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Seed Germination and Seed Bank of Scutellaria Species

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Seed Germination and Seed Bank of *Scutellaria* Species

by

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ABSTRACT

Florida skullcap, *Scutellaria floridana* Chapm. (Lamiaceae), is a federally threatened species found in the Florida Panhandle. Its habitat is a fire-prone, longleaf pine forest dominated by *Aristida stricta* Michx. var. *beyrichiana* (wiregrass). Other *Scutellaria* species used in the study are found in southeastern U.S., including two within the Florida Panhandle. The objective of this study was to compare several techniques to germinate seeds of *Scutellaria incana*, *Scutellaria lateriflora*, *Scutellaria ovata* ssp. *ovata*, and *Scutellaria floridana*, and to access the presence of a seed bank for *S. floridana*. For the germination study, *Scutellaria lateriflora* and *S. ovata* ssp. *ovata* were stratified for one and two months at 4^{o}C, whereas *S. floridana* was stratified for 1.5 months. All four species were scarified with concentrated sulfuric acid for various times (15 to 60 minutes). Smoke treatments were: Wright's® Natural Hickory Seasoning (1:100 to 1:1000) for all species, and also plant debris smoke (half and full strength) for *S. floridana*. Only *S. floridana* seeds were soaked in gibberellic acid (500 mg/L GA_3) and tested for seed viability (40%). For each treatment, 3 replications of 10, 25 or 50 seeds per Petri dish with moistened filter paper were used depending on the species. After dusting with Thiram to prevent fungal contamination, dishes were placed in colorless tubs in a seed germination chamber with 16 hours light at 17 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and 25^{o}C. Germinated seeds were counted for 30 days. Arcsine transformation of germination percentages were analyzed with a one-way analysis of variance at 5% level. The best treatment for all species, excluding *S. floridana*, was 15 minutes acid scarification for *S. lateriflora* with all other treatments yielding 13% germination or less for the 3 species. Control seeds of *S. floridana* germinated significantly higher than seeds with

stratification, scarification, and plant debris smoke solutions, but were similar with Hickory Smoke Seasoning and GA_3. For the control of *S. floridana*, 83% of viable seeds germinated. For the seed bank study, soil samples were collected in 2 years from 8 different sites with *S. floridana* in Florida by scraping the soil surface near plants to collect 600 mL per site. The first seed bank soils were collected in February 2012 and the second seed bank soils in December 2012 – January 2013. Soil texture particles varied from 0-100% sand, 0-88% silt, and 0-38% clay. Soil pH ranged from 4-5, and soil moisture was between 17-63%. Planting pots were filled with sterilized sand and a layer of soil on top, and placed in a growth chamber at 25^{o}C with 16 hours light at 90 µmol/m^2/sec for 64 days. Six replications per site were used. Emergence was recorded as either monocot or dicot seedlings. A total of 11 dicots and 7 monocots emerged from all three sites in the first study. For the second study, 73 monocots and 34 dicots emerged from the 7 sites. No seedlings of *S. floridana* emerged after 64 days. Overall, seeds of *S. floridana* responded differently than the other three *Scutellaria* species. Based on lack of dormancy, seeds of *S. floridana* are likely to germinate, but its seedlings were not observed in the seed bank, suggesting no seeds were present or seeds were not able to germinate and emerge in our conditions.

INTRODUCTION

Study Species

Florida skullcap, *Scutellaria floridana* Chapm. (Lamiaceae), is a federally threatened perennial forb in the Florida Panhandle where only a few populations exist, including only seven populations that are under protection (U.S. Fish and Wildlife Service 2009). Bay, Gulf, Franklin, and Liberty are counties within Florida that contain this species. The reason for the decline in populations of *S. floridana* is due to habitat destruction and modification such as urban development, timbering, and fire suppression (U.S. Fish and Wildlife Service 2009).

Scutellaria floridana can be found in grassy, longleaf pine forests and savannas, once frequented by fires and also can be found in bogs (U.S. Fish and Wildlife Service 2009; U.S. Fish and Wildlife Service 1994). Prior to settlement, regular fires throughout the Florida Panhandle resulted in a less intense fire. Several factors of fires are unknown, but a fire creates disturbance in an area allowing new plant growth (Glitzenstein *et al.* 1995). Fires also reduce shrub and new tree seedling growth, thus reducing competition from species such as *Cyrilla racemiflora* (swamp titi) (U.S. Fish and Wildlife Service 2009; Hessl and Spackman 1995). Few nutrients are found in the acidic, sandy, wet soil where *S. floridana* is found. This species grows in full sun to light shade conditions (U.S. Fish and Wildlife Service 2009). The main understory vegetation in the habitat of *S. floridana* is *Aristida stricta* Michx. var. *beyrichiana* (Trin. & Rupr.) D.B.Ward (wiregrass) with an overstory containing several pine species (Estill and Cruzan 1999).

Scutellaria floridana has unbranched roots extending from rhizomes. Stems of *S. floridana* are purple at the base of the stem and gradually change to green near the top of the stem (Kral 1983). The opposite leaves of this species do not have the mint smell

commonly noted with other species in this family. The mint family, Lamiaceae, is also characterized by square stems and has flowers with disproportionate lips (Springer 1997). *Scutellaria floridana* blooms in the spring and summer with blue/purple flowers having a white middle lower lip (U.S. Fish and Wildlife Service 1994). Muricate nutlets develop and mature in June (Campbell and Peterson 2007).

Similar to *S. floridana*, hoary skullcap, (*Scutellaria incana* Biehler), blue skullcap, (*Scutellaria lateriflora* L.), and heartleaf skullcap, (*Scutellaria ovata* Hill ssp. *ovata*) were other species used in this study for comparison to the focal species (*S. floridana*). These species are found in southeastern U.S., with two species found in Florida (USDA, NRCS 2013). Due to the similarity of habitats and relatedness of these species (*S. incana*, *S. lateriflora*, and *S. ovata* ssp. *ovata*), techniques performed on these 3 species where seed is more abundant, may provide insight relative to *S. floridana* where seed is limited.

Seed Germination

Due to the rarity of *S. floridana*, it is important to understand some aspects of its seed biology including if the seeds of this species have dormancy and if they are persistent in its habitat. Techniques commonly used to break seed dormancy include: stratification, scarification, smoke treatments (for species in fire prone habitats), and gibberellic acid (GA_3). Stratification is a common technique used in seed ecology studies where seeds of some species need a cold, moist treatment to simulate winter conditions. The winter conditions can cause the expanding and contracting of the seed coat. Exposing seeds of *Galeopsis speciosa* Mill. (Lamiaceae) to stratification increased seed germination where long periods of stratification yielded the highest germination

percentages (Karlsson *et al.* 2006). Another method which can increase seed germination is scarification. By damaging the seed coat, or breaking the seed coat, germination may be induced. Seeds from *Callicarpa americana* L. (Lamiaceae) were submerged in concentrated sulfuric acid for various lengths of time which yielded higher germination than the control (Contreras and Ruter 2009).

Other techniques which could be beneficial for *S. floridana* are the use of smoke treatments. Because *S. floridana* inhabits areas where fire historically occurs, liquid smoke or smoke from burning plant debris could increase germination. Several studies have shown that using a dilution of liquid smoke was beneficial for germination of seeds in Lamiaceae (Carlsward *et al*. 2009; Todorovic *et al*. 2007).

Another way to simulate fire is by creating an aqueous solution infiltrated with plant debris smoke (Finn *et al*. 2010). This technique can be performed by burning plant debris found in the habitat of *S. floridana*. Brown and van Staden (1997) noted in several studies that aqueous smoke solutions had a positive response on germination. Another technique to break dormancy relates to plants producing a plant hormone called gibberellic acid (GA_3, $C_{19}H_{22}O_6$) which promotes germination. By soaking seeds in different concentrations of GA_3, germination percentages can increase in Lamiaceae (Todorovic *et al*. 2007; Karlsson *et al*. 2006) as well as in other families (Raeber and Lee 1991). When seeds do not germinate, triphenyltetrazolium chloride (TTC) is used to determine if cellular respiration is occurring in a seed. This technique helps distinguish if seeds are dead or dormant. Different concentrations of TTC are created to determine seed viability by staining the cotyledon and/or embryo (Sharma and Sharma 2010; Karlsson *et al*. 2006). The objective of the seed germination study was to

compare several techniques to germinate seeds of *S. incana*, *S. lateriflora*, *S. ovata* ssp. *ovata*, and *S. floridana*.

Seed Bank

Seed banks are a good way to examine the presence of seeds of different species in the soil. In order to determine if seeds are not only transient in the soil, but are also persistent, it is important to collect soil samples during winter (Baskin and Baskin 1998). Moreover, seeds can be trapped between soil particles as well as in leaf litter. Depending on seed size, the ability to hold seeds in soil is correlated with soil particle size. Large particles of soil are better for holding larger seeds and small soil particles are better for holding smaller seeds (Baskin and Baskin 1998). *Scutellaria parvula*, a species related to *S. floridana*, was found to have seeds in soils at 5-25 cm depth (Baskin and Baskin 1982). Once soil samples are collected, two different techniques are used for determining the seed bank of a species: either counting seeds found in the collected soil or counting seedlings that germinate from the soil. Baskin and Baskin (1998) suggest the latter of the two techniques is preferred because it also includes seed viability.

Another important facet to seed bank studies is percent moisture of the soil. Water content in the soil can affect seed germination in soils. If differences in amount of water occur during seed development, it may affect viability or vitality of seeds (Baskin and Baskin 1998). After shedding from mother plant, seeds may not germinate if the soil is too dry; while if percent moisture is higher, seeds may begin to germinate. The objective of the seed bank study was to determine the presence of viable, nondormant seeds of *S. floridana* and other monocots and dicots found in its habitat.

METHODS

Seed Germination

Scutellaria incana seeds were purchased from Missouri Wildflowers Nursery (Jefferson City, MO), *Scutellaria lateriflora and Scutellaria ovata* ssp. *ovata* seeds were purchased from Prairie Moon Nursery (Winona, MN), and *Scutellaria floridana* seeds were collected from ANF4 (Liberty County, Florida) on 14 April 2012 or were donated from Bok Tower Gardens (Lake Wales, FL) who collected them on 15 August 2007 (ambient stored) and on 20 May 2007 (cold stored at 7^{o}C). The donated seeds were from the National Collection Beds at Bok Tower Gardens, but the seeds in these beds were offspring from collections taken in Bay County and Gulf County. Because *S. floridana* is a federally listed species, codes are used instead of location names to protect this species in its native habitat.

For all seed germination treatments, 3 replications (dishes) were used (Table 1). Seeds were placed in Petri dishes (100 x 15 mm, Fisher Scientific, Pittsburg, PA) lined with three sheets of moistened filter paper (90 mm, Whatman #1, Fisher Scientific, Pittsburg, PA). All dishes were moistened with 5 mL of appropriate solutions (distilled water or smoke solutions). Tetramethylthiuram disulfide (50% Thiram) (Loveland Industries, Loveland, CO) was dusted over the seeds in the Petri dishes to reduce mold growth. Dishes were sealed with parafilm and kept within a colorless plastic container (30.5 x 21.6 x 16.5 cm). The tub was placed in a Percival Scientific seed germination chamber (Perry, IA) with 16 hours light from fluorescent lamps (17.0 ± 2.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8 hours dark at 25.0 ± 0.0°C.

For all treatments, unless otherwise stated, *S. incana*, *S. ovata* ssp. *ovata*, and *S. floridana* had 10 seeds per replicate. *S. lateriflora* had 50 seeds for all replicate except for stratification, which had 25 seeds per replicate. Germination was recorded when the radicle broke through the seed coat every day for *S. incana*, *S. lateriflora*, and *S. ovata* ssp. *ovata* and every other day for *S. floridana* over a period of 30 days. Once seeds germinated, those seeds were removed and placed in Fafard Growing Mix #2 (Earth City, Missouri) combined with soil from Florida (*S. floridana*) to continue seedling growth. The other three species of *Scutellaria* were placed in Fafard Growing Mix #2 (Earth City, Missouri) only. Germination percentage was recorded and arcsine transformations of these values were analyzed with a one-way analysis of variance (ANOVA) using SPSS 19, followed by Duncan's Multiple Range test at a 5% level.

Stratification

Seeds of each species were placed in a folded sheet of Whatman #1 filter paper (90 mm, Fisher Scientific, Pittsburg, PA), wrapped in cheesecloth, and soaked in Captan solution (5 g/L, Bonide Products, Inc., Yorkville, NY) for one minute. A mixture of 15 g of sphagnum peat moss and 220 g of sand were moistened with distilled water. The cheesecloth bundles were placed in the sphagnum moss and sand mixture for one and two months (*S. lateriflor*a and *S. ovata* ssp. *ovata*) or for 1.5 months (*S. floridana*). The remaining Captan was distributed over the mixture to prevent fungal growth. The container was stored in an Isotemp laboratory refrigerator (Fisher Scientific, Pittsburg, PA) at 4^{o}C.

Scarification

Seeds of all species were submerged in concentrated sulfuric acid (95-98%) for 30 and 60 minutes, except *S. floridana* was only scarified for 30 minutes. An additional 15 minutes trial was used for *S. lateriflora* and *S. ovata* ssp. *ovata*. After soaking, acid was removed from the seeds by rinsing them under running water for 5 minutes.

Wright's® Natural Hickory Seasoning

Wright's® Natural Hickory Seasoning (B & G Foods, Inc., Roseland, NJ) was diluted to different concentrations to simulate the burnt vegetation in natural areas. For *S. incana*, *S. lateriflora*, and *S. ovata* ssp. *ovata*, concentrations of 1:100, 1:500, and 1:1000 (mL hickory seasoning: mL distilled water) were used. The smoke seasoning for *S. floridana* was a concentration of 1:100.

Plant Debris Smoke

For *S. floridana*, dried plant debris was burned to create smoke, which was passed through distilled water. Plant debris was collected from sites in Florida around *S. floridana* populations (Table 2). From each collection site (THSF1 and THSF2), plant debris (100 g), which was air-dried on newspaper, was placed inside a bee smoker (GloryBee Foods, Inc., Eugene, OR) and burned. The bellows of the bee smoker were pumped continuously until plant material was burned. A 25.4 mm OEM heater Thermold® radiator hose was held by hand to the opening of the bee smoker. The other end of the hose was inserted into a 1000 mL Erlenmeyer flask containing 300 mL of distilled water to bring smoke into the water. The side arm of the flask was attached to a small hose to create a water vacuum within the system (Finn *et al*. 2010). After burning, remaining ash was cooled and weighed for sites THSF1 (6.0 g) and THSF2 (4.9 g).

pH Smoke Solutions

The pH of smoke solutions (Wright's® and plant debris smoke) was determined using a Bluelab soil pH meter (Bluelab Corporation Ltd., Tauriko, New Zealand). The probe was placed in a 25 mL sample of the smoke solutions. The pH strips (EMD Chemicals Inc., Darmstadt, Germany) were placed in the water/soil mixtures for 5 minutes, and the color of the strip was compared to the color codes on the packaging. The pH of the smoke soultions ranged from 3.5-4.0 (Table 3).

Gibberellic Acid

Scutellaria floridana seeds were wrapped in miracloth and submerged in 500 mg/L gibberellic acid (Sigma-Aldrich, St. Louis, MO) for 24 hours. The seeds were then removed from the solution and plated.

Tetrazolium

A tetrazolium test was used to determine seed viability of the seeds of *S. floridana* from Bok Tower Gardens (ambient and cold) and from ANF4. Seeds collected at different times and locations had 3 replications of 5 seeds each, except seeds that were imbibed from ANF4 which had 2 replications of 5 seeds each. *Scutellaria floridana* seeds were imbibed with distilled water for 24 hours. Seeds were cut in half, placed on moistened filter paper, and 2-3 drops of 1% (1 g/100 mL) triphenyltetrazolium chloride (TTC) were placed on the embryo and cotyledon. Intensity of redness was determined 5 times over 24 hours. Colors were ranked on each seed as no color, light pink, pink/red or red.

Soil Analysis

Seed Bank

In February 2012, soil samples were collected on 26 February at SJBP2a and SJBP2b and on 25 February 2012 at LB3. Using a hand trowel, three samples of 500 mL each of soil were collected in the vicinity where the species was recorded to occur. Bags of soil from each site were mixed. Keeping sites separated, soils were air-dried for 3 days before placing inside pots. Soil samples also were collected at SJBP2ab on 1 January 2013 at ANF4 and ANF5 on 31 December 2012, and at LB3, LB6, and LB9 on 24 January 2013 (Table 4). Only one sample of 600 mL was collected based on amounts needed from earlier collections. Small plastic pots (8.9 cm x 7.6 cm) were lined with a sheet of Whatman #1 filter paper to prevent sand from falling through the holes. Sterilized natural fine play sand (Sakrete, Charlotte, NC) was used to fill the pots over halfway (3 cm), and about 100 mL (2.5 cm) of air-dried soil was layered on top of the sand. Six replicates (pots) for each location were used and these pots were placed in rectangular plastic trays. These pots were watered daily. Seedling emergence was recorded for 64 days as monocot or dicot. Emergence of *S. floridana* also was observed. For seed bank study 2, a one-way analysis of variance followed by Duncan's Multiple Range test at a 5% level was used for number of monocots, number of dicots, and total number of monocots/dicots. An ANOVA was not used for seed bank study 1 because data was collected per site, not per replicate per site.

Soil Texture

For each soil collection, 10 mL of soil were added to 60 mL jars with 50 mL of distilled water. Three drops of texture dispersing reagent (LaMotte Chemical Products Co., Chestertown, MD) were added to the jars and shaken vigorously. The jars were not disturbed for a week to determine the texture of the soil by measuring the heights of sand, silt, and clay. These numbers were converted to percentages and soil texture was determined using a soil texture triangle.

Soil Moisture

Fresh mass was taken on a Scout ProSP 601 (Ohaus, Pine Brook, NJ) and then soil was placed in a Despatch Model LBB1-69A-1 oven (Despatch Industries, Minneapolis, MN) at 100^{o}C overnight. Once dry, samples were weighed again and the difference between fresh and dry mass was used to calculate percent moisture (difference between fresh and dry mass divided by dry mass then multiplied by 100). Percent moisture was completed for all soil samples.

Soil pH

The pH of soils collected was determined using a Bluelab soil pH meter (Bluelab Corporation Ltd., Tauriko, New Zealand). From each site, 10 g were mixed with 25 mL of distilled water and left overnight to settle. Samples were stirred the next day, strained with a metal strainer, and allowed to rest for 15 minutes before using the pH meter. The pH strips were placed in the water/soil mixtures for 5 minutes. The color of the strip was compared to the color codes on the packaging.

RESULTS

Scutellaria floridana

Stratification

Seeds of *S. floridana* which were stratified for 1.5 months at 4°C showed were significantly higher than the control (Table 5). After 7 days of imbibition, the control seeds started to germinate, whereas, the stratified seeds did not start to germinate until 19 days (Figure 1). Germination stopped on day 17 for the control. Once seeds germinated on day 19 of the stratification treatments, no more seeds germinated.

Scarification

Acid scarification for 30 minutes did not result in germination of any *S. floridana* seeds, which was significantly lower than for the control (Table 5).

Wright's® Natural Hickory Seasoning

With Wright's® Natural Hickory Seasoning, *S. floridana* germination began on day 5 and continued until day 25 (Figure 1). The control began germinating on day 7 and ceased on day 17. The germination percentages with Wright's Hickory Smoke Seasoning® and the control were similar (Table 5).

Plant Debris Smoke

For the plant debris smoke treatments, both half strength and full strength solutions significantly inhibited germination for *S. floridana*. After 30 days of imbibition, no germination occurred in either of the solutions, although the responses of the control and plant debris smoke treatments were significantly different (Table 5).

Gibberellic Acid

Soaking seeds in GA_3 for 24 hours resulted in some germination of *S. floridana*. Both control and GA_3 began germinating on day 7. However, germination did not halt for GA_3 until day 27 (Figure 1). By day 30, germination for seeds in GA_3 and the control were not significantly different (Table 5).

Tetrazolium

The seeds used in this study, collected in May 2007 and stored at 7^{o}C, had 40.0% seed viability based on tetrazolium staining (Table 6). Seeds collected in August 2007 and stored at ambient conditions at Bok Tower Gardens had a viability of 26.7%. Seeds collected from ANF4 in April 2012 and stored at 4^{o}C or imbibed at 25^{o}C had 0.0% seed viability.

Seed Bank Studies

Emergence: After 64 days, a total from all 3 sites included 7 monocots and 11 dicots (Figure 2). The most dicots (9) were found growing in soil from SJBPa, which had only dicots and no monocots, while the most monocots (5) were found growing in soil from LB3. SJBP2b had the least seedlings growing with only 2 monocots and 1 dicot. For the second seed bank study (December 2012 – January 2013), after 64 days, 73 monocots and 34 dicots emerged (Figure 3). Both SJBP3 (4) and ANF4 (4) had the least amount of seedlings emerge from the soil collections, while ANF5 (29) had the most seedling emergence. ANF5 had significantly higher emergence for monocots and combined (monocot and dicot), while ANF4 had significantly lower emergence for monocot and combined (monocot and dicot) (Table 7). The highest emergence of dicots was from soil

collected at LB3 (Table 7). No seedlings of *S. floridana* were found in either of the soil studies.

Soil Texture, Moisture, and pH: In seed bank study 1, all soils had sand as a component of the soil. Silt was the major soil component in all 3 sites (Table 8). Clay was only found in soils from LB3. Soil texture for SJBP2a was silt loam, SJBP2b was silt, and LB3 was silt loam. Soil components ranged from 12-33% sand, 56-88% silt, and 0-11% clay (Table 8). In seed bank study 2, all sites contained sand and silt (Table 8). ANF5, ANF4, and LB9 contained more sand than silt or clay. SJBP2ab was composed mainly of silt. Both SJBP3 and SJBP2ab soils did not contain clay. Soil textures for sites from the second seed bank study had mostly silt and loam. Soil components ranged from 29-50% sand, 38-71% silt, and 0-12% clay (Table 8).

The largest percent moisture from seed bank study 1 was from soil collected at SJBP2b at 63.4% moisture (Table 8). SJBP2a had 32.7% moisture and was the intermediate, followed by LB3 at 16.8% soil moisture. For seed bank study 2, soil moisture was highest at the ANF sites. ANF4 (50.8%) had the highest water content followed by ANF5 (43.2%) (Table 8). SJBP sites were intermediate having between 31-33%. LB sites had the lowest water content in the soil ranging from 18-22% moisture.

Soils collected for seed bank study 1 were acidic. SJBP2a and LB3 were the most acidic of the soils collected at a pH of 4.4 (Table 8). SJBP2b had a pH of 5.0. Soils collected for the second seed bank study were acidic. LB9 had a pH of 4.4 (Table 8). SJBP2ab, SJBP3, and ANF5 had soil pHs of 4.0.

Other Scutellaria species

Stratification

Scutellaria lateriflora and *S. ovata* ssp. *ovata* were stratified for 1 and 2 months, but there was no significant difference. *Scutellaria lateriflora* had 2.0% and 1.3% germination after stratification for one and two months, respectively (Table 5). *Scutellaria ovata* ssp. *ovata* had no germination for the control, 1 month stratification or 2 months stratification.

Scarification

Scutellaria incana was scarified for 30 and 60 minutes. Seeds with broken seed coats also were analyzed in the same study. The control and broken seed coat did not result in germination. After 30 minutes in acid, *S. incana* had a germination percentage of 10.0% (Table 5). Sixty minutes scarification only yielded 3.3% germination. *Scutellaria lateriflora* and *S. ovata* ssp. *ovata* were scarified for 15, 30, and 60 minutes. After scarifying *S. lateriflora* seeds for 15 minutes, the germination percentage was significantly higher (69.3%) than all other treatments (Table 5). The control and 60 minutes scarification resulted in germination percentages at 1.3%. Thirty minutes scarification resulted in 5.3% germination for *S. lateriflora*. *Scutellaria ovata* ssp. *ovata* did not have any germination by the end of the scarification study, except for 60 minutes scarification.

Wright's® Natural Hickory Seasoning

All three species were used in this study. *Scutellaria lateriflora* had the same germination for concentrations of 1:100 and 1:500 at 0.7% germination (Table 5). The control for *S. lateriflora* and the concentration of 1:1000 had no germination after 30

days. *Scutellaria ovata* ssp. *ovata* did not germinate in the control or when the seeds were exposed to the hickory smoke seasoning at concentrations of 1:100, 1:500 or 1:1000. For *S. incana*, the only treatment which resulted in germination was the 1:1000 concentration (3.3%) compared to other concentrations of 1:100 and 1:500.

DISCUSSION

This study determined that an array of seed treatments have different outcomes for different *Scutellaria* species. Unlike the Karlsson *et al*. (2006) study for *Galeopsis speciosa*, seeds of *Scutellaria incana*, *Scutellaria lateriflora*, *Scutellaria floridana*, and *Scutellaria ovata* ssp. *ovata* did not respond to stratification for 1, 1.5 or 2 months. *Galeopsis speciosa* is found in Canada, whereas, species used in this study are found in the U.S. as far south as Florida. For *S. floridana*, germination was decreased by stratification at 4^{o}C. Seeds found in warmer climates may not respond to stratification because soil temperatures during winter may never reach temperatures slightly above freezing (4^{o}C). Stratifying seeds at slightly warmer temperatures could result in higher germination (Baskin and Baskin 1998).

Scarification significantly increased germination in *Callicarpa americana* after soaking the seeds in sulfuric acid for 15 and 30 minutes (Contreras and Ruter 2009). In this study, the only species to have significantly increased germination by scarification was *S. lateriflora* after a 15 minutes soak in concentrated sulfuric acid. The shorter the time the seeds were in contact with acid, the higher the germination percentage for this species. *Scutellaria incana*, *S. lateriflora*, and *S. ovata* ssp. *ovata* at longer submergence times resulted in similar percentages to the control. Reducing the time submerged in acid could result in higher germination for all of the species in this study, except *S. ovata* ssp.

ovata, which may need longer times. Both *S. incana* and *S. ovata* seemed to respond to acid scarification, but the responses in the scarification and control treatments were similar. For *S. floridana*, 30 minutes scarification reduced germination relative to the control. No seed coat remained on the majority of the seeds and decreased germination suggests seed damage after scarification, for *S. floridana*. Like *S. incana* and *S. lateriflora*, a shorter acid scarification of 5-15 minutes could be beneficial for these species. For all species used, additional studies with acid scarification for different submergence times might increase germination.

According to Todorovic *et al*. (2007), varying concentrations of liquid smoke (Zesti Eurosmoke) increased germination in *Nepeta rtanjensis* (Lamiaceae). In our study, smoke was used to stimulate germination as the *Scutellaria* species' habitat is fire-prone. In this smoke study, neither Wright's Hickory Smoke Seasoning® nor the plant debris smoke (only tested with *S. floridana*) solutions resulted in higher germination percentages in any of the 4 species. The lack of effect on germination is not quite clear because for *S. incana*, *S. lateriflora*, and *S. ovata* ssp. *ovata*, several concentrations were used. Species in different families have responded to plant debris smoke (Brown and van Staden 1997). My study shows water soluble compounds created by burning plant debris actually decreased germination for *S. floridana*. Concentrations used in this study could have been too concentrated and more dilute solutions may increase germination. The lack of germination also could show *S. floridana* responds to the heat created from a fire in its habitat and could be examined in further studies. The other 3 species were not used for the plant debris smoke study because dried plant material was not collected from each of their habitats.

Gibberellic acid (GA_3) is a plant growth regulator known for increasing germination in Lamiaceae and other families (Todorovic *et al*. 2007; Karlsson *et al*. 2006; Raeber and Lee 1991). *Scutellaria floridana* was the only species soaked in GA_3 for this study. Seeds of *S. floridana* were not inhibited by GA_3, but germination percentages were not similar to the control. Concentrations of GA_3 in Raeber and Lee (1991) ranged from 10-500 ppm (mg/L) and were used on *Penstemon parryi* seeds, with the highest germination at 500 ppm (mg/L). Their study found germination occurred faster when seeds were presoaked than seeds placed on filter paper moistened with GA_3. Also, the higher the concentration of GA_3 used, the higher the germination of *P. parryi* (Raeber and Lee 1991). In my study, we used a 500 mg/L GA_3 presoak, but further studies could use higher concentrations of GA_3.

In order to determine seed viability of *S. floridana*, TTC was dripped over seeds. Knowing the viability of seeds is important because not all seeds are viable in a seed lot. TTC test indicated about 40% of the seeds received from Bok Tower Gardens (collected in May 2007) were viable. Germination percentages of *S. floridana* ranged from 0-33.3%. Compared to the viability of the seed lot used (40%), the control had 83% germination of the viable seeds. Hickory smoke seasoning (1:100) had 42% germination and stratification (1.5 months) had 17% germination of the viable seeds.

The seed bank study was conducted to determine if seeds of *S. floridana* are present in soils within its habitat. Baskin and Baskin (1998) suggest the best time to collect soil samples to determine if seeds are present and persistent is in winter. After allowing 64 days for seeds to germinate and seedlings to emerge, neither collections contained seedlings of *S. floridana*. These plants had flowered the previous year at ANF4

so seeds should have been present in the soil as long as viable seeds were set and dispersed. Several different factors could affect emergence of *S. floridana*. Seeds may not be present in the soil or conditions, such as after-ripening, may not have been adequate for germination and emergence of this species or seeds. Because seeds used in the germination studies were stored since 2007 and were not tested to determine after-ripening effects, seeds present in the soil for the seed bank study may need months-years to germinate.

Only a few monocots and dicots emerged from the February 2012 seed bank, but several emerged from the December 2012 – January 2013 seed bank. This difference could result from a vast array of different factors, such as different collection periods, different soil collection sites, or different soil textures. Soil textures in 3 sites collected in February 2012 had a range of sand from 44-100%, silt from 0-56%, and clay from 0-38%. February 2012 seed bank had a pH range from 4.4-5.0. For the 7 soil samples collected December 2012 – January 2013 seed bank, sand ranged from 0-44%, silt from 10-87.5%, and clay 10-20%. This seed bank had a pH range from 4.0-4.9. From the February 2012 collection, the largest percent moisture was from SJBP2b (63.4%) with LB3 having the lowest moisture in the soil. The highest percent moisture from December 2012 – January 2013 was from ANF4 with LB6 being the driest soil. The soil from all sites and from both collections tends to be sandy, acidic, and moist. Differences in soil moisture could be due to different locations in Florida and from different soil types or different rainfalls prior to sampling.

Further studies on *Scutellaria* species should include variations on experiments in this study. By varying (warmer) temperatures for stratification, varying (shorter or

longer) lengths for acid scarification, and varying (lower and higher) concentrations for smoke solutions and gibberellic acid solutions could impact germination. For seed bank studies, collecting soil samples throughout the year (i.e, after seed set or before seedling emergence) or placing only native soil in planting pots could result in the appearance of *S. floridana* in soil samples, but physical presence of the seed should be verified.

The results from this study can be used for restoration purposes for *S. floridana*. In order to maintain *S. floridana* in its native habitat, the insight gained from this study is beneficial for the survival of this species. As an aspect of the 5-Year Review: Summary and Evaluation devised by the U.S. Fish and Wildlife Service, this study can be used for the delisting of this federally threatened species.

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REFERENCES

Baskin, C.C. and J.M.Baskin. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press: San Diego, USA.

Baskin, J.M. and C.C. Baskin. 1982. Ecological life cycle and temperature relations of seed germination and bud growth of *Scutellaria parvula*. *Bulletin of the Torrey Botanical Club* 109(1): 1-6.

Brown, N.A.C. and J. van Staden. 1997. Smoke as a germination cue: a review. *Plant Growth Regulation* 22: 115-124.

Campbell, C.C. and C.L. Peterson. 2007. Seed collection and research on eight rare plant species of the Florida Panhandle Region. Final Report for USFWS Grant Agreement No. 401815G173.

Carlsward, B., J. Coons, and N. Coutant. 2009. Comparative seed anatomy of Illinois tallgrass prairie species with and without reported smoke-induced germination. Final Report for Illinois Wildlife Preservation Fund Grant Agreement #09-005. Illinois Department of Natural Resources, Springfield, IL.

Contreras, R.N. and J.M. Ruter. 2009. *Callicarpa americana* L. (Lamiaceae). *Native Plants* 10(3): 283-286.

Estill, J.C. and M.B. Cruzan. 1999. Phytogeography of rare plant species endemic to the Southeastern United States. *Castanea* 66(1/2): 3-23.

Finn, D.J., S.D. Prosser, J.M. Coons, N.E. Coutant, and B.A. Lawrence. 2010. Burning system to collect water soluble compounds from the smoke of plant materials. Transactions of the Illinois State Academy of Science 103 (supplement):29. Poster.

Glitzenstein, J.S., W.J. Platt, and D.R. Strong. 1995. Effects of fire regime and habitat on tree dynamics in North Florida longeleaf pine savannas. *Ecological Society of America* 65(4): 441-476.

Hessl, A. and S. Spackman. 1995. Effects of fire on threatened and endangered plants: An annotated bibliography. *National Biological Service Information and Technology Report 2*. Boulder, Colorado.

Karlsson, L.M., J. Ericsson, and P. Milberg. 2006. Seed dormancy and germination in the summer annual *Galeopsis speciosa*. *European Weed Research Society* 46: 353-361.

Kral, R. 1983. A report on some rare, threatened, or endangered forest-related vascular plants of the South. USDA Forest Service Technical Publication. R8-TP2; Atlanta, Georgia.

Raeber, A.C. and C.W. Lee. 1991. Gibberellic acid, temperature, light, stratification, and salinity affect germination of *Penstemon parryi* seed. *HortScience* 26(12): 1535-1537.

Sharma, S. and R.K. Sharma. 2010. Seed physiological aspects of pushkarmool (*Inula racemosa*), a threatened medicinal herb: Response to storage, cold stratification, light and gibberellic acid. *Current Science* 99(12): 1801-1806.

Springer, L. 1997. Mints with a twist. *Horticulture* 94(3): 36.

Todorovic, S., S. Zivkovic, Z. Giba, and D. Grubisic. 2007. Basic seed germination characterisitics of the endemic species *Nepeta rtanjensis* (Lamiaceae). *Plant Species Biology* 22: 205-210.

U.S. Fish and Wildlife Service. 2009. *Scutellaria floridana* (Florida skullcap). *5-Year Review: Summary and Evaluation*. Panama City, Florida.

U.S. Fish and Wildlife Service. 1994. Four plants of the lower Apalachicola region, Florida: *Euphorbia telephioides* (Telephus spurge), *Macbridea alba*, *Pinguicula ionantha* (Godfrey's butterwort), and *Scutellaria floridana* (Florida skullcap). Atlanta, Georgia.

USDA, NRCS. 2013. The PLANTS Database (http://plants.usda.gov, 24 February 2013). National Plant Data Team, Greensboro, NC 27401-4901 USA.

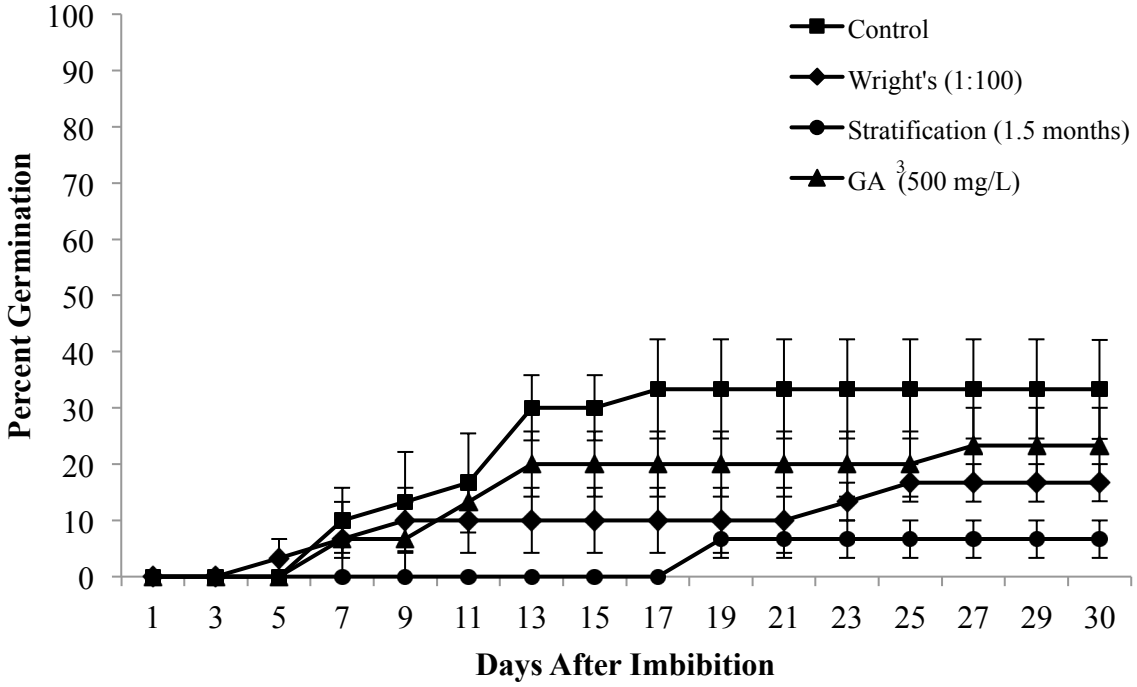

Figure 1. Germination rates over 30 days for *Scutellaria floridana* seeds stratified for 1.5 months at 4°C, or imbibed in Wright's Hickory Smoke Seasoning® at 1:100, or soaked for 24 hours in 500 mg/L gibberellic acid (GA_3), or a control with no treatment. Means ± standard error.

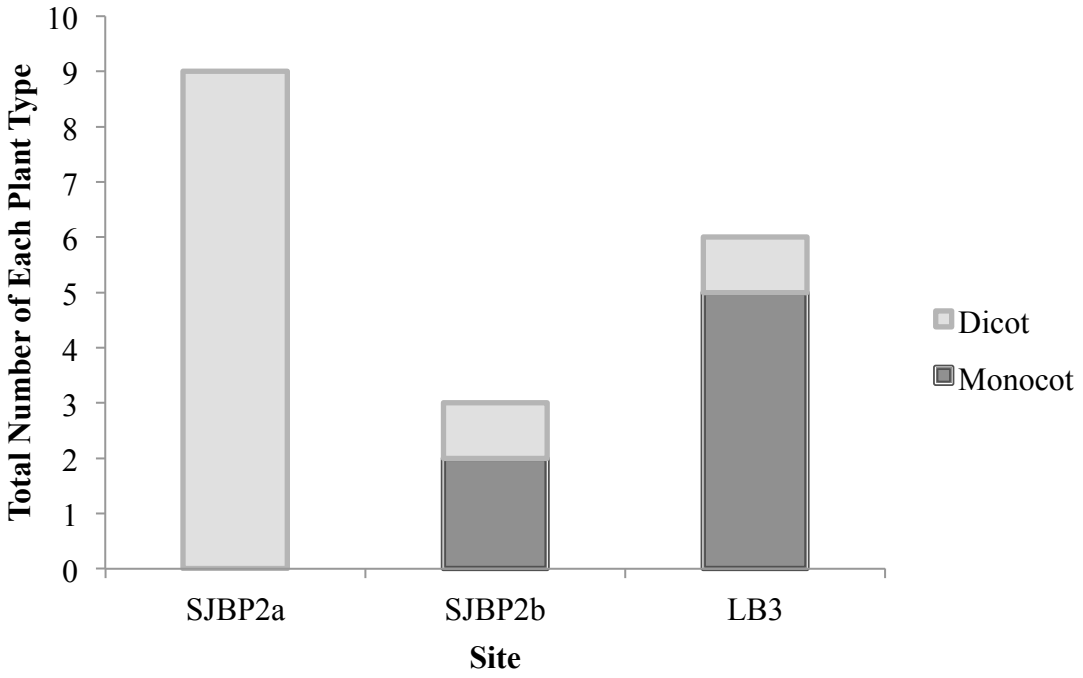

Figure 2. Number of monocots and dicots emerged from seed bank of soil samples collected from 3 sites in February 2012. Emergence expressed per 600 mL of native soil.

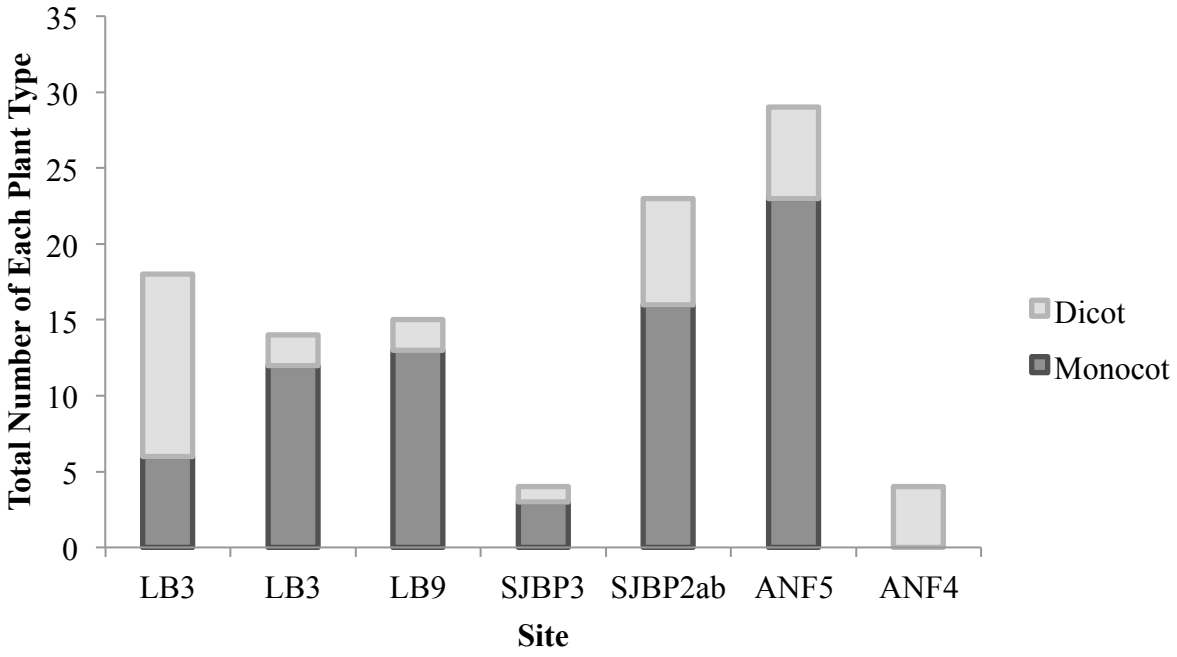

Figure 3. Number of monocots and dicots emerged from seed bank of soil samples collected from 7 sites in December 2012 and January 2013. Emergence expressed per 600 mL of native soil.

Table 1. Summary of treatments used for *Scutellaria* species.

Treatment	*S. incana*	*S. lateriflora*	*S. ovata* ssp. *ovata*	*S. floridana*
	x	1 month	1 month	x
Stratification	x	x	x	1.5 months
	x	2 months	2 months	x
	x	15 mins	15 mins	x
Scarification	30 mins	30 mins	30 mins	30 mins
	60 mins	60 mins	60 mins	x
	1:100	1:100	1:100	1:100
Wright's Hickory Smoke	1:500	1:500	1:500	x
	1:1000	1:1000	1:1000	x
Plant Debris	x	x	x	half strength
Smoke	x	x	x	full strength
Gibberellic Acid (GA_3)	x	x	x	500 mg/L

(x) Indicates treatment not used for *Scutellaria* species

Table 2. List of species in plant debris used for smoke study.

Scientific Name	Common Name	THSF1	THSF2
Andropogon sp.	Bluestem	x	
Aristida stricta Michx. var. *beyrichiana*	Wiregrass	x	x
Aristida sp.	Wiregrass		x
Cyrilla racemiflora	Titi	x	
Dichanthelium sp.	Panicgrass or Rosette Grass		x
Drosera sp.	Sundew		x
Gaylussacia sp.	Huckleberry		x
Hypericum sp.	St. John's Wort		x
Ilex coriacea	Large or Sweet Gallberry		x
Ilex myrtifolia	Myrtle-leaf Holly		x
Pinus elliottii	Slash Pine	x	x
Pinus palustris	Longleaf Pine	x	x
Rhexia sp.	Meadow Beauty	x	
Sarracenia flava	Yellow Pitcher Plant or Trumpets	x	
Smilax sp.	Greenbrier or Catbrier		x
Taxodium ascendens	Pond Cypress		x
Vaccinium corymbosum	Highbush Blueberry	x	

Table 3. pHs for solutions used in smoke studies for *Scutellaria floridana* using a pH meter.

Solutions	**pH**
Wright's 1:100	3.5
Plant Debris Smoke - Half Strength	4.0
Plant Debris Smoke - Full Strength	3.8

Table 4. Sites for *Scutellaria floridana* seed bank studies.

Population	FL County	Collection Date	Study 1	Study 2
ANF5	Liberty	12/31/2012		x
ANF4	Liberty	12/31/2012		x
SJBP3	Gulf	1/1/2013		x
SJBP2ab	Gulf	1/1/2013		x
SJBP2a	Gulf	2/26/2012	x	
SJBP2b	Gulf	2/26/2012	x	
LB3	Bay	1/24/2013		x
LB6	Bay	1/24/2013		x
LB9	Bay	1/24/2013		x
LB3	Bay	2/25/2012	x	

Table 5. Germination (%) of 4 *Scutellaria* species with different treatments after 30 days.

Treatments		***S. incana*[y,z]**	***S. lateriflora***	***S. ovata* ssp. *ovata***	***S. floridana***
Stratification	Control	x	0.0 ± 0.0a[y,z]	0.0 ± 0.0a	33.3 ± 8.8a
	1 month	x	2.0 ± 1.2a	0.0 ± 0.0a	x
	1.5 months	x	x	x	6.7 ± 3.3b
	2 months	x	1.3 ± 0.7a	0.0 ± 0.0a	x
Scarification	Control	0.0 ± 0.0a	1.3 ± 1.3 b	0.0 ± 0.0a	33.3 ± 8.8a
	15 mins	x	69.3 ± 8.1a	0.0 ± 0.0a	x
	30 mins	10.0 ± 5.8a	5.3 ± 3.5b	0.0 ± 0.0a	0.0 ± 0.0b
	60 mins	3.3 ± 3.3a	1.3 ± 1.3b	13.3 ± 8.8a	x
Wright's Hickory Smoke	Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	33.3 ± 8.8a
	1:100	0.0 ± 0.0a	0.7 ± 0.7a	0.0 ± 0.0a	16.7 ± 3.3a
	1:500	0.0 ± 0.0a	0.7 ± 0.7a	0.0 ± 0.0a	x
	1:1000	3.3 ± 3.3a	0.0 ± 0.0a	0.0 ± 0.0a	x
Plant Debris Smoke	