of the property. Researcher J.S. Glitzenstein previously identified patches of the species and, in 2010, assisted by S.M. Hermann, the pair walked approximately 1-2 meters apart and noted visible stems in each patch. Estimated stem numbers for each patch were rounded to the nearest ten. The 15 patches known to have had *S. americana* present in 2010 were searched for live stems in the summers of 2014, 2015, and 2016 using the same approach used in 2010. These were then censused completely, rather than estimating and rounding to the nearest ten. The census conducted in the summer of 2014 revealed just over a hundred live stems. *S. americana* were found in five of the previously recorded fifteen patches. For simplicity the five were labeled Patches A-E (Figure 1) and are the source of data used in this chapter.

Canopy closure (also called canopy cover or canopy density) can be defined as percent of sky blocked by tree canopy at a point on the ground and so provides a metric related to shading. Canopy cover can be estimated by using a spherical densiometer. A spherical densiometer (Forestry Suppliers, Inc.) is a polished mirror having the curvature of a 15¼ cm wide sphere. A grid is etched on the surface, forming squares (Lemmon 1956). Each quarter-square was recorded as 1) reflecting branches/needles in the canopy or 2) reflecting only sky. Four readings were averaged for each patch and were recorded 6 m north, south, east and west of the approximate center of the patch (Lemmon 1956). Readings were recorded February 28, 2016. The overstory of every patch was composed exclusively of conifers. Figure 1 provides a map of the patches.

Patch A was approximately 20 m by 20 m. The patch was burned in 2014 and late March of 2016. *S. americana* in this patch were under *Pinus echinata* Mill. and *Pinus taeda* L. No pine regeneration and few hardwood stems were present in the mid-story. The patch was 4.5 m upslope from a dirt road in heavy use. Patch A canopy closure was 81%.

Patch B was approximately 30 m by 20 m. The patch was burned in 2014, 2015, and late March of 2016. *S. americana* at this patch were adjacent to a drainage ditch, with *P. echinata* and *P. taeda* on the

north side and a large open area on the south side. Some hardwood stems were present in the mid-story with pine regeneration beginning to occur in the understory. Roller-chopper tracks were discovered in 2016 directly adjacent to the patch of *S. americana*. Canopy closure was 49%.

Patch C was approximately 50 m x 50 m in 2010 (J.S. Glitzenstein, unpublished data), however by 2014 there were only two plants 1 m apart. The patch was burned in 2014 and late March of 2016. *S. americana* at this patch were directly adjacent to a drainage ditch under *P. echinata* and *P. taeda*. Many oak saplings had become established in the drainage ditch with some pine regeneration occurring upslope. Canopy closure was 85%.

Patch D was approximately 45 m by 20 m. The patch was burned in 2014 and late March of 2016. *S. americana* in this patch were under *Pinus palustris* Mill. and some *P. echinata*. Many oak saplings had become established in the mid-story. *Pteridium aquilinum* (L.) Kuhn was abundant in the understory, with minimal pine regeneration. Roller-chopper tracks were discovered in 2016 that cut through the patch and over five marked *S. americana*. Canopy closure was 60%.

Patch E was approximately 40 m by 20 m. The patch was burned in late March in 2016. Forty-six new stems were discovered at Patch E in 2016. However, old stems from 2015 were present for many plants, indicating that some were alive in 2015 as well. It was not possible to determine how many were active in 2015. Therefore, Patch E could not be included in discussion of site size and demographic trends. The *S. americana* that were observed in 2015 occurred in a small opening with few to no mid-story hardwood stems. Pine regeneration was starting to occur near the patch. The overstory was *P. echinata* and *P. taeda*. Canopy closure was 61%.

Plants initially were marked with pin flags. Permanent tags were added later, and each plant was revisited to collect census data. Surveys were conducted three times in 2014, once at the end of July and twice in August, which is later in the growing season than the dates of the 2015 and 2016 censuses. Census data were recorded every two weeks in 2015 from June 15th to October 23rd for all patches. The census date with the greatest number of stems for all patches in 2015 was June 29th. Consequently, data from this date were used in summaries of patch size and demography included below. Census data were

recorded twice a month in 2016 from March to June. The census date with the greatest number of stems in 2016 was June 10th. Data from this date were used in summaries of patch size and demography (Table 1).

Leaf size was one of the metrics used in developing stage class categories for *S. americana*. In previous work on *S. americana*, Kirkman et al. (1996) described criteria for assigning size classes to leaves of the species and those protocols were used in the current work. The length of the largest (longest) leaf on each plant was measured and plants were assigned to the following leaf size categories: 1) Repro (stems with flowers or seed capsules present); 2) VegL (large leaves >1.0 cm long; 3) VegM (medium leaves >0.5 cm and ≤1.0 cm long, and 4) VegS (small leaves ≤0.5 cm long). Reproductive plants always have large leaves (Kirkman et al. 1996).

Initially the demographic structure of patches of *S. americana* was explored using number of individual stems in the size and reproductive categories described above. As noted in Chapter 1, this species is often multi-stemmed, with damage to stems apparently resulting in basal sprouting (K. Fuller, observation, June 15, 2014). Then, in an effort to provide a basis for an elementary viability model, patches were evaluated based on status of individual plants. Clusters of stems were considered a single plant if all stems were within 2 cm of each other. Plants were assigned to one of three categories: reproductive, non-reproductive (vegetative), or absent. Plants with at least one stem with a flower or a seed capsule were considered reproductive. Simple matrices were constructed using information based on year-to-year shifts in plant status (see Menges 2010). This approach provided the basis for a simple site viability model based on individual plants.

The matrices used to calculate size and demographic trends of the Bullock County site are based on stage class transition matrices described in Caswell (1989). Numbers of absent plants were added to the matrices as a way to account for *S. americana*'s ability to remain dormant during the growing season. This permitted inclusion of more observations in descriptions of site trends.

The absent category was made up of plants that were not observed during an entire growing season. Recruits, new individuals in a population (Eriksson and Ehrlén 2008), were included in the number of plants that were absent in 2015 and active (reproductive or vegetative) in 2016. This number

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also included previously established individuals that were dormant in 2015. Once cotyledons disappear there is no way to determine the age of a plant, recruits may not be distinguishable from established individuals that were dormant the previous year.

Relative proportions of 2015 reproductive plants that underwent each transition (reproductive to reproductive, reproductive to vegetative, and reproductive to absent) were calculated for 2016. The relative proportions that describe the transitions between 2015 to 2016 stages were then applied to plant numbers in 2016 to predict 2017 demography. The same was done for vegetative and absent plants.

Results

The number of stems spanning 2014-2016 for Patches A-D and 2016 for Patch E is presented in Table 1. The estimated total number of stems in 2010 for each patch is included as a basis of comparison. By inspection, Patch A had a small but consistent increase in number of stems from 2014 to 2015, and from 2015 to 2016 (Table 1). Number of stems at Patch B increased from 2014 to 2015, and in 2016 the total number of stems returned to approximately the number observed in 2014 (Table 1). Patches C and D had a small but consistent decrease in number of stems from 2014 to 2016 (Table 1). Patch C supported just 4 stems in 2014 and 2015, and none appeared in 2016, while stem number in Patch D varied from a low of 43 in 2015 to a high of 50 in 2016. Over a longer term (from 2010-2016) there was a decrease in the numbers of stems in Patches D and E: Patch D dropped from ~200 to 42 stems and Patch E from ~100 to 50 stems.

The number of plants in each stage class transition category (from 2015 to 2016) is found in Table 2. Vegetative to Vegetative was the category with the most plants (24 / 69 or 34.8%) summed over all patches. That stage class transition category was also the dominant one in three of the four patches (Table 2). Patch C was the only one that did not follow that trend. However, there were only two plants found on that patch and both transitioned from vegetative to absent (Table 2). In 2016, a total of 20 plants were reproductive; 13 had transitioned from being vegetative in 2015 and three had been absent during the same year. Only four plants that were reproductive in 2015 were also reproductive in 2016 (Table 2).

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Between 2015 and 2016, 33% of plants that were reproductive in 2015 and 18% of plants that were vegetative in 2015 were absent (dormant or dead) in 2016 (Table 3a); however it should be noted that the number of individuals absent was similar and relatively small each year (10-11 plants). In addition, three plants were absent in 2015 that were reproductive in 2016, and twelve plants were absent in 2015 that were vegetative in 2016 (Table 3a).

If probabilities associated with transition among reproductive, vegetative, and absent stages remain constant for 2016 to 2017, site stage classes are expected to show the trends presented in Table 3b. This model results in an increase of two reproductive individuals in 2017, a decrease of five to six non-reproductive plants, and an increase of two to three absent plants (Table 3b). Among-year comparisons are summarized in Table 3c. It should be noted that the data set available for this exercise was small; development of a more robust model requires more data and observations over a longer time period.

Discussion

In Bullock County, Alabama, *Schwalbea americana* has undergone a significant reduction in numbers since 2010, or perhaps a large portion has gone dormant due to adverse conditions such as drought (Norden and Kirkman 2004). Since 2014, the total number of stems observed in Patches A-D has been relatively constant, and the number of plants has also been relatively constant. However, in 2010 15 patches were documented, and in 2014 only five of them appeared to have retained plants (Table 1). In 2016 that number dropped to four patches with active plants (Table 1). If *S. americana* has been eliminated from the patches for which no plants were found in 2016, the probability of extirpation from the entire site is high and of conservation concern.

Exploration of stage class transitions of individual plants from 2015 to 2016 (Tables 2-3c) and projected from 2016 to 2017 (Table 3b) also indicates the need for more information on the species. Although the projected transitions (from 2016 to 2017) suggested a moderately stable, short-term result for the remaining patches of *S. americana,* the data from 2015 to 2016 also revealed the need to better understand potential long-term dormancy of the species.

In some patches at the Bullock County property insect herbivory of *S. americana* is noticeable (Fuller and Hermann unpublished data) and may be a contributor to the apparent decline of this rare species. Fire can alter the spatial distribution of herbivorous insects, with the interior of a burned area often exhibiting less herbivory than the edge (e.g. Knight and Holt 2005). Fire may therefore be an important regulating agent for limiting herbivory and maintaining plant health. Because *S. americana* is often associated with frequent-fire maintained ecosystems (e.g. Kirkman et al. 1998) more information on the effects of individual burns may be important. Total number of stems in four of the patches increased slightly in 2015, when only one of the patches was burned. Lack of fire may have resulted in basal sprouting due to increased herbivory. If so, number of stems is not necessarily a good indicator of patch status. It is likely more accurate to use number of plants than number of stems, since number of stems may fluctuate more than the number of associated plants from one year to another (K. Fuller, observation, August 24, 2015).

The descriptions of patch size and demographic trends based on stage classes presented here are based on two to three years of data in an ecosystem with a two to four year fire return interval (e.g. Guyette et al. 2012). Re-emerged plants may become active as a result of fire application (Kirkman et al. 1998). Precipitation also has been shown to affect the inducing and breaking of vegetative dormancy in other species (Epling and Lewis 1952, Thomas et al. 1981, Lesica and Steele 1994). Future research should examine this relationship for *S. americana.*

Long-term monitoring is needed to shed light on the conservation concerns of *S. americana* in Bullock County, specifically the cause of the inactivity of 10 out of 15 historic patches. In addition, within-patch differences over time warrant more study. In the current work, moderately long-term comparison of observations made on five patches in 2010 estimated ~370 stems compared to just 150 stems in 2016. Improved understanding of prolonged dormancy may be an important component of improving conservation efforts. Additional monitoring may help answer some basic questions researchers still have about *S. americana* ecology and management. Future studies should closely follow *S. americana* responses to roller chopping and feral hog damage to assess potentially harmful long-term

effects (Kral 1983). Of greatest importance for conservation is for researchers to determine what

management strategies could be used to improve the viability of the one known Alabama site supporting

S. americana.

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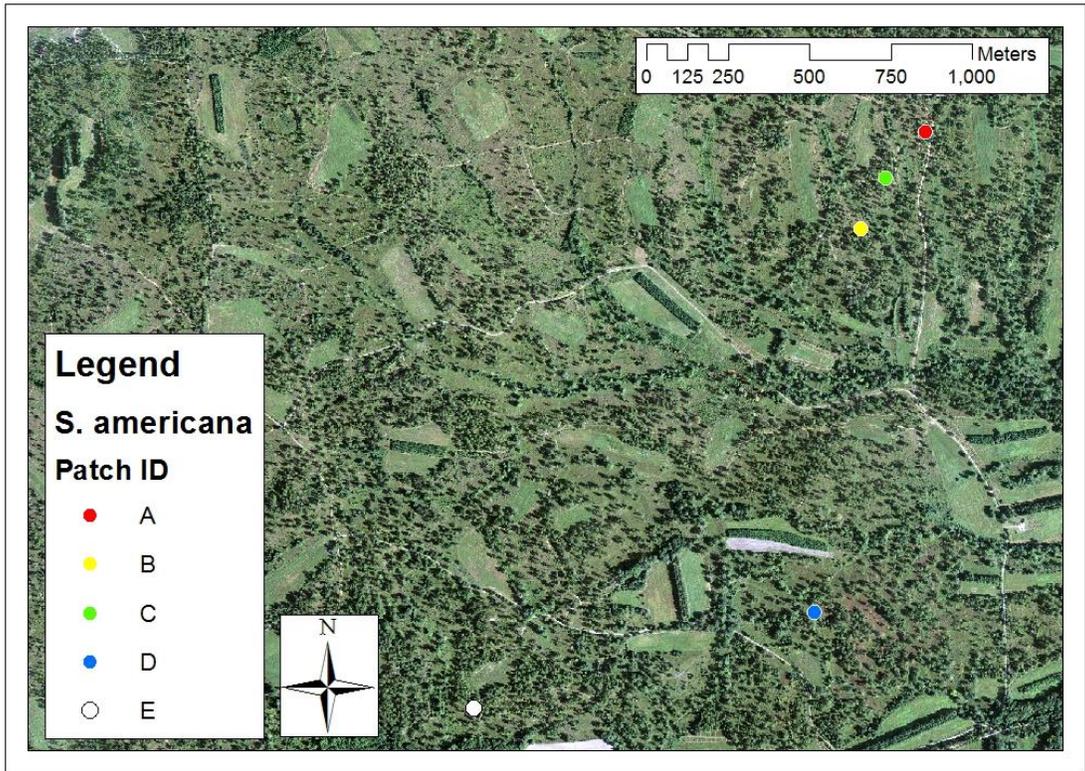
Figure 1. Map of the *S. americana* patches in Bullock County, Alabama. Patches are clusters of plants separated by 100 m or more.

Table 1. Numbers of stems per patch from 2010 to 2016 of the *S. americana* site in Bullock County, Alabama. Number of stems in 2010 recorded by S.M. Hermann and J.S. Glitzenstein (unpublished data), when no reproductive category was included. Patches are clusters of plants separated by 100 m or more. 'Repro' are plants with flowers or seed capsules present. 'VegL' are non-reproductive plants with leaves longer than 10 mm. 'VegM' are non-reproductive plants with leaves longer than 5 mm and shorter than or equal to 10 mm. 'VegS' are non-reproductive plants with leaves shorter than or equal to 5 mm.

Year	Patch	Repro	VegL	VegM	VegS	Total
2010						
	A					~10
	B					~40
	C					~20
	D					~200
	E					~100
	Total					~370
2014						
	A*	2	9	2	0	13
	B	6	30	0	0	36
	C	2	1	0	1	4
	D	14	33	2	0	49
	E**	-	-	-	-	-
2015						
	A	6	14	0	0	20
	B	4	51	0	0	55
	C	0	3	1	0	4
	D	0	36	6	1	43
	E**	-	-	-	-	-
2016						
	A	6	14	1	0	21
	B	10	27	0	0	37
	C	0	0	0	0	0
	D	6	26	10	0	42
	E	7	42	1	0	50
	Total 2016	29	109	12	0	150

* In 2014, sampling in Patch A did not begin until late in the growing season. Numbers observed in different stage classes for Patch A are presented. However, they are not compared to other years in Patch A or among other patches.

**Patch E not censused completely in 2014 and 2015, data not shown.

Table 2. Numbers of plants per patch associated with stage-class transitions from 2015 to 2016. Stages are: Reproductive (with at least one stem supporting a flower or seed capsule), Vegetative (no reproductive structures), and Absent (dead or dormant). There may have been Absent plants during both years, however no assessment of that transition was possible based on observations spanning just two years.

Stage-Class Transitions: from 2015 to 2016	Patch A	Patch B	Patch C	Patch D	Total
Reproductive to Reproductive	2	2	0	0	4
Reproductive to Vegetative	1	1	0	0	2
Reproductive to Absent	2	1	0	0	3
Vegetative to Reproductive	3	5	0	5	13
Vegetative to Vegetative	4	8	0	12	24
Vegetative to Absent	1	0	2	5	8
Absent to Reproductive	2	0	0	1	3
Absent to Vegetative	0	5	0	7	12
Total Number of Plants	**15**	**22**	**2**	**30**	**69**

Tables 3. Demographic trends of all known patches of *S. americana* in Bullock County, Alabama. 'Reproductive' are plants with flowers or fruits. 'Vegetative' are non-reproductive individuals. 'Absent' refers to dead or dormant (unobserved) plants not observed that year. Numbers in parentheses indicate the proportion of plants that transitioned among corresponding stages.

Table 3a. Number and proportion of plants that transitioned between stage categories from 2015 to 2016 for the *S. americana* in Bullock County, Alabama.

| To 2016 | From 2015 | | |
	Reproductive	Vegetative	Absent
Reproductive	4 (0.44)	13 (0.29)	3 (0.20)
Vegetative	2 (0.22)	24 (0.53)	12 (0.80)
Absent	3 (0.33)	8 (0.18)	*

* Indicates the transition of Absent to Absent, a shift that is not possible to detect among only two years.
  Format based on table format in Menges (2000).

Table 3b. Numbers of plants predicted for stage class transitions from 2016 to 2017 for *S. americana* in Bullock County, Alabama, based on observed trends in transitions from 2015 to 2016.

| To 2017 (projected) | From 2016 | | |
	Reproductive	Vegetative	Absent
Reproductive	8.8 (0.44)	11.0 (0.29)	2.2 (0.20)
Vegetative	4.4 (0.22)	20.1 (0.53)	8.8 (0.80)
Absent	6.6 (0.33)	6.8 (0.18)	*

Table 3c. Numbers of *S. americana* plants in three stage classes over three successive years (2015-2017) in Bullock county, Alabama. Projected number of plants in 2017 is based on observed trends from 2015 to 2016.

Year	Reproductive	Vegetative	Absent
2015	9	45	10
2016	20	39	11
2017 (projected)	22.0	33.3	13.4

Chapter 3

Evaluating responses of the federally endangered *Schwalbea americana* L. (Orobanchaceae) to host presence and shade

Introduction

Schwalbea americana (American chaffseed) is a federally endangered hemiparasitic plant (USFWS 1992). It is thought to be adapted to open, sunny areas and has even been described as "shade intolerant" (USFWS 1992). The basis for this assumption is the fact that natural populations typically reside in open areas and that *S. americana* responds with increased flowering following removal of surrounding vegetation by clipping or burning (Kirkman et al. 1998; Norden and Kirkman 2004). However, while some studies reference degree of canopy closure, there are no published data on the effect of the amount of shade on the height of *S. americana* stems- apart from its effects on flowering (Norden and Kirkman 2004).

A hemiparasite's ability to photosynthesize may negate the need to get carbon from a host. As a result, hemiparasites tend to attach to host xylem (Těšitel 2010). Additionally, the benefit of hemi-parasitism, as opposed to holo-parasitism, increases as availability of light increases. Since parasitism allows for sequestering of mineral nutrients from host tissue rather than the soil, the benefit of any form of parasitism increases as availability of soil nutrients decreases (Těšitel 2010). Therefore a host's ability to obtain mineral nutrients while not limiting light for the parasite is important in order for conditions to favor the hemiparasite. Xylem-feeders also tend to be generalists compared to phloem-feeders because phloem is living tissue and requires biochemical compatibility for attachment (Thorogood and Hiscock 2010). Due to its hemiparasitic nature and lack of host specificity, *S. americana* likely attaches to host

xylem only, taking up mineral nutrients and water but not much photosynthate (Thorogood et al. 2009; Těšitel et al. 2010). A small amount of photosynthate absorption could confer some shade tolerance if *S. americana* is able to supplement its own photosynthate with that of a host's.

Hemiparasites are thought to be sensitive to shade, especially early in life when they must invest in infrastructure aboveground for photosynthesis and belowground for acquiring hosts (e.g. Mardoian and Borowicz 2016). Obee and Cartica (1997) noted that *S. americana* stem height and leaf size were positively correlated with plant survival after out-planting. However, robust *S. americana* plants have been observed being overtopped by the herbaceous layer at natural sites in Alabama and South Carolina (K.J. Fuller, personal observation). This suggests that there is a need for additional information on the effect of shading in the conservation of this rare species.

This chapter describes preliminary greenhouse studies that lack traditional replication necessary for statistical analysis (e.g. Hurlbert 1984). However, there is increasing exploration of ways to utilize information derived from unreplicated research (e.g. Davies and Gray 2015; Millar and Anderson 2004). One approach suggested by Davies and Gray (2015) is: 1) to explore whether pseudoreplication is likely to be associated with confounded effects; if not, 2) statistically assess data but be cautious about attributing cause and effect, or 3) consider if assessment of the unreplicated information suggests new hypotheses that warrant testing. To date this approach primarily has been suggested for large, landscape-level data sets (Davies and Gray 2015), however it may also be applicable to small-scale studies.

The work described in this chapter explored the effect of shade and presence of a host plant on *S. americana* stem height and leaf size. If shaded conditions were associated with larger *S. americana* individuals, then growing *S. americana* under shade may be preferred if the goal is eventual out-planting. If plants in full sun are larger, full sun may be preferable. Shade cast by a host may affect the relationship between plant size and shade level. Shade at the level of *S. americana* stems is increased with host presence. However, hosts also provide mineral nutrient support, which likely increases plant size and may confer some degree of shade tolerance. Host presence is therefore expected to interact with the effect of shade level on plant size.

The current chapter describes two studies that targeted effects of shade on size of *S. americana.* The first (Shade-Host Study) was based on a nested design with two variables: 1) 33% shade generated by shade-cloth versus no shade (described below), and, 2) presence versus absence of a host plant. The host was assumed to have multiple influences including generating some shade (e.g. Keith et al. 2004). Norden and Kirkman (2004) demonstrated that shade applied at 80% of full sun reduced flowering in *S. americana*. The 33% shade treatment used in the current study was intermediate and selected because it was thought to create conditions that may be more similar to those generated by overtopping vegetation at natural sites. Many plant species exhibit larger leaves when shoots develop in shadier conditions (Niinemets and Kull 1994; Weijschedé et al. 2006; Xu et al. 2009). It is also common for vertically-oriented plants to exhibit more elongated shoots in shadier conditions (Ballaré et al. 1994; Huber and Hutchings 1997; Weijschedé et al. 2006). Increases in shade are therefore expected to be positively correlated with increases in stem height and leaf size.

There were three questions of interest associated with the Shade-Host Study. Question 1: Do *S. americana* plants have greater leaf length and height when grown under 33% shade compared to plants grown without shade? Question 2: Do *S. americana* plants grown in the presence of a host have greater leaf length and height compared to those grown without a host? Question 3: Do *S. americana* with taller hosts have greater leaf length and height?

The second study (Shade Only) was implemented to gather additional information on effects of shade and was based on three shade intensities: no shade, 33% shade, and 61% shade (described below). The 61% shade treatment may be close to the limit of what *S. americana* can tolerate. As a result, seedlings grown in 61% shade may exhibit taller stems because they have elongated internodes and larger leaves, but have fewer nodes per stem. This would not necessarily be a positive outcome, as more shaded conditions could theoretically result in fewer leaves and less total leaf area despite individual leaves being larger. Average internode lengths and numbers of nodes were therefore compared among treatments to gather information on the effect of shade level on stem structure.

The Shade Only Study explored three questions of interest. Question 1: Do *S. americana* plants grown under 61% shade exhibit greater leaf length and height than those grown under 33% shade or without shade? Question 2: Do *S. americana* plants grown under 33% shade exhibit greater leaf length and height compared to those grown without shade? Question 3: Do *S. americana* plants grown under 61% shade exhibit greater average internode lengths and fewer nodes than those grown under 33% shade or without shade?

Methods

The two shade studies took place in a greenhouse at the Plant Science Research Center in Auburn, Alabama. Light intensity readings were measured with an Apogee Instruments Inc. Quantum Meter to record Photosynthetic Photon Flux. With no obstruction to natural light and with supplemental 24-hour fluorescent lighting, readings at the greenhouse bench (1440-1621 μmol/m^{2}s) were 14.2-22.5% less than readings recorded in a nearby gravel lot (1860-1890 μmol/m^{2}s). One shade hut was constructed for the 33% shade treatments and one for the 61% shade treatment using PVC tubing and plastic shade cloth. The 33% shade hut measurements (925-1150 μmol/m^{2}s) were 29.0%-35.8% of readings recorded at a greenhouse bench without shade cloth. The 61% shade hut measurements (520-682 μmol/m^{2}s) were 57.9%-63.9% of readings recorded in the greenhouse without shade cloth. Shade greater than 61% was not included in this study, since a previous study demonstrated that 80% shade has a detrimental effect on recruitment (Norden and Kirkman 2004).

As noted above, these preliminary studies were not replicated. A single shade hut, each approximately 1.5 m long, was constructed for each shade treatment and a similar space was used for no shade treatments. This relatively small space was uniform and was centrally located relative to overhead lights. The area was hand-watered, and fertilizer was prepared as a single batch; there were few if any differences related to water pressure, nutrient availability, etc. In addition, there appeared to be no confounding effects among the unreplicated small treatment areas. Also see description of statistical approach (below).

Seedlings were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina. These were germinated in a 99-cell flat by J.S. Glitzenstein with about fifty seeds per cell. Seed population sources were mixed and not tracked. From June 25th through June 29th, 2015, the seedlings in each cell were carefully unearthed and separated from the rest of the cell. Seedlings were transplanted into 45-cell flats, one plant per cell, containing 50% peat, 25% vermiculite, and 25% perlite and moved to full-sun greenhouse space.

Shade-Host Study

The Shade-Host Study included four or five *S. americana* per flat, with twelve flats in total. Flats had 45 cells each. Cells were 4.5 cm in diameter by 10.75 cm in height. Hosts were transplanted into half of all the flats while the other flats received no hosts. Helton et al. (2000) determined that *Pityopsis graminifolia* (Michx.) Nutt. was a preferred host for *S. americana* compared to four other species common to *S. americana* habitat, so it was selected as the host for the Shade-Host Study. *P. graminifolia* seedlings were germinated from seeds collected in South Carolina and were grown in the same conditions as the *S. americana* seedlings but in a flat without cells. *P. graminifolia* seedlings were separated and transplanted into the flats on August 4th, 2015. Flats with and without *P. graminifolia* were placed in the greenhouse either under 33% shade or without shade (Figure 1) on August 6th, 2015. The nested treatments were: 1) 33% shade with and without a host, and 2) no shade with and without a host. Thirteen seedlings were assigned to each treatment (Figure 1).

Determann et al. (1997) demonstrated that fertilization keeps *S. americana* alive when seedlings are not attached to a host plant. Although half the *S. americana* in the Shade-Host Study had a host plant, all seedlings were fertilized every other day to avoid confounding the treatments. Neptune's Harvest Organic Fish Fertilizer (Neptune's Harvest, Gloucester, New York) was used, as recommended by D.J. Gustafson (pers. comm., March 30, 2015). Each cell received 7.4 ml of an 11.23 g/L dilution (225 mg/L N) of Neptune's Harvest Organic Fish Fertilizer with N:P:K ratio of 2:4:1. Once a week from October 8th,

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2015 to January 31st, 2016 (the Shade-Host Study), the length of the largest leaf and the height of *S. americana* seedlings were measured, in addition to the heights of *P. graminifolia* seedlings.

<div align="center">*Shade Only Study*</div>

The Shade Only Study included 12 *S. americana* plants per flat and did not include host plants. On August 19th, 2015, 45-cell flats were placed in the greenhouse either without shade, under 33% shade, or under 61% shade with 36 seedlings (three flats) per treatment (Figure 2). From October 25th, 2015 to January 31st, 2016 the length of the largest leaf and the height of *S. americana* seedlings were measured once a week. Number of nodes per stem was recorded on December 7th, 2015. Height was divided by the number of nodes to calculate average internode length.

<div align="center">Statistical Analysis</div>

All data were analyzed using R 3.2.3 statistical software (R Core Team 2013). To account for potential pseudoreplication and autocorrelation due to repeated measures, the cell number of each plant was used as a random variable, and a correlation structure was included. Differences among flats were not significant for any of the analyses, and including flat number as a random variable did not improve the fit of the models. Therefore, it was not included in any of the final analyses.

<div align="center">*Shade-Host Study*</div>

To examine the effect of shade, presence or absent of a host, and the potential interaction between these two variables, leaf length and height of *S. americana* were analyzed using two-way analysis of variance (ANOVA; NLME package). To examine the effect of *P. graminifolia* height on size of *S. americana*, the leaf length and log-transformed height of *S. americana* were analyzed with regression (NLME package), including only *S. americana* with a host present. Heights of *S. americana* were log-transformed to meet the assumption of a linear relationship between the continuous input and output variables.

To examine the effect of light intensities on *S. americana* size and stem structure, *S. americana* height, leaf length, mean internode length, and number of nodes were analyzed using ANOVA with post hoc Tukey's honestly significant difference test (STATS package).

Results

The high frequency of watering and fertilizing was likely the cause of growth patterns in the greenhouse not observed in natural patches in Alabama or South Carolina. By December 10th, 2015 many *S. americana* stems were flimsy and did not stand erect. In an effort to create more natural conditions, fertilizing was reduced to once a week and watering was reduced gradually over December 2015 and the first half of January 2016. An unexpected result was the subsequent die-back of almost all *S. americana* and some *P. graminifolia* plants. Resprouted plants were more erect, exhibiting growth forms that were observed in natural settings.

In the Shade-Host Study, shade level was not significantly correlated with leaf length (p=0.78) or height of *S. americana* plants (p=0.37). Presence or absence of a host did not significantly correlate with leaf length (p=0.94) or height (p=0.80) of *S. americana*. No significant interaction was found between shade treatment and host presence for either leaf length (p=0.53) or height of *S. americana* (p=0.56). When only *S. americana* with a host present were considered, each 1 mm increase in *P. graminifolia* height was correlated with a 0.03 mm (±0.007, 95% C.I.) increase in leaf length (p= 0.0001; Figure 3) and a 0.003 mm (±0.0005, 95% C.I.) increase in the log-transformed height of *S. americana* (p=0.0001; Figure 4).

In the Shade Only Study, there was no significant difference in leaf length among the shade treatments. *S. americana* grown under 33% shade were 14.6 mm (±14.7, 95% C.I.) taller than *S. americana* grown without shade (p=0.050; Figure 5). *S. americana* grown under 61% shade had mean internode lengths 0.39 mm (±0.30, 95% C.I.) longer than *S. americana* grown without shade (p=0.028; Figure 6). *S. americana* grown under 33% shade did not differ significantly in internode length from

those grown either without shade or under 61% shade. *S. americana* grown under 61% shade had 5.5 nodes (±2.1, 95% C.I.) fewer than *S. americana* grown under 33% shade (p=0.03) and 6.1 nodes (±2.1, 95% C.I.) fewer than *S. americana* grown without shade (p=0.02; Figure 7).

Discussion

The current chapter describes preliminary greenhouse studies that lack traditional replication. There appeared to be no confounding effects among the unreplicated treatments. However, the data should be used to indicate possible avenues for future research and cannot be used to draw definite conclusions. The two studies detailed here explored the influence of level of shade and presence of a host plant on *S. americana* stem height and leaf size.

The Shade-Host Study examined the effect of treatment combinations of two shade levels and presence or absence of a host on *S. americana* leaf length and height. In this study, there was a positive correlation between *P. graminifolia* height and the leaf length and log-transformed height of *S. americana*. This supports the idea that a host's ability to provide nutrients improves parasite health. However, no significant effect of shade on *S. americana* leaf size or height was found in the Shade-Host Study regardless of host presence or absence. This may be a result of small sample sizes. Also, the study may have been confounded by some photosynthate being taken up by attached *S. americana* through host xylem (Těšitel et al. 2015). No significant effect of shade was detected for unattached *S. americana* in this study either, but removing attached *S. americana* from the model reduced the sample size by half. If *S. americana* receives some photosynthate from its host, then attached *S. americana* are likely somewhat less affected by shade levels because of their ability to supplement their photosynthate with photosynthate obtained from a host. Sample sizes may have been too small to detect an interaction between shade levels and host presence. However, the hypothesis that host presence alters *S. americana*'s response to different levels of shade should be examined in future studies.

The Shade Only Study examined the effect of three levels of shade on *S. americana* leaf length and height. In this study, the 33% shade treatment yielded *S. americana* stems that were taller than those

grown without shade and had more nodes than those in the 61% shade treatment, but it did not produce significantly different average internode lengths from those grown either without shade or under 61% shade. The 61% shade seedlings had somewhat elongated internodes compared to seedlings grown without shade. Seedlings grown under 61% shade also had fewer nodes than seedlings grown either without shade or grown under 33% shade, without an increase in leaf size or height. This suggests that *S. americana* benefits from more light than is available under 61% shade (520-682 μmol/m^{2}s). The data from this study support the assumption that *S. americana* does best in high light environments. Future studies should examine the hypothesis that *S. americana* plants grown under moderate shade (925-1150 μmol/m^{2}s) exhibit the greatest leaf size and height without a reduction in leaf area.

Future propagation efforts may benefit from using moderate shade for growing *S. americana*, as increased seedling size has been shown to improve out-planting success (Obee and Cartica 1997). This suggests that out-planting sites should be chosen in part by the level of shade cast by both the canopy and surrounding understory vegetation. Plants that died back after a reduction in watering and fertilizing grew back with improved growth forms. Allowing stems to die back may therefore be beneficial if researchers encounter the same presumably unnatural growth form of the *S. americana* stems observed in this study. Future research should examine the effect of fertilizer on the relationship between *S. americana* and a host, as well as the effect of shade. Specifically, studies should focus on how each of these relationships relates to survival after out-planting.

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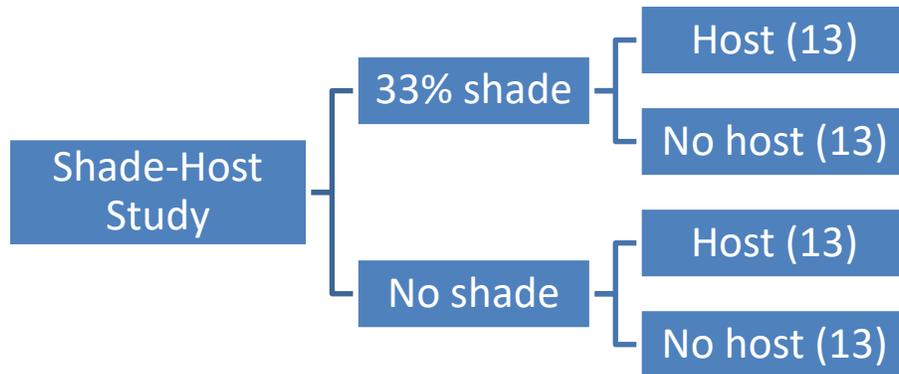
Figure 1. The Shade-Host Study using *S. americana* and *P. graminifolia* with a nested design without replication (see methods). The number in parentheses corresponds to the number of *S. americana* in each treatment group. *P. graminifolia* served as the host for this study. 33% shade was generated using a hut of plastic shade cloth. Photosynthetic photon flux was recorded to be 29.0%-35.8% of readings recorded at a greenhouse bench without shade cloth. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.
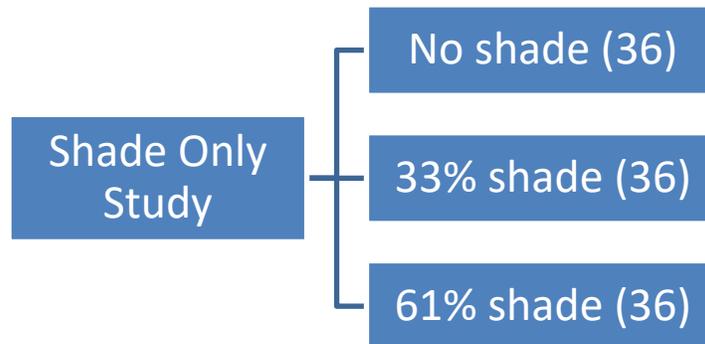
Figure 2. The Shade Only Study using *S. americana* with a randomized design without replication. The number in parentheses corresponds to the number of *S. americana* in each treatment group. Shade was generated using two huts of plastic shade cloth: one for 61% shade and one for 33% shade. Photosynthetic photon flux readings for the 33% shade hut were 29.0%-35.8% of readings recorded at a greenhouse bench without shade cloth. Photosynthetic photon flux readings for the 61% shade hut were 57.9%-63.9% of readings recorded at the greenhouse bench without shade cloth. See Figure 1 for description of plant origin.
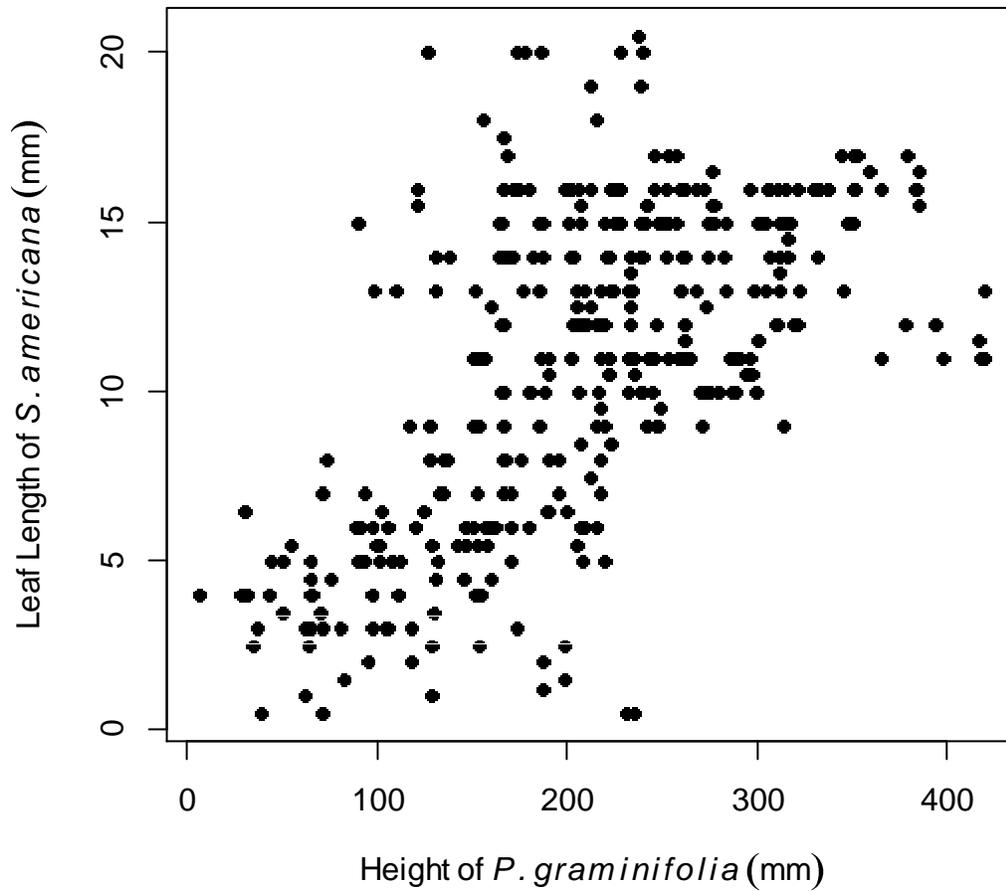
Figure 3. Length of the largest leaf on *S. americana* stems (mm) as a function of *P. graminifolia* height (mm), including only *S. americana* with *P. graminifolia* present. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.
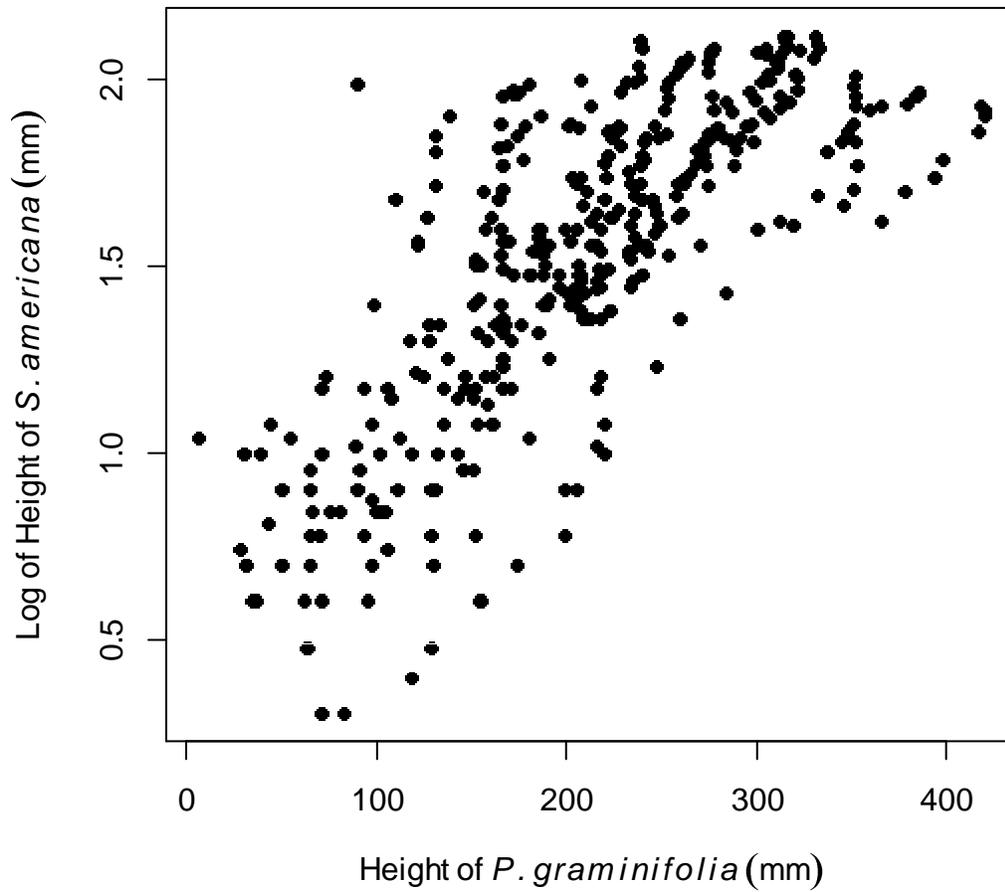
Figure 4. Log of *S. americana* height (mm) by *P. graminifolia* height (mm) for the Shade-Host Study, including only *S. americana* with *P. graminifolia* present. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.
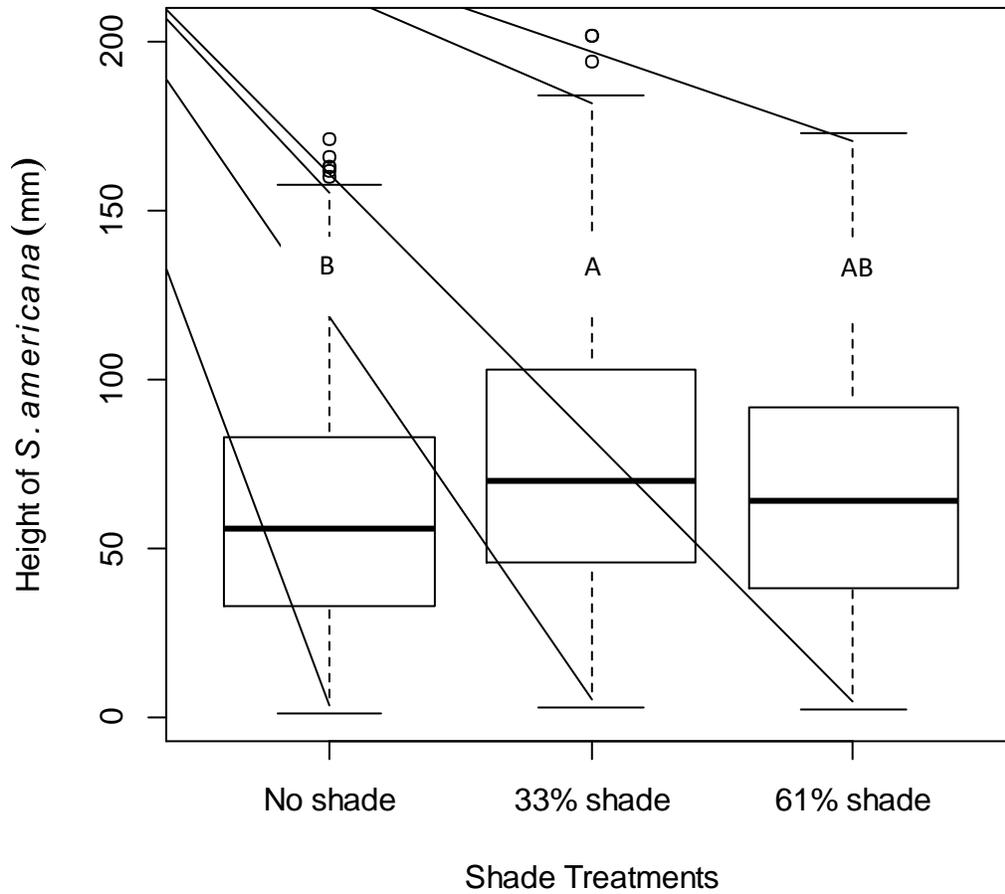
Figure 5. *S. americana* height (mm) by shade treatment for the Shade Only Study. Unique letters above bars indicate significantly different results based on an α value of 0.0503. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.
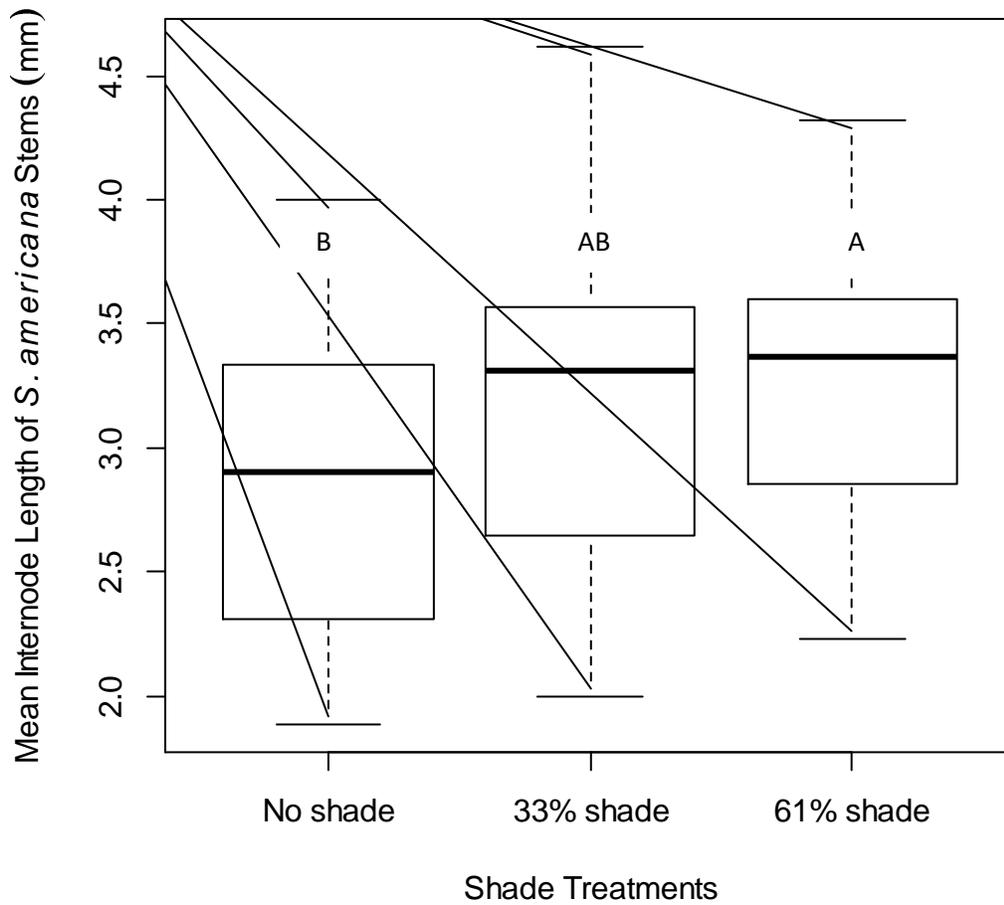
Figure 6. Mean internode length (mm) of *S. americana* stems by shade treatment for the Shade Only Study. Unique letters above bars indicate significantly different results based on 95% confidence. See Figure 1 for description of plant origins and Figure 2 for descriptions of shade treatments and experimental design. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.
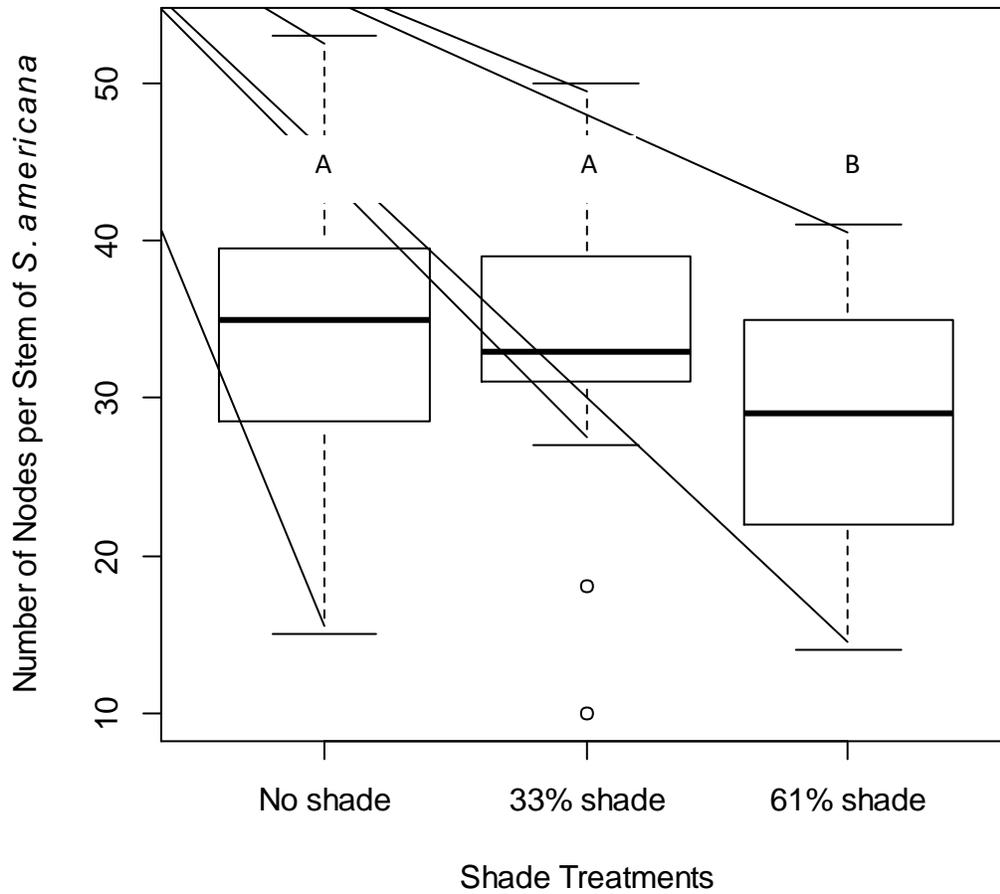
Figure 7. Number of nodes per stem of *S. americana* by shade treatment for the Shade Only Study. Unique letters above bars indicate significantly different results based on 95% confidence. See Figure 1 for description of plant origins and Figure 2 for descriptions of shade treatments and experimental design. *S. americana* and *P. graminifolia* plants were germinated from seeds collected from two populations: in Williamsburg County and Lee County, South Carolina.

Chapter 4

Preliminary Findings on Three Topics Important to Safeguarding *Schwalbea americana* L. and

Recommendations for Future Research

Introduction

  Schwalbea americana is a federally endangered root hemi-parasite, known to attach to a variety

of species (Musselman and Mann 1977; USFWS 1992; Helton et al. 2000). Attempts to safeguard the

species are made difficult by the fact that no clearly defined protocols exist for propagating and out-

planting. Only one published study has reported results from attempts to out-plant previously germinated

S. americana and success was limited (Obee and Cartica 1997). Below are described three preliminary

greenhouse studies; these results may improve future out-planting efforts. The three studies focus on *S.*

americana's response to five potential host species (Study 1), addition of fertilizer (Study 2), and a

treatment that combined smoke and propagation using cuttings rather than direct seeding (Study 3).

Preliminary Study 1: Host species and seedling attachment

  Schwalbea americana is known to use a range of host plant species (cf. Helton et al. 2000).

Glitzenstein observed that *S. americana* seedlings growing with *Eupatorium capillifolium* (Lam.) Small

appeared to be larger than those attached to other volunteer species (pers. comm., May 25, 2015).

Because *E. capillifolium* readily germinates and is easy to maintain (Gilreath 1986), it was evaluated for

suitability as a host species for propagation of *S. americana.* In Preliminary Study 1, *E. capillifolium* was

compared to four other common herbaceous species (*Phyllanthus urinaria* L., *Eclipta prostrata* (L.) L.,

Cerastium glomeratum Thuill., and *Youngia japonica* (L.) DC.) that could serve as host species for *S.*

americana. All five hosts used in this study are ruderals but only *Eupatorium capillifolium* and *E. prostrata* are native species.

Methods used to determine host preference (or selectivity) of parasitic plants vary. One method compared frequency of parasitic attachment to a host species with the availability of the host species in the parasite's habitat (Gibson and Watkinson 1989). Another evaluated host species based on the number of host seedlings with physical attachments (termed haustoria) to *S. americana* in a greenhouse setting (cf. Helton et al. 2000). Some authors have defined a preferred host as the host that supports optimal growth (cf. Musselman and Press 1995; Press and Phoenix 2005), and height of attached parasites is commonly used to identify preferred hosts (Helton et al. 2000; Ren et al. 2010). To date no published study has compared host species using the number of attached parasites per host or number of leaves on attached parasites as a way to identify preferred hosts. However, number of attached parasites could signify ease of penetration of a host's roots, or how much nutrient support a host provides. In Preliminary Study 1, number of leaves on attached parasites was measured as an additional size parameter to stem height.

Preliminary Study 1 was designed to address two questions. Question 1: Does one of the five host species support more *S. americana* individuals compared to the other potential hosts? Question 2: Does any attached host species result in *S. americana* with greater height and/or number of leaves compared to other hosts?

Methods: Host species and seedling attachment

S. americana seeds used in this study originated from two naturally occurring populations in Williamsburg County and Lee County, South Carolina. No more than one capsule per plant was collected in the fall of 2014, fifty from each location. Capsules were dried, and seeds were extracted at the USFS National Tree Seed Laboratory in Macon, GA and then grown outdoors by J.S. Glitzenstein in southern Leon County, Florida in a 99-cell flat containing 50% peat, 25% vermiculite, and 25% perlite. Cells are defined here as individual compartments in a flat. Approximately fifty *S. americana* seeds were sown per

cell in the spring of 2015, and ruderal plants were allowed to seed into the flat. From June 25th, 2015 through June 29th, 2015, cells were carefully excavated. *S. americana* seedlings and their hosts were separated from all other plants and substrate. Clusters (groups of hosts and parasites physically connected by haustoria) were examined and substrate was washed away from their roots for photographic records and host species identification. The number of *S. americana* seedlings attached to each host was recorded. *S. americana* height and number of leaves were also recorded. *S. americana* data were averaged for each cluster. Host species were compared based on the number of attached *S. americana*, and the mean height and mean number of leaves of their attached parasites.

Statistical Analysis: Host species and seedling attachment

All data were analyzed using R 3.2.3 statistical software (R Core Team 2013). To examine the effect of host species, number of attached parasites per host was analyzed using one-way Analysis of Variance (ANOVA; NLME package) with post hoc Tukey's honestly significant difference test (STATS package). Host height was used as a random variable to account for the variation in the amount of nutrients supplied by different sized hosts. To further examine the effect of host species, mean *S. americana* height and number of leaves were analyzed using one-way ANOVA with post hoc Tukey's honestly significant difference test (STATS package). Number of attached *S. americana* per host was used as a random variable to account for the variation in demand placed on the host by differing numbers of parasites.

Results: Host species and seedling attachment

The number of attached *S. americana* did differ by host species. There was no significant difference in number of attached *S. americana* between *E. capillifolium* and *Y. japonica,* however *E. capillifolium* had significantly more attached *S. americana* than three trial host species but was similar to Y. japonica (Table 1, Figure 1). In addition, host species *E. capillifolium* and *E. prostrata* were both associated with taller *S. americana* compared to two host species, C. glomeratum and P. urinaria (Table 2,

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Figure 2). Again, Y. japonica was intermediate (Figure 2). *S. americana* associated with *P. urinaria* had significantly fewer leaves compared to other trial host species (Table 3 and Figure 3).

Discussion: Host species and seedling attachment

This preliminary study on potential preference for five host species by *S. americana* revealed that host species are associated with some metrics with potential for influencing establishment of the hemiparasite. *P. urinaria* supported significantly fewer *S. americana* than *E. capillifolium. S. americana* attached to *P. urinaria* were significantly shorter than those attached to all other hosts and had significantly fewer leaves than those attached to *E. capillifolium, C. glomeratum,* and *Y. japonica.* Judging from these results, *P. urinaria* should not be used as a host for out-planting projects or as a host species in future *S. americana* studies. *E. capillifolium* was associated with significantly taller attached *S. americana* than *P. urinaria* and out-performed *P. urinaria* in the other analyses as well. *E. capillifolium* also had a greater number of attached *S. americana* than *C. glomeratum* and *E. prostrata*. As stated previously, number of attached *S. americana* per host may not be a reliable method of examining host preference; however a significantly greater number of attached *S. americana* on *E. capillifolium* may indicate greater ease of host root penetration or greater nutrient support for attached parasites. *E. capillifolium* is also relatively easy to maintain in a greenhouse setting (Gilreath 1986) and may be useful as a host for growing *S. americana* for out-planting.

Preliminary Study 2: Fertilizer only and fertilizer-host

In a previous study, increased height and leaf size were correlated with increased survival after out-planting of *S. americana* (Obee and Cartica 1997). Although Determann et al. (1997) demonstrated that germinated seeds are able to survive and expand when maintained in the laboratory on growth medium, to date there is no published information on the effect of fertilizer on growth rates of greenhouse seedlings. If fertilization increases seedling size, it may indirectly improve out-planting success. Additionally, fertilizing has been shown to increase the number of parasite attachments (haustoria) to

hosts in five other genera of root hemi-parasitic plants (Mann and Musselman 1981). If this is the case for *S. americana*, increasing the number of haustoria prior to out-planting may aid in surviving the shock of transplanting to natural but less fertile conditions.

Two fertilizer studies were pursued: a Fertilizer Only Study and a Fertilizer-Host Study. In the Fertilizer Only Study, no host was included, and the following question was addressed: Will fertilized *S. americana* be taller and/or have greater leaf length than unfertilized *S. americana*? For this study, seedlings germinated from seeds collected in Alabama and South Carolina were studied separately, in order to account for possible site-based differences in response to fertilizer treatments. In the Fertilizer-Host Study, a host was included and the following question was asked: Will fertilized *S. americana* seedlings have greater height and leaf length, and more haustorial attachments compared to unfertilized *S. americana*? Helton et al. (2000) found *Pityopsis graminifolia* (Michx.) Nutt. to be a preferred host for *S. americana* compared to four other host species common to its habitat, so it served as the host for the Fertilizer-Host Study.

Methods: Fertilizer only and fertilizer-host

The two fertilizer studies were conducted in a greenhouse at the Plant Science Research Center (PSRC) at Auburn University in Auburn, Alabama. South Carolina seeds for both fertilizer studies came from the same two populations as Preliminary Study 1. Alabama seeds were collected from a site in Bullock County, Alabama. Capsules were dried and seeds extracted at the USFS National Tree Seed Laboratory in Macon, GA. The duration of the drying treatment and subsequent storage conditions were not recorded. Alabama and South Carolina seeds were placed on moist filter paper in glass petri dishes for wet-cold stratification prior to germination. Seeds were maintained at 5 to 7°C from March 31st, 2015 to April 14th, 2015 at Auburn University. Petri dishes were checked daily and rewetted about every other day to maintain desired moisture levels. Seedlings were then transferred to 48-cell flats of sandy loam soil. The flats were placed in a germination chamber at 22°C at the PSRC in indirect light to germinate. However, no germination resulted from April 14th, 2015 to May 16th, 2015, likely due to insufficient light.

Flats were then moved into a cooler for further stratification from May 16th, 2015 to June 16th, 2015 at 4ºC, then to a greenhouse at the PSRC. Neptune's Harvest Organic Fish Fertilizer (2:4:1 N:P_2O_5:K_2O Neptune's Harvest, Gloucester, New York) was used, as recommended by D.J. Gustafson (pers. comm., March 30, 2015). Each cell received 7.4 ml of an 11.23 g/L dilution (225 mg/L N).

<p style="text-align:center;">*Fertilizer Only Study*</p>

Alabama seeds for the Fertilizer Only Study came from a site in Bullock County, Alabama (described in Chapter 2). Seed capsules from nineteen *S. americana* plants were collected in August, 2014. The number of capsules collected was contingent upon the number of capsules produced per stem. No capsules were collected from stems that produced only one capsule. One capsule was collected from stems that produced two to four capsules, two capsules were collected from stems that produced five to nine capsules, three capsules were collected from stems that produced ten to fifteen capsules, four capsules were collected from stems that produced sixteen to twenty capsules, and five capsules were collected from stems that produced greater than twenty capsules. Capsules were placed in a paper bag in a 35% humidity room for 18 days at the USFS National Tree Seed Laboratory in Macon, GA. The seeds were then extracted from capsules and stored in vials in the dark at 21ºC from October 6th, 2014 to March 31st, 2015 at Auburn University. Light varied somewhat as a result of periodic opening and closing of the storage cabinet.

Information on the duration of the drying treatment and subsequent storage of South Carolina seeds was not recorded and likely differed from the treatment of the Alabama seeds. However, methods of stratification and germination following storage were the same for both seed sources and are described in the methods section above.

Thirty-six Alabama seedlings and 90 South Carolina seedlings were transplanted into four 45-cell flats on August 31st, 2015 and September 1st, 2015, respectively. Flats contained 50% peat, 25% perlite and 25% vermiculite and were placed in full-sun greenhouse space. Two flats were fertilized and two were unfertilized (see experimental design in Figure 4). Fertilizer was applied to all flats once to

minimize mortality in the unfertilized treatment on September 2nd, 2015, then once a week to the flats in the fertilized treatment. Once a week, the height and the length of the largest leaf were measured for each *S. americana* seedling.

Fertilizer-Host Study

For the Fertilizer-Host Study, *P. graminifolia* seeds were collected from Auburn, Alabama. Seeds were sown on a substrate of 50% peat, 25% perlite and 25% vermiculite then lightly covered with horticultural grit according to directions in Midgley (2006) on September 26th, 2015. The treatment of *S. americana* seeds is described above.

One hundred and twenty six South Carolina seedlings were transplanted into seven 18-cell flats of 50% peat, 25% perlite and 25% vermiculite from October 18th, 2015 to October 21st, 2015 at the Plant Science Research Center. *P. graminifolia* seedlings were then transplanted into the same cells from October 22nd, 2015 to October 25th, 2015. Half of the cells in each flat received fertilizer once a week (see experimental design in Figure 5). Fertilizer was applied once on November 9th, 2015 to all flats to minimize mortality in the unfertilized treatments. Once a week, the height and the length of the largest leaf were measured for each *S. americana*. On February 2nd, 2016 seven cells were excavated, and soil was washed away from the roots to record occurrence of haustorial attachment. However, few seedlings were attached, and the data are not reported below.

Statistical Analysis: Fertilizer only and fertilizer-host

All data were analyzed using R 3.2.3 statistical software (R Core Team 2013). In order to examine the effect of fertilizer, *S. americana* height and leaf length were analyzed using one-way ANOVA for both studies. For the Fertilizer Only Study, the South Carolina and Alabama data were analyzed separately. To account for pseudoreplication and autocorrelation due to repeated measures, the cell number of each plant was used as a random variable and a correlation structure was included. The

relationship between *P. graminifolia* and *S. americana* height was not examined because of the collinear relationship between *P. graminifolia* height and the fertilizer treatments.

Results: Fertilizer only and fertilizer-host

Fertilizer Only Study

Fertilized *S. americana* from South Carolina were 4.60 mm taller than unfertilized *S. americana* from South Carolina (p= 0.002; Figure 6). Fertilized *S. americana* from South Carolina had 0.92 mm greater leaf length than unfertilized *S. americana* from South Carolina (p= 0.008; Figure 7). *S. americana* from Alabama also exhibited a trend toward greater height when fertilized (p= 0.051; Figure 8). However Alabama seedlings did not exhibit significantly greater leaf length when fertilized.

Fertilizer-Host Study

In the Fertilizer-Host Study, *S. americana* were 1.95 mm taller than unfertilized *S. americana* (p= 0.043; Figure 9). Leaf length did not differ significantly between treatments (p= 0.26).

Discussion: Fertilizer only and fertilizer-host

The fertilizer studies examined the relationship between fertilization and *S. americana* height and leaf length. Seedlings from different seed sources were analyzed separately. *S. americana* from the South Carolina populations responded to fertilizer with greater height regardless of host presence. Leaf length was also larger in the Fertilizer Only Study for South Carolina plants. This suggests that fertilizer does increase seedling size, and is therefore recommended. *S. americana* from Alabama exhibited a trend of greater height with the addition of fertilizer and although the sample size of Alabama plants was less than that for South Carolina, this could still signify a difference in response due to seed source. Mortality in other greenhouse studies was always greater for *S. americana* from Alabama compared with South Carolina (Fuller 2015, unpublished data). The basis for this difference between seed sources is not clear but it may influence responses to treatments.

Preliminary Study 3: Smoke and propagation by stem cuttings

In the greenhouse studies described in previous chapters, *S. americana* grown in the greenhouse were flimsy and did not stand erect. This may have been the result of overwatering and fertilizing. However, when the stems died back due to reduced watering and fertilizing, some regrew more vigorously with more upright growth forms (K.J. Fuller 2015, unpublished data). Some stems had also changed leaf arrangement from opposite-leaved to alternate-leaved. Juvenile individuals are apparently opposite, maturing to alternate-leaved before flowering (J.S. Glitzenstein, per. comm. Fed. 7, 2016). However this observation does not appear in the literature. Stem die-back may be only one method of initiating this shift. Cutting stems at the root collar and allowing them to regrow may also initiate a change in growth form and a shift to alternate leaves.

Natural populations of *S. americana* may experience similar stimuli to the die-back described above as a result of periodic natural or prescribed fires. As fires pass through their habitat, *S. americana* stems are consumed, and they regrow. They may shift to alternate-leaved arrangement in response to fire. Furthermore, smoke treatments often increase growth rates of fire-dependent plant species (e.g. Daws et al. 2007; Abdelgadir et al. 2013). Therefore, applying smoke before cutting stems at the root collar may increase the percentage of plants that regrow and result in alternate-leaved individuals.

In the greenhouse studies in Chapter 3, there were two instances in which a stem of *S. americana* was cut by accident. These cuttings were planted with the hope that they would root. One was placed under misters and rooted without any hormone application. The one without misters did not root. If cuttings can be successfully rooted they may provide an easy, inexpensive method of increasing the numbers of individuals for out-planting. Although this would not increase genetic diversity, it may be an important option for propagators, given *S. americana*'s low survival rate after out-planting (Obee and Cartica 1997).

Methods: Smoke and propagation by stem cuttings

S. americana seedlings from the Fertilizer-Host Study were used in this experiment. After the previous study was completed a total of 76 *S. americana* remained. There appeared to be no confounding effects due to the previous treatments. Four of the seven flats, containing a total of 44 *S. americana* seedlings, were placed in black trash bags filled with smoke on March 15, 2016. Three of the flats, containing 32 plants, were not exposed to smoke. Smoke was generated using *Pinus palustris* Mill. (longleaf pine) needles and a beekeeper's smoker. The bags were tied off and left for twelve hours overnight. The smoke had dissipated or been absorbed by the time the bags were opened the next morning.

All 76 stems from both treatments were then cut at the root collar on March 16, 2016 including the *P. graminifolia* seedlings. *S. americana* stems greater than 2 cm in height were planted in a substrate of 50% peat, 25% perlite and 25% vermiculite and placed under misters that ran three times a day for 3 min. Stems under 2 cm were discarded. The original flats containing the below ground tissue of 76 plants and the 48 planted cuttings were fertilized on March 18, 2016, then again every two weeks until May 15th, 2016. No statistical analyses were applied to the results of this preliminary study.

Results: Smoke and propagation by stem cuttings

By April 4, 2016, 49 out of the 76 *S. americana* in the original flats had regrown, including 27 out of 44 that were smoked (61.4%) and 22 out of 32 that were not smoked (71.9%). Regrown individuals did exhibit more upright growth forms but none were alternate-leaved.

Cuttings averaged 3.85 cm in height at planting. Of the planted cuttings, 42 out of 48 stems showed new stem growth after 19 days, including 20 smoked (86.7%) and 22 not smoked (88.0%). Plants were not unearthed to assess root growth. Overall, the experiment yielded a 15 stem (19.7%) increase in the total number of *S. americana*. Similar values were found for growth rates of smoked and not smoked individuals, and for pre-cutting growth rates and post-cutting growth rates.

Discussion: Smoke and propagation by stem cuttings

Preliminary Study 3 examined the relationship between smoke and the response of *S. americana* seedlings to loss of aboveground biomass and propagation by stem cuttings. Smoke did not increase the percentage of *S. americana* plants that regrew from roots or the percentage of cuttings that put on new growth, so it appears that this treatment is unlikely to benefit propagation efforts. A lack of detectable effect related to smoke in this study parallels the finding of Kirkman et al. (1998) and Norden and Kirkman (2004) that smoke does not increase *S. americana* flowering. Regrown individuals were more upright and less flimsy, so removing above-ground biomass is recommended if researchers encounter similar issues with unstable and flimsy stems and want to improve plant growth forms. However regrown individuals were not alternate-leaved. This may be a result of the plants being younger and smaller than those in the previous greenhouse studies, and therefore less mature. Following planting, cuttings exhibited new stem growth within 19 days without rooting hormone. This resulted in a net gain of stems, despite some loss from the original flats. In order to avoid these losses, propagators could leave part of the original stem intact, replanting just the top 2 cm. This could greatly improve propagation efforts by creating new individuals more rapidly than is possible from seed.

Summary

In Preliminary Study 1, *P. urinaria* exhibited significantly shorter attached *S. americana* than any other host and was out-performed in other analyses as well. It should not be used as a host for *S. americana*. *E. capillifolium* exhibited significantly taller attached *S. americana* than *P. urinaria* and had a greater number of attached *S. americana* than *C. glomeratum* and *E. prostrata*. This suggests that *E. capillifolium* may be worth using as a host for growing *S. americana*.

In Preliminary Study 2, *S. americana* from the South Carolina populations responded to fertilizer with greater height regardless of host presence. South Carolina plants also responded with greater leaf length when fertilized in the Fertilizer Only Study. *S. americana* from Alabama exhibited a trend toward

greater height with the addition of fertilizer (p=0.051) but no difference in leaf length. This suggests that fertilizer does increase seedling size, and is therefore recommended in future production efforts.

In Preliminary Study 3, smoke did not increase the percentage of regrown *S. americana* or the percentage of rooted cuttings, so it is not recommended for future propagation efforts. Planted cuttings exhibited new growth without rooting hormone. This resulted in a net gain of stems, despite some loss from the original flats. Cuttings are therefore recommended as they could be used to improve propagation efforts by rapidly creating new individuals.

The preliminary results from this chapter should be examined further with future studies. Five potential hosts for *S. americana* were examined. Among them *E. capillifolium* appears to be preferred. Future studies should compare *E. capillifolium* with *P. graminifolia*, which is commonly used in *S. americana* propagation. The results showed that fertilizer increases the size of *S. americana* plants. Future studies should examine how fertilizer affects a seedling's tendency to seek out a host or its likelihood to survive after out-planting. In Preliminary Study 3, *S. americana* cuttings rooted successfully. Future research may wish to compare plants originating from seed and those originating from cuttings in regard to host attachment, vigor and survival after out-planting. The results from the above studies provide questions for future research that may benefit out-planting efforts of *S. americana*.

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Table 1. ANOVA p-values comparing number of *S. americana* attached to individuals of five test host species. * Indicates significantly different number of *S. americana* attached per individual host plant.

Host species	*C. glomeratum*	*E. capillifolium*	*E. prostrata*	*P. urinaria*	*Y. japonica*
C. glomeratum	---------------	---------------	---------------	---------------	---------------
E. capillifolium	0.0001*	---------------	---------------	---------------	---------------
E. prostrata	0.9999	0.0012*	---------------	---------------	---------------
P. urinaria	0.1800	0.0002*	0.8580	---------------	---------------
Y. japonica	0.0737	0.2088	0.3057	0.4531	---------------

Table 2. ANOVA p-values comparing mean height of *S. americana* attached to individuals of the above species. * Indicates significantly different mean height of *S. americana*

Host species	*C. glomeratum*	*E. capillifolium*	*E. prostrata*	*P. urinaria*	*Y. japonica*
C. glomeratum	---------------	---------------	---------------	---------------	---------------
E. capillifolium	0.0001*	---------------	---------------	---------------	---------------
E. prostrata	0.3249	0.2963	---------------	---------------	---------------
P. urinaria	0.0081*	0.0001*	0.0067*	---------------	---------------
Y. japonica	0.2098	0.3006	0.9999	0.0019*	---------------

Table 3. ANOVA p-values comparing mean number of leaves per stem of *S. americana* attached to individuals of the above species. * Indicates significantly different mean numbers of leaves per stem of *S. americana*

Host species	C. glomeratum	E. capillifolium	E. prostrata	P. urinaria	Y. japonica
C. glomeratum	---------------	---------------	---------------	---------------	---------------
E. capillifolium	0.1659	---------------	---------------	---------------	---------------
E. prostrata	0.9335	0.8032	---------------	---------------	---------------
P. urinaria	0.0035*	0.0013*	0.1202	---------------	---------------
Y. japonica	0.3040	0.9968	0.9306	0.0.0026*	---------------
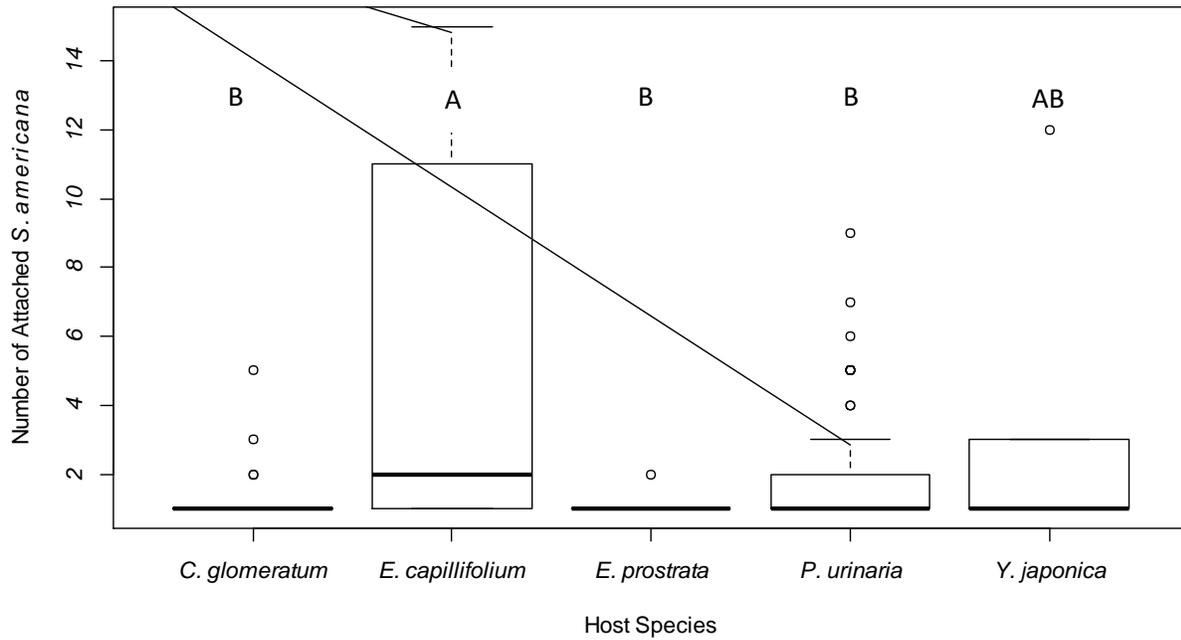
Figure 1. Number of attached *S. americana* per host by host species. Unique letters above bars indicate significantly different results based on 95% confidence.
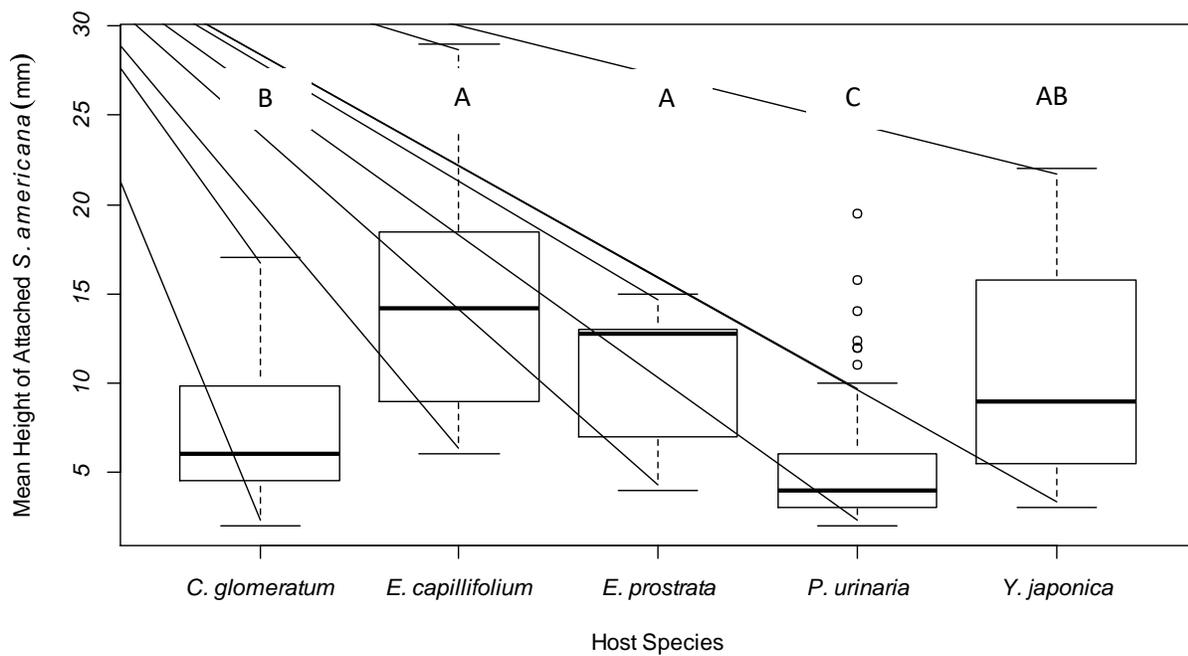
Figure 2. Mean height of attached *S. americana* by host species. Unique letters above bars indicate significantly different results based on 95% confidence.
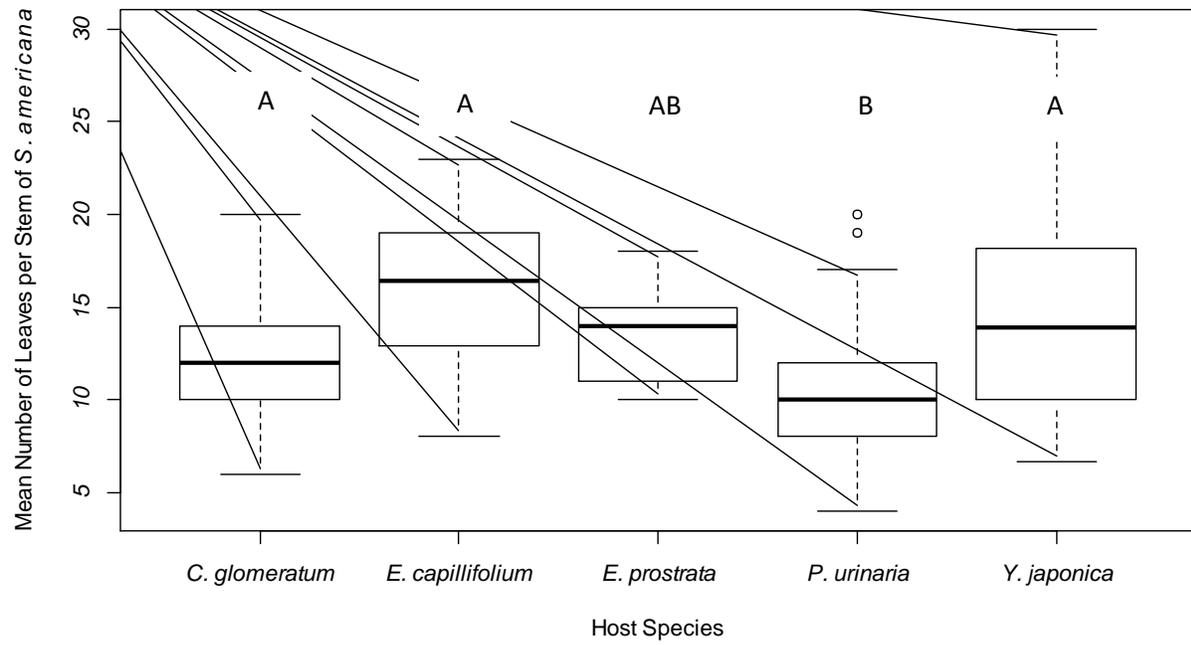
Figure 3. Mean number of leaves per stem of *S. americana* by host species. Unique letters above bars indicate significantly different results based on 95% confidence.
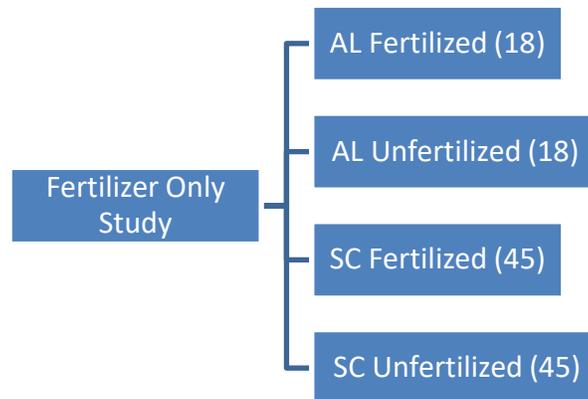
Figure 4. The Fertilizer Only Study using *S. americana* with a nested design without replication. The number in parentheses corresponds to the number of *S. americana* in each treatment group. Each plant in the fertilized groups received 7.4 ml of an 11.23 g/L dilution of Neptune's Harvest Organic Fish Fertilizer with N:P:K ratio of 2:4:1.
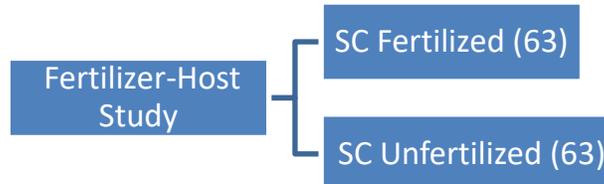

Figure 5. The Fertilizer-Host Study using *S. americana* and *P. graminifolia* with a randomized design without replication. The number in parentheses corresponds to the number of *S. americana* in each treatment group. Each plant in the fertilized group received 7.4 ml of an 11.23 g/L dilution of Neptune's Harvest Organic Fish Fertilizer with N:P:K ratio of 2:4:1.
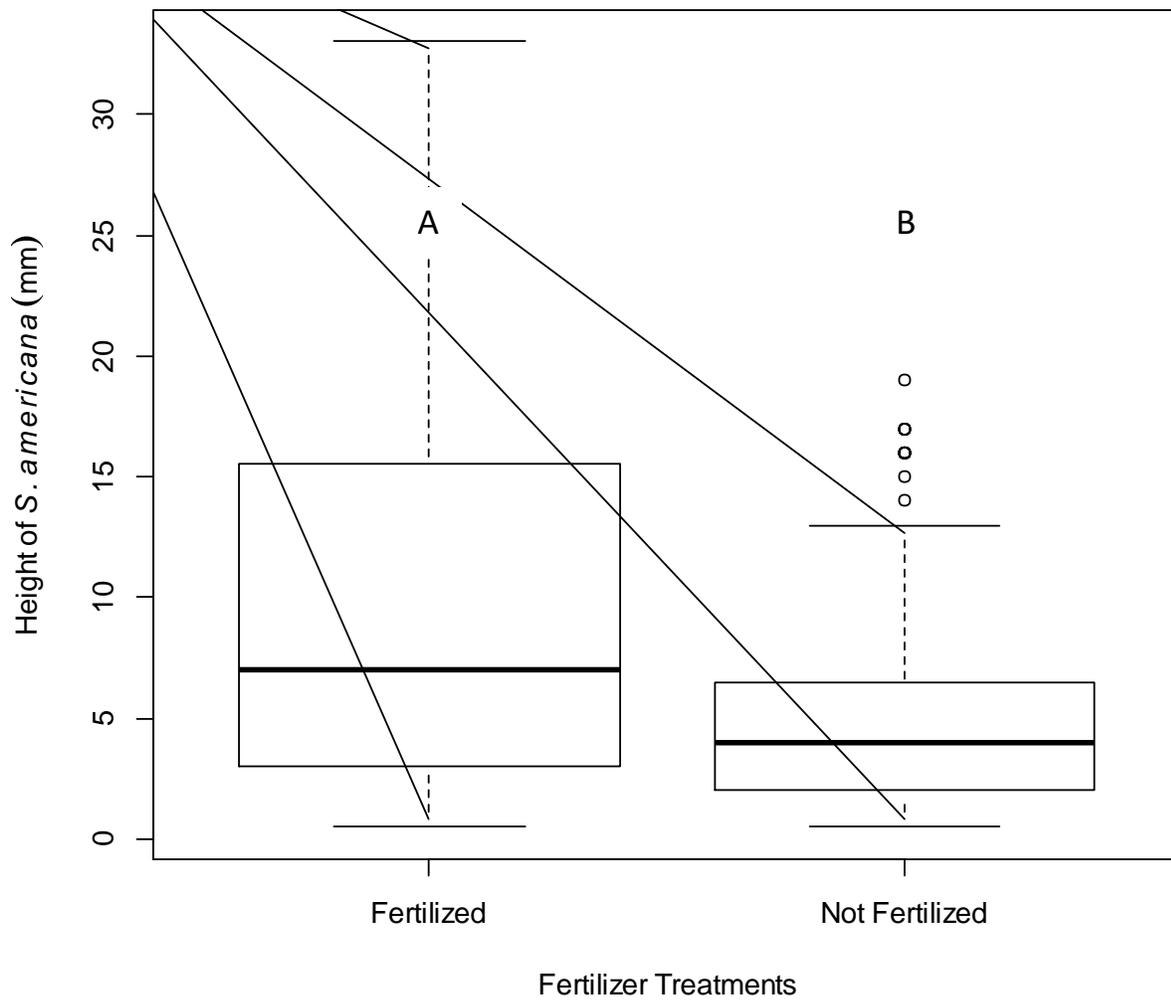
Figure 6. Height of *S. americana* from South Carolina by fertilizer treatment for the Fertilizer Only Study. Unique letters above bars indicate significantly different results based on 95% confidence.
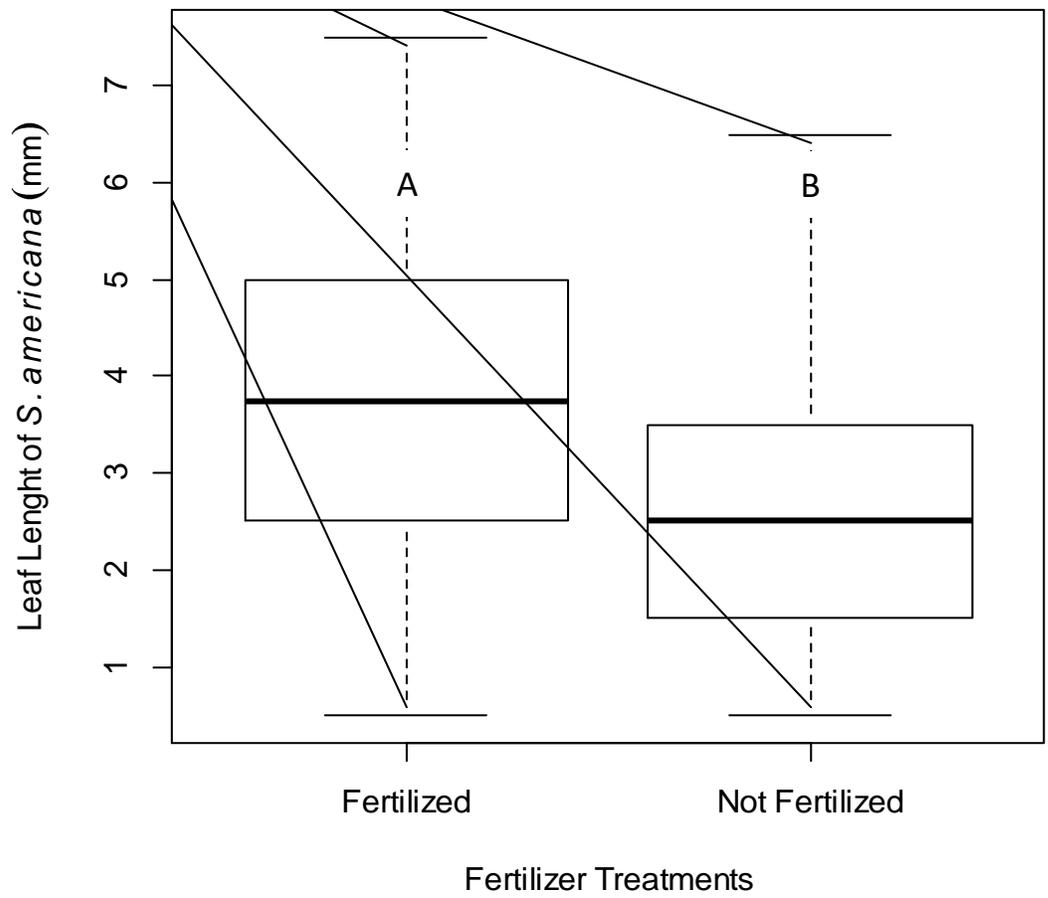
Figure 7. Leaf length of *S. americana* from South Carolina by fertilizer treatment for the Fertilizer Only Study. Unique letters above bars indicate significantly different results based on 95% confidence.
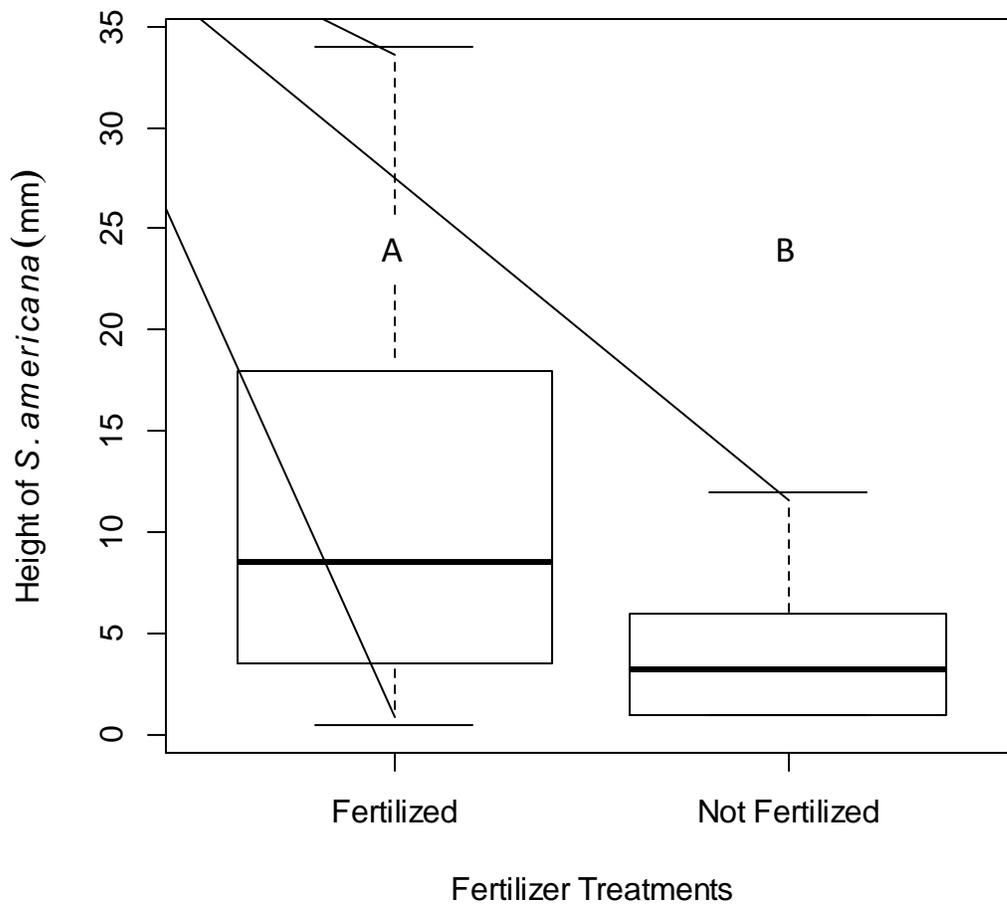
Figure 8. Height of *S. americana* from Alabama by fertilizer treatment for the Fertilizer Only Study. Unique letters above bars indicate significantly different results based on 95% confidence.
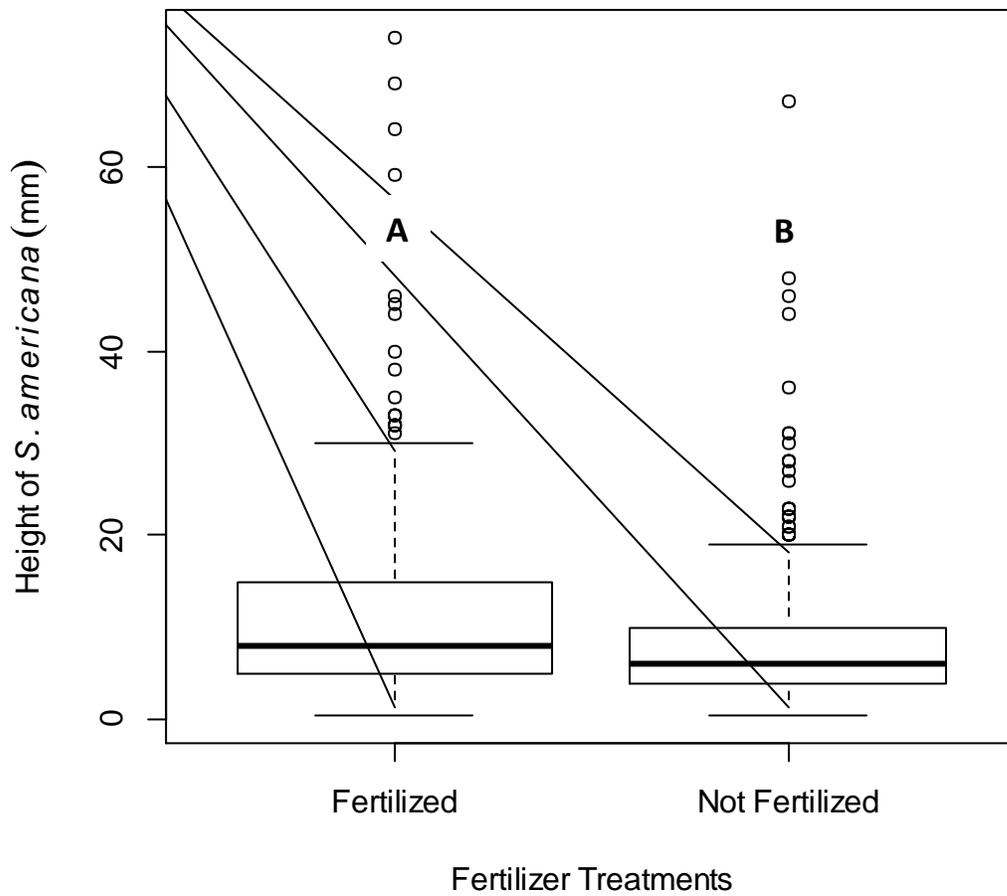
Figure 9. *S. americana* height by fertilizer treatment for the Fertilizer-Host Study. Unique letters above bars indicate significantly different results based on 95% confidence.