Cooperative Research Agreement # 29-1013
Final Report

Dispersal and disturbance as factors limiting the distribution of rare plant species at the Savannah River Site and the Carolina Sandhills National Wildlife Refuge

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Richard Primack
Biology Department
Boston University
5 Cummington St.
Boston, MA 02215

Joan Walker
USDA FS Southern Research Station
Department of Forest Resources
Clemson University
Clemson, SC 29634

Introduction

This report documents the findings of a study conducted under a Cooperative Research Agreement # 29-1013 established with Dr. Richard Primack, Boston University. An Establishment Report for this project was submitted to the Savannah River Site research management staff in 1994.

The report is in manuscript form, but will require revision and additional analyses before submitting to a peer-reviewed journal. The data may be appropriate for submission to *Ecological Restoration*, and may be combined with follow-up studies conducted at the Carolina Sandhills National Wildlife Refuge and funded by the Bureau of Land Management. The combined results of several studies will yield a more significant candidate for publication, for example for submission to *Natural Areas Journal* or *Restoration Ecology*.

Brief Administrative History

The Savannah River Site (SRS) natural resources management staff personnel originally approached Dr. Primack after hearing a research report about his work on establishing new populations of rare plants in deciduous forest of New England. About that time Joan Walker joined the Southern Research Station RWU-4201 (SRS 4201), and was asked to facilitate this agreement. Because Dr. Primack was located in Boston and was not familiar with management in longleaf pine forests in the sandhills, we developed this project in conjunction with another project that was being developed at the time (“Effects of fire regime, midstory removal, and historical land uses on understory composition”). Further, we worked collaboratively to develop the methods and conduct the fieldwork. Seed collections (including locating suitable seed sources) and locating and establishing field plots were completed by SRS-4201 personnel. Boston University and Arnold Arboretum staff completed germination and seedling growth work. Both organizations planted the study; SRS personnel conducted most follow-up monitoring. SRS-4201 personnel prepared the data set for analysis and sent it to Primack’s lab where the analyses included in this report were completed. Dr. Primack and associates prepared the initial draft of the report; Joan Walker revised and submitted the report.

Joan L. Walker
Research Plant Ecologist
USDA FS Southern Research Station

Experimental Establishment of New Populations of 13 Rare Plant Species in Upland Longleaf Pine Forest in South Carolina Sandhills

Richard Primack¹ and Joan L. Walker²

¹ Biology Department, Boston University, 5 Cummington St. Boston, MA 02215.

² Corresponding Author; USDA FS Southern Research Station, Department of Forest Resources, Clemson University, Clemson, SC 29634.

Summary

We conducted an experiment to identify effective methods of creating new populations of herbaceous species in managed upland longleaf pine forest at two locations in the Fall-line Sandhills of South Carolina. We included thirteen species and a variety of site treatments. All sites were burned and lightly raked prior to planting. Sowing seeds on untreated or fertilized treatments resulted in the lowest establishment of all treatments. Digging the planting area to remove belowground plant structures and using hardware cloth cages to exclude potential mammalian seed predators and herbivores led to increased establishment of target species. Establishment was higher using seedling transplants compared to seeds. Success rate was highly variable among sites so population establishment efforts should try to incorporate many sites initially to find the sites that give the greatest chance of success, or increase efforts to carefully identify species' habitat requirements and screen potential sites accordingly. Some species showed very low rates of success despite the variety of methods used; for such species additional work is required on their basic ecology, in particular germination biology and site requirements, as part of a restoration project. The overall low rate of establishment success emphasizes the need to protect and manage existing populations of uncommon Sandhills species, and to recognize that establishing large, long-term, reproducing populations of such species will be difficult.

Introduction

Numerous efforts are currently underway to create new populations of rare species. Progress in this field has been hampered by a short-term perspective, a focus on one species at a time, and a lack of an experimental approach (Falk et al. 1996, Primack and Drayton 1997, Glitzenstein et al. 2001). The present account reports on a 4-year study using 13 species and 7 site preparation treatments at two locations in the Fall-line sandhills of South Carolina. The purpose of using multiple species and methods was to develop general rules regarding the most effective way to create new populations that could guide future efforts to restore desirable native herb populations.

Humans have used longleaf pine forests throughout the Southeastern United States extensively and intensively (Frost 1993). Use by Native Americans and early European populations included agriculture concentrated in the bottomlands, and burning, which undoubtedly affected most of the landscape. By the early twentieth century, much of the original forest had been cleared; natural regeneration was slow and intensive reforestation projects began, on private as well as newly acquired public lands. Many contemporary sandhills landscapes bear evidence of reforestation during the past 70-80 years, and in many cases show the effects of plantation establishment and fire suppression: increased abundances of hardwood and pine, often loblolly or slash pine. There is a widespread perception that forests and woodlands have lost native species especially in the ground layer vegetation, and consequently forest management on many federal lands has shifted to an emphasis on conserving biological diversity and restoring habitat for native species. Additionally, Federal land managers have responsibilities for recovering species protected under the federal Endangered Species Act, for example, the red-cockaded woodpecker (RCW), and are actively managing to restore desirable longleaf pine forest structure, open pine forests, little hardwood mid-story, and diverse herbaceous ground layers. Establishing new populations of uncommon herbaceous species may present unique challenges, such as lack of seed sources or exacting establishment requirements.

This study was designed to determine effective methods to create new populations of uncommon herbaceous species at two federal properties in the Fall-line sandhills of South Carolina: the Savannah River Site (SRS), a National Environmental Research Park, and the Carolina Sandhills National Wildlife Refuge (CSNWR) (Figure 1). The study focuses on 13 species that are characteristic of longleaf pine woodlands in the Sand Hills region (Griffith et al. 2002) (Table 1).

The study had two primary objectives: first to determine whether it was more effective to use seeds or well-established (several-month-old) seedlings to establish new populations. Gathering and planting seeds most closely approximates the natural way in which new populations become established, and may be more economical than producing stock for out-planting. Planting large seedlings has the advantage of bypassing the vulnerable stage of seed germination and initial seedling growth. The second objective was to determine which site treatment would be most effective at increasing the rate of success for establishing new populations. Selected site treatments are simple and are similar in effects to site treatments carried out during silvicultural operations.

Materials and Methods

Study areas

Figure 1 shows the locations of Savannah River Site and the Carolina Sandhills National Wildlife Refuge, occupying the geographic extremes of the Sand Hills ecoregion in South Carolina (Griffith et al. 2001, 2002).

Figure 1. Location of seed collection and planting sites.

Experiments were installed in two compartments at SRS: Compartment 23, Stand 54; Compartment 4, Stand 8. Both were about 30 year old longleaf pine plantations. Hardwood stems were thinned from the sites prior to this study, and the residual pine basal areas was about 50 square feet/ acre of pole timber. Species removed included *Quercus laevis*, *Q. phellos*, *Q. stellata*, *Q. marilandica*, *Q. incana*, *Nyssa sylvatica*, *Sassafras albidum*, *Crataegus* sp., and *Carya tomentosa*. Soils are mapped as the Blanton-Lakeland Association, characterized as excessively drained with a loamy subsoil (as in Compartment 4) or sandy throughout (as in Compartment 23) (Rogers 1990). Herbaceous cover was sparse, owing perhaps to historical agricultural uses and dense accumulation of longleaf pine straw since plantation establishment.

We also set up experimental blocks in two stands at the CSNWR. Sparse, 70-80 year-old longleaf pines dominated both sites, and sparse to dense *Quercus laevis* stems dominated the understory. Oak stems were removed from experimental planting sites. The residual pine canopy was very open, and experimental sites received virtually no shade. There was exposed mineral soil, and a sparse ground cover dominated by *Aristida stricta* and *Gaylussacia dumosa*. Soils in CSNWR sites are mapped as Alpin soils, very deep, excessively drained, moderately permeable entisols on uplands. The stands were naturally established, and it is unlikely that they had been intensively farmed. Turpentine and woodland grazing are more likely historical land uses.

Study sites in both SRS and CSNWR had burned under prescribed conditions in the dormant season preceding planting the experiment (winter 1994).

Seed collection and handling

The study focuses on 13 species that are characteristic of longleaf pine woodlands in the Sand Hills ecoregion (Table 1). All but three, *Polygonella polygonum*, *Amorpha herbacea* var. *herbacea*, and *Eriogonum tomentosum*, are considered “rare” (Kartesz 1999) in some part of their range, but none are protected by federal regulations anywhere. We tried to include species that exhibited a range of ecological characteristics: perennials and annuals; autotrophic and parasitic or hemiparasitic species; upright and trailing habits, strictly herbaceous and woody species. Final selections were guided by seed availability.

We planted all seeds within their natural geographic range, though distances between collection and planting sites varied among species (Table 2). The longest distance between seeds collection sites and planting sites were for *Pityopsis*, *Amsonia* (part of the *Amsonia* seed was also collected at SRS and the two collections were pooled before planting), and *Stylisma* collected at the North Carolina Sandhills Gamelands (NCSGL) and planted at SRS. Except Fort Stewart, seed collection sites were located in the Sand Hills ecoregion (Figure 1) within the Middle Atlantic Coastal Plain. Fort Stewart is located on the Sea Island Flatwoods of the Southern Coastal Plain (Omernik et al. 2001), and only *Seymeria pectinata* was collected there. *Seymeria* grows on well-drained sands in both the Sand Hills and more coastal ecoregions; the only flowering and fruiting populations we could find at the onset of this study were

Block: 2.5 x 25 m; 3 blocks/ stand; 2 stands/ site; 2 sites: Savannah River Site, Carolina Sandhills NWR.

Figure 2. Experimental design.

located at Fort Stewart. Although we would prefer to sow seed from the nearest collection sites possible, we judged that planting Fort Stewart seed for this species in well-marked research sites would not pose any undesirable genetic consequences to any existing populations.

Seeds were collected from natural populations in South Carolina and Georgia during the summer of 1994, and stored dry, under laboratory conditions until planting in field sites in October (at CSNWR) or November (at SRS) 1994; *Seymeria* was planted in March 1995.

Seeds of all species were germinated at Boston University to determine viability, and to produce seedlings for outplanting. In October 1994, seeds were sown onto a sandy soil mixture and placed in growth chambers with 14-hour days at 23°C and 10-hour nights at 15 °C with high humidity. A sample of seeds was also cold stratified in moist sand in the dark at 4 °C. *Nolina*, *Aureolaria*, *Baptisia*, *Phaseolus*, *Amorpha*, *Pityopsis*, and *Eriogonum* exhibited high germination rates (greater than 30%) without cold stratification. Following cold-stratification, a high germination was seen also in *Polygonella* and *Amsonia*. A low germination rate (1-10%) was seen in *Warea*, *Stylisma*, *Hypericum*, and *Seymeria*, and was not improved by cold stratification. Note that *Stylisma* has a hard seed coat characteristic of the Convolvulaceae (Baskin and Baskin 1998), and may have benefited from physical scarification, which was not tested. Germination requirements for *Seymeria*, a rooted hemiparasitic species in the Scrophulariaceae are not reported in the literature. Contact with a host species may be necessary for its germination, but other hemiparasitic genera in the family (for examples, *Agalinis* and *Aureolaria* in our study) are known to germinate without host contact (Baskin and Baskin 1998). We did not attempt to germinate the seeds in the presence of possible hosts or in native soil.

Following germination, seedlings were transplanted to individual “root trainers” to develop an elongate root form suitable for transplanting into a sandy, dry habitat. Starting shortly after germination, seedlings were watered and fertilized minimally to promote the development of “tough” tissues that are likely to survive in natural habitats.

Experimental design and treatments

All sites had prescribed surface fires during the winter (February) of 1994. In October 1994, blocks of experimental quadrats were established in 2 stands at SRS and in 2 stands at CSNWR. Three blocks were set up in each stand; blocks were associated with burn treatments in a companion study, but the burn treatments were not a part of this species establishment study. Thus, at each geographic location six experimental blocks were established. Within each block we established 12 1-m x 2.5-m quadrats (Figure 2); treatments were applied at the quadrat scale and assigned randomly. In most blocks all 12 quadrats were aligned in a contiguous block and separated by 1-m buffers; in several, the blocks were divided but in close (within 5 m) proximity. Each quadrat received one of six soil preparation treatments (treatments were applied two times in each block as insurance against unforeseen disturbance to any one quadrat) for a total of 12 replicates/treatment at SRS and 12/treatment at CSNWR. In addition, about 10 m from each block, we

established two additional quadrats with the midstory layer intact (12 quadrats with intact midstory at SRS; 12 at CSNWR). Each quadrat was divided into 10 0.5-m x 0.5-m subquadrats. We planted each subquadrat with a randomly assigned single species. Because the intent of this work was to evaluate establishment and long-term persistence, and because success rates were very low, we present seedling establishment results only from the final observation date, May 1998. Some additional observations on germination and mortality are included in the narrative text.

Seven experimental (including the intact midstory treatment) applied at the quadrat level are as follows:

1. Control: Light raking to remove leaf litter, followed by planting of seeds.
2. Dig: Light raking, followed by thorough digging with a mattock to a depth of 20 cm. Soil clumps were broken up, and roots and grass removed; the soil was raked again, smoothed and gently patted down, and finally planted.
3. Fertilize: The area was raked, followed by the application of 6 tablespoons of slow release fertilizer pellets (18-6-12, “Osmocote”) per quadrat, an application half of that recommended for fertilizer-sensitive plants. After fertilizer application, the quadrat was lightly raked and planted.
4. Cage: The quadrat was lightly raked and planted. A wire mesh enclosure with the bottom edge buried 10 cm deep into the soil was installed over the planted subquadrat.
5. Dig, Fertilize, and Cage: The area was cultivated as for the “dig” treatment, lightly raked, slow-release fertilizer pellets applied, and planted. The enclosure was installed as above.
6. Seedlings: Several month-old seedlings were planted in the lightly raked “seedling” subquadrat in March 1995. There was little ground disturbance except for the planting hole.
7. Midstory: These quadrats were located in adjacent areas where the midstory oak layer was not removed. The area was lightly raked, and seeds were planted.

Planting consisted of scattering seed, then gently pressing them into the raked and smoothed surface to a depth approximating the diameter of the seed. For *Eriogonum*, *Pityopsis*, and *Stylisma*, the dispersal units included fruit and flower parts; these rough-cleaned propagules were counted for distribution, and at planting time they were crumbled by hand and scattered into planting sites. *Hypericum* fruits were rolled between fingers to break them open and scatter the tiny seeds. Cleaned seeds were planted for the remaining species.

In total 13 species were planted, but not all species were planted at both study areas; 9 species were planted at CSNWR and 10 at SRS. The choice of which species to plant at each site was determined by the availability of seeds and the desire to plant species near to the original seed source (Table 2). *Amsonia*, *Hypericum*, *Pityopsis*, *Seymeria*, *Stylisma*, and *Warea* were planted at both SRS and CSNWR; *Amorpha*, *Eriogonum*, and *Phaseolus* were planted at CSNWR only; and *Aureolaria*, *Baptisia*, *Nolina*, and *Polygonella* were planted at SRS only. The number of seeds planted on each subquadrat was determined by the availability of the seeds, from a high of 700 seeds per subquadrat for *Aureolaria*, to a low of 4 fruits plus contents (# of seeds not determined) per subquadrat for *Seymeria*, with most species in the range of 40 to 100 seeds (Table 3).

Seedlings were planted in March 1995, in the assigned quadrats, which like the seed quadrats had been burned, had the oak understory removed, and had been lightly raked. The number of seedlings planted per subquadrat varied from 1 to 10 depending on the availability of seedlings. For *Amsonia*, which used the most seedlings, there were 10 seedlings per subquadrat, 20 seedlings per block, and 120 seedlings per site, and 240 seedlings for both sites combined. Seedlings were thoroughly watered immediately following transplanting. During the next two weeks, seedlings were again watered one time when there were several days without rain.

Following the initial site treatments, no further treatments were carried out. The sites were monitored during 1995-1998 for the number of seedlings and plants, and their size and reproductive condition. As the purpose of this project was to determine how the treatments affect the ability to develop new permanent populations, the seedling establishment results from 1998 are presented.

Results

Germination in experimental sites was observed as early as December 28, 1994, when 71 *Pityopsis* seedlings were observed in 17 sub-quadrats at SRS. Other germinants were observed, but identities could not be confirmed. On December 29 at CSNWR, 58 seedlings of *Eriogonum* were observed in 19 subquadrats, and were easily recognized by their large flattened cotyledons. Additional germinants included 3 *Pityopsis* in 3 sub-quadrats, 6 *Amorpha* in 6 sub-quadrats, 1 *Amsonia*, 3 *Phaseolus*, 3 *Stylisma*, 1 *Warea* individual, and 1 *Hypericum*. Earlier germination at CSNWR than at SRS may be related to higher light and warmer soil conditions during the autumn. Comparatively warmer conditions might be expected in the absence of a canopy in the CSNWR experimental sites. By April 1995 *Amsonia*, *Nolina*, *Polygonella* and *Pityopsis* were germinating despite a 5-week drought, and *Baptisia* was just beginning to germinate at SRS. At CSNWR, *Amsonia* and *Amorpha* germination continued; most *Amorpha* seedlings germinated where turkey oak leaves covered the quadrats, suggesting a need for constant moisture conditions, perhaps for prolonged periods, for successful germination. *Eriogonum* seedlings established in March showed signs of stress and some mortality, possibly associated with the preceding 5 weeks without precipitation. Although most germination was complete by May 1995, *Stylisma* continued to germinate through October 1995, a year after sowing. *Stylisma* is a member of the Convolvulaceae, known for hard seed coats (Baskin and Baskin 1998); long exposure to wetting and drying may be required to break down the seed coat before germination can proceed. These observations

confirm the viability of planted seeds and the presence of suitable safe sites for germination, though not necessarily successful establishment. The late fall germination of some species suggests a lack of dormancy mechanisms, or that dormancy requirements were met prior to or during storage, in a variety of sand hills species, consistent with observations of others (Walker and van Eerden, unpublished data) and with greenhouse germination trials for this study.

By May 1998, remarkably few subquadrats were occupied. At CSNWR, there were no individuals of *Amorpha*, *Phaseolus*, *Seymeria*, or *Warea*, and only a single plant of *Hypericum* (Table 3). Considering just *Amorpha*, a total of 7200 seeds were planted in 72 subquadrats, and 72 seedlings planted in 12 subquadrats, but there were no surviving plants in 1998. For *Pityopsis*, nine of the 12 subquadrats planted with seedlings still had plants in 1998, but none of the quadrats planted with seeds had plants. For *Stylisma* and *Eriogonum*, most of the quadrats occupied came from planted seedlings, though a few came from seeds. Only in *Amsonia* were there large numbers of plants from planted seeds.

The results at SRS are similar, with many species including *Aureolaria*, *Baptisia*, *Hypericum*, *Seymeria*, and *Warea* with a very low rate of success. Somewhat higher success is evident for *Amsonia*, *Nolina*, and *Polygonella*, with *Pityopsis* and *Stylisma* intermediate. In general, the rate of success at SRS is somewhat higher than at CSNWR. Seedlings at SRS persisted longer, perhaps because site conditions at SRS were less severe with a pine canopy to ameliorate air and soil temperatures, and finer textured soils with incorporated organic matter to retain moisture.

Certain treatments were more effective than others are for establishing plants from seed (Figures 3 and 4). Simply sowing seeds onto the ground, with or without the mid-canopy intact did not create suitable conditions for seed germination and growth. The most effective treatment was to remove the mid-canopy and dig up (cultivate) the soil. This was more effective than only fertilizing the soil surface or only excluding seed predators with exclosures. These results demonstrate that nutrients and herbivore-pressure were not limiting factors at these sites. In addition, combined digging, fertilizer, and exclosures did not stimulate germination beyond digging up the surface itself. Although cultivation benefited establishment from seeds, establishment from seed was low even in this treatment, and varied among species. For *Amsonia*, the most successful species, only 14 of 24 quadrats were occupied, with only about 5 seedlings per quadrat. For *Nolina*, the second most successful species, only 5 of the 12 quadrats were occupied, with 5 seedlings per quadrat.

For some species, installing cages was associated with improved establishment. For *Nolina*, planting in the exclosure resulted in 9 quadrats occupied and 9 seedlings per quadrat. For *Polygonella*, the combined effects of digging, fertilizer, and exclosure stimulated the most seedling establishment. The exclosure (cage) effect may represent escape from seed predators and herbivores; however, we note that installing the cages required digging a trench (10-15 cm deep) to bury the bottom of the cage. The small-scale “trenching” may have produced the same effect as cultivating the planting site.

Figure 3a. % Occupancy by treatment in May 1998 of subquadrats planted at Savannah River Site. Control conditions and soil treatments are as described in the text. 'All' refers to the combination of digging, caging, and fertilization. 'Midstory' refers to sites with an intact oak midstory. The 'seedling' treatment are plots where greenhouse-grown seedlings were out-planted.

Figure 3b. % Occupancy by treatment in May 1998 of subquadrats planted at Carolina Sandhills National Wildlife Refuge. Control conditions and soil treatments are as described in the text. 'All' refers to the combination of digging, caging, and fertilization. 'Midstory' refers to sites with an intact oak midstory. The 'seedling' treatment are plots where greenhouse-grown seedlings were out-planted.

Overall, the number of subquadrats occupied in 1998 was far greater for subquadrats planted with seedlings than subquadrats planted with seeds. Out of 108 subquadrats planted with seedlings in 1995 at CSNWR, 26 still had seedlings in 1998; by contrast only 9 out of 108 subquadrats dug up and planted with seeds had seedlings in 1998. This same pattern holds true at SRS; 34 out of 120 quadrats planted with seedlings in 1995 still had seedlings in 1998. In contrast, success was lower for the treatments in which seeds were used.

Survival of planted seedlings varied greatly among species. Seedling survival was greatest for *Pityopsis* at SRS at 63% and *Amsonia* at SRS at 22.5%. In some species, no seedlings survived by 1998. Two days of unseasonably cold weather about 2 weeks after outplanting in March likely killed outright or top-killed some seedlings. A few *Phaseolus* seedlings, topkilled in March at CSNWR, resprouted from the base by mid-April. These large bean seedlings were the only species observed re-sprouting at this time. *Amorpha* seedlings planted at the CSNWR were among the first to succumb, coinciding with the 5 weeks of drought following March planting. Seedlings at SRS persisted longer, perhaps because site conditions at SRS were less severe with a pine canopy to ameliorate air and soil temperatures, and finer textured soils with incorporated organic matter to retain moisture.

The ultimate measure of success in creating new populations is the growth of plants to reproductive size and the formation of a second generation of plants. An experiment lasting four years is still too short to determine if any of the species will form permanent populations at these sites, however there are some indications of success. At SRS, several of the *Amsonia* plants on the digging treatments were large and multi-stemmed; these plants were large enough to flower, although they were not observed to do so. Three *Pityopsis* plants resulting from planted seedlings were in flower in 1998. At SRS, small plants of *Pityopsis*, *Polygonella*, *Amsonia*, and one *Hypericum* plant occur well outside of the subquadrat where they were planted either due to dispersal from reproducing plants or movement of seeds planted in 1994.

Sites were monitored monthly during the 1995 growing season, and once or twice each year from the spring 1996 to May 1998. The counts in 1995 were greatest, with large numbers of small seedlings recorded. By 1998, the numbers had become considerably reduced. However, in general, the subquadrats with the most plants in 1998 were the ones in which there were many seedlings in 1995. For example, for *Amsonia* at CSNWR, for the control, dig, fertilizer, exclosure, dig-fertilizer-exclosure, and mid-canopy intact treatments, the numbers of seedlings were 0, 27, 4, 15, 37, and 5 in 1995, and 0, 27, 0, 9, 21, and 0 in 1998. In general, subquadrats with few seedlings in 1995 had no plants in 1998, and quadrats with many seedlings in 1995 tended to still have seedlings in 1998. For example, for *Amsonia*, 5 of the 6 quadrats that had fewer than 10 seedlings in 1995 had no seedlings in 1998; in contrast the four quadrats with the most seedlings in 1995 all still had large numbers of seedlings in 1998.

Discussion

This study identifies several basic principles to consider in any attempt to create new populations in upland Sand Hills habitats, or more generally in other habitats with rare plant species.

First, it is extremely difficult to create new populations of rare species, as has been seen in other projects (Pavlik 1996, Primack 1996, Glitzenstein et al. 2001). When the objective is to establish new populations in unoccupied sites, it is sometimes useful to understand the causes for the target species' absence. In our study sites, as in many places, the causes for target species' absence and the limits to establishing populations are unknown. There are no historical records for the presence of any of the target species in the experimental sites. If they previously occupied the site, historical land uses, likely cultivation for agriculture and livestock grazing, may have eliminated them. Without a local seed source, establishment would not be possible. Even with seed the current site conditions may not provide necessary safe sites for germination and establishment. The observed persistence of greenhouse grown seedlings suggest that site conditions may support adults, but the safe sites for germination were lacking, except as created by selected treatments for selected species. In only a few species and sites did significant numbers of plants grow to a large size and flower after four years. Only a few subquadrats indicate a possible second generation of seedlings. In light of similar results, Falk and Olwell (1992) concluded that creating new populations is so uncertain that it should only be the strategy of last resort, with highest conservation priorities assigned to protecting existing populations of rare and endangered species.

Different sites have very different rates of success, and success varied among species. Overall, plantings at SRS were more successful than at CSNWR, suggesting a better match between target species habitat requirements and site conditions. Even within each site, certain blocks had better success than others did. In particular, the failure of the legumes, *Amorpha* and *Baptisia*, even when germination rates were high, may indicate unsuitable site conditions. In upland sandhills communities, legumes typically are associated with loamier soils, and often in swales or flats where moisture and organic matter can accumulate (Peet and Allard 1993, Hains et al. 1999, James 2000). Notably, the natural stands surrounding experimental blocks in the CSNWR, lacked legumes except *Galactia regularis* and *Baptisia cinerea*, typical of the most xeric sites at CSNWR (James 2000). A variety of legumes, including *Lespedeza* spp., *Desmodium* spp., and *Galactia* spp., were growing near the SRS sites, suggesting more moderate soil conditions. Careful attention must be directed toward understanding the site factors that determine successful establishment. These factors include, for example, the degree of shade, degree of competition, soil characteristics, soil moisture, herbivory, and level of disturbance (Fiedler and Laven 1996). The use of indicator species can help to identify potential sites for new populations. However, even with the best background work it may be difficult to identify potentially suitable sites with a high degree of certainty. As a result, it is probably best to select numerous sites for an initial planting, followed by a more intensive planting in those sites that give some initial success.

Results will be species specific, and some species are very difficult to work with. In this project *Amsonia*, *Nolina*, and *Polygonella* gave fairly good levels of seed germination in the field and in controlled growth chambers, and transplanted seedlings survived well outside. Other species, such as *Amorpha*, *Aureolaria*, *Phaseolus*, *Hypericum*, *Seymeria*, and *Warea* showed none or very few seedlings in quadrats where seeds were sown and had very low rates of survival of transplanted seedlings. Species that failed included the annuals, *Warea* and *Seymeria*, also hemi-parasitic. *Aureolaria*, another hemi-parasitic annual, survived at low levels (1 or 2

individuals) in most treatments at SRS. Its success is difficult to judge, however, because a resident population was discovered after the experiment was installed. Surviving individuals may have established from local seed inputs, rather than from the intentionally planted seeds or seedlings. Annuals must successfully produce viable seeds that must germinate and resulting seedlings establish successfully, if the species is to persist. Multiple introductions in consecutive years may be necessary for establishing annuals. The failures of *Seymeria* and *Aureolaria* (as planted seedlings) may represent the failure to establish haustorial connections with host species that include a variety of common grasses and pines. Although hosts were likely present in the site, introduced seeds or seedlings may not have been in the proximity necessary for successful association. Species with special nutritional needs or dependent on other species (for examples, mycorrhizal fungi, pollinators, and dispersal agents) are likely to be difficult to establish. Species may be “difficult” if habitat requirements are difficult to achieve. As previously noted, legume failure may be related to unsuitable site conditions, rather than low seed germinability. For example, *Amorpha* seeds germinated very well in controlled conditions, but of 7,200 seeds sown at CSNWR, not a single seedling resulted. Moreover, out of 72 seedlings planted, not one was alive in 1998. A similar low rate of success was seen for *Phaseolus* despite the high germination in controlled conditions. In contrast, two of the most successful species, *Nolina* and *Polygonella*, are abundant in disturbed locations at Fort Gordon, just 60 km northwest of SRS (personal observation). For these two species, seed availability may be main factor determining distributions at SRS.

Where success is needed for a difficult species, two obvious courses are possible. One, increase the knowledge and understanding of the species’ biology, particularly the topics of seed germination, early seedling ecology, and population ecology (Guerrant 1996). The second option is to overcome low rates of success by using more individuals (more seeds, seedlings, or adults), or more sites, thereby increasing the likelihood that habitat requirements will be met somewhere.

Planting young plants gives a higher rate of success than sowing seeds. The most successful method of establishing new individuals was using seedling transplants. Approximately 25% of quadrats on which seedlings were transplanted still had plants present three years later. There was a lower rate of success using seeds planted at the site. The success of each seedling planted was vastly greater than each seed planted; for example, out of 120 *Amsonia* seedlings planted at SRS in 1995, 27 (about 25%) were still alive three years later. In contrast, out of 3,384 seeds planted, 89 seedlings (about 3%) were present in 1998. For *Stylisma*, out of 108 seedlings planted, 22 (about 20%) were alive in 1998; in contrast, of 7,200 seeds planted, only 19 seedlings resulted, a success rate of around 0.3%. From a restoration perspective, we note that although outplanting seedlings may result in “populations” of target species in the short term, failure to regenerate will ultimately doom any new populations.

Despite the generally lower rate of success using seeds to start new populations, certain site treatments can dramatically enhanced the successful establishment of plants using seeds. Sowing seeds on undisturbed bare soil is not an effective way to create a new population, even a site that has been burned and lightly raked; fertilization does not increase success. Of the tested methods, digging to remove coarse plant debris and roots, followed by light raking to smooth the surface maximized success. Increased establishment may result from increased availability of soil moisture after competing roots were removed. This result is consistent with Harrington

and Edwards (1999) who showed that trenching to reduce belowground competition enhanced survival and growth of out-planted greenhouse-grown seedlings under a young pine stand. Our results suggest increased soil moisture may also promote establishment from seeds. In some cases exclosures also enhance seedling germination; the reasons for this are not certain but in addition to protecting seeds from predation, the exclosures may prevent herbivory from rabbits and deer, may provide some shade from the direct sun, and the trenching associated with installing the cages may have reduced belowground competition, similar to the digging treatment.

Conclusions

The purpose of this project was to investigate the most effective methods for re-establishing uncommon native wildflower species in Carolina Sandhills longleaf pine communities. Based on results we make the following recommendations:

1. First, establishing new populations of uncommon sandhills species is not easy. The success rate is so low it is better do everything possible to protect, manage, and expand existing populations. The success rates for certain species are so low, that considerable effort should be focused on learning about the biology of the species as part of the recovery effort, particularly in germination ecology and site requirements.
2. The chances of success are highly variable among locations. Careful site selection may improve the chances of success, but it is useful to choose many sites initially and focus more efforts on those sites that give some initial success.
3. Success rates are far greater using plants rather than sowing seeds (Vasseur and Gagnon 1994). Planting several month old seedlings bypasses the highly vulnerable germination and establishment phases. However, unless sites provide opportunities for future establishment, new populations will not persist past the first generation.
4. Site treatments can enhance establishment of some species (Guerrant and Pavlik 1998), specifically, in this study, by digging up the soil (removing above- and below-ground competition) and excluding mammalian herbivores or seed predators. While not tested in the experiment, the initial burning that removed the litter layer, raking the site, and removal of sub-canopy including shrubs probably also facilitated seedling establishment.
5. Large numbers of individuals are needed. The goal of a reintroduction is to establish a viable, self-sustaining, reproducing population. If the goal is 500 individuals, which is reasonable for a perennial plant species, then how many seeds or seedlings should be planted? If the success rate of seeds developing into plants is around 0.3 to 3%, then 15,000 to 150,000 seeds should be planted. For seedlings with a 10 to 30% rate of becoming established plants, 1,500 to 3,000 plants should be planted.

6. The final point to make again is that establishing new populations of uncommon sandhills perennial herbs is extremely difficult. Effective site selection, appropriate site treatment, and use of large samples of seeds and plants can increase the success. However, protecting and managing extant populations of these species should receive the highest priority.

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Table 1. Names and growth habits of plants and sites of seed collections used in the study. South Carolina collection sites included Carolina Sandhills National Wildlife Refuge (CSNWR), Savannah River Site (SRS), and Peachtree Rock Preserve (PTR); in North Carolina, the NC Sandhills State Gamelands (NCSGL); in Georgia, Yuchi Wildlife Management Area (YWMA), Fort Gordon (FG), and Fort Stewart (FS). Taxonomy follows Kartesz (1999).

Species (Family)	Common Name	Growth Habit	Seed Source
<i>Amorpha herbacea</i> var. <i>herbacea</i> (Fabaceae)	Cluster-Spike Indigo-bush	Suffrutescent perennial	CSNWR
<i>Amsonia ciliata</i> (Apocynaceae)	Fringed Blue Star	Perennial herb	SRS, NCSGL
<i>Aureolaria pectinata</i> (Scrophulariaceae)	Comb-leaf Yellow False Foxglove	Annual, parasitic or hemiparasitic herb	SRS
<i>Baptisia lanceolata</i> (Fabaceae)	Yellow Wild Indigo; Gopherweed	Perennial herb	SRS, FG, YWMA
<i>Eriogonum tomentosum</i> (Polygonaceae)	Sandhill Wild Buckwheat	Perennial herb	SRS
<i>Hypericum lloydii</i> (Clusiaceae)	Sandhill St. John's-wort	Low evergreen shrub	PTR
<i>Nolina georgiana</i> (Liliaceae)	Georgia Bear-grass	Perennial herb	YWMA
<i>Phaseolus polystachios</i> var. <i>sinuatus</i> (Fabaceae)	Sandhill Bean	Trailing, herbaceous perennial vine	NCSGL
<i>Pityopsis pinifolia</i> (Asteraceae)	Taylor County Silk-grass	Perennial herb	NCSGL
<i>Polygonella americana</i> (Polygonaceae)	Southern Jointweed	Evergreen sub-shrub	SRS
<i>Seymeria pectinata</i> (Scrophulariaceae)	Comb Black-senna	Annual, parasitic or hemiparasitic herb	FS
<i>Stylisma pickeringii</i> var. <i>pickeringii</i> (Convolvulaceae)	Pickering's Dawnflower	Trailing, herbaceous perennial vine	NCSGL
<i>Warea cuneifolia</i> (Brassicaceae)	Carolina Pineland Cress	Annual herb	FG

Table 2. Seed collection sites. Species (designated by genus name; Table 1) collected at each site and distances (km) from each collection site to both planting sites are given. Seeds for the same species with multiple collection sites were mixed before planting (*Amsonia*, *Baptisia*).

Collection Site	Species collected	Distance to:	
		SRS, km	CSNWR, km
SRS	<i>Amsonia</i> , <i>Aureolaria</i> ¹ , <i>Baptisia</i> ¹ , <i>Polygonella</i> ¹	0	197
CSNWR	<i>Amorpha</i> ²	197	0
Yuchi WMA	<i>Baptisia</i> ¹ , <i>Nolina</i> ¹	22	219
Ft. Gordon	<i>Warea</i>	60	231
Ft. Stewart	<i>Seymeria</i>	142	318
Peachtree Rock Preserve	<i>Hypericum</i>	76	122
NC Sandhills Gamelands	<i>Amsonia</i> , <i>Phaseolus</i> ² , <i>Pityopsis</i> , <i>Stylisma</i>	272	76

¹ Species planted only at Savannah River Site.

² Species planted only at Carolina Sandhills National Wildlife Refuge.

Table 3. Number of subquadrats occupied and numbers of seedlings established as of 1998. For each species (designated by genus; see Table 1 for scientific names) and site, the number of propagules (rough-cleaned seeds, some including flower and fruit parts) sown and greenhouse grown seedlings out-planted per sub-quadrat are given. For each treatment the number of quadrats with at least 1 seedling in 1998 and the number of seedlings (in parenthesis) are reported.

A. Carolina Sandhills National Wildlife Refuge

Species	Propagules Planted	Treatment						Seedlings Planted	Seedlings
		Control	Dig	Fertilize	Cage	Dig, fertilize, cage	Mid-canopy		
<i>Amorpha</i>	100	0	0	0	0	0	0	6	0
<i>Amsonia</i>	45	0	6(27)	0	0(3)	3(21)	0	6	7(16)
<i>Eriogonum</i>	50	0	1(2)	0	0	1(1)	0	8	5(12)
<i>Hypericum</i>	50	0	0	0	0	0	0	1	1(1)
<i>Phaseolus</i>	15	0	0	0	0	0	0	10	0
<i>Pityopsis</i>	50	0	0	0	0	0	0	2 or 3	9(14)
<i>Seymeria</i>	4	0	0	0	0	0	0	2 or 2	0
<i>Stylisma</i>	50	0	2(4)	0	1(1)	0	0	4 or 5	4(10)
<i>Warea</i>	50	0	0	0	0	0	0	0 or 1	0
Site Total		0	9(33)	0	4(10)	4(22)	0		26(53)

B. Savannah River Site

Species	Propagules Planted	Treatment						Seedlings Planted	Seedlings
		Control	Dig	Fertilize	Cage	Dig, fertilize, cage	Mid-canopy		
<i>Amsonia</i>	47	0	8(38)	4(15)	4(15)	4(21)	0	10	7(27)
<i>Aureolaria</i>	700	2(2)	1(1)	1(2)	1(1)	0	1(1)	5	0
<i>Baptisia</i>	45	0	2(3)	0	2(3)	1(1)	0	5	1(1)
<i>Hypericum</i>	50	0	1(2)	0	0	0	0	1	0
<i>Nolina</i>	100	1(1)	5(27)	0	9(81)	4(6)	1(3)	10	7(21)
<i>Pityopsis</i>	50	0	4(12)	0	0	3(4)	0	2 or 3	6(19)
<i>Polygonella</i>	100	2(5)	4(13)	2(13)	3(28)	4(50)	2(14)	10	8(23)
<i>Seymeria</i>	4	0	0	0	0	0	0	1 or 2	0
<i>Stylisma</i>	50	0	3(3)	0	2(9)	1(2)	0	4 or 5	5(12)
<i>Warea</i>	50	0	0	0	0	1(1)	0	1 or 2	0
Site Total		5(8)	28(99)	7(30)	21(137)	18(85)	4(18)		34(103)

Figure 1. Locations of experimental locations and seed collection sites.

Figure 2. Experimental design.

Figure 3a. Proportions of experimental subquadrats planted in spring 1995 at Savannah River Site occupied by established seedlings in May 1998. For each treatment 12 sub-quadrats of each species were planted.

Figure 3b. Proportion of experimental subquadrats planted in spring 1995 at Carolina Sandhills National Wildlife Refuge occupied by established seedlings in May 1998. For each treatment 12 sub-quadrats of each species were planted.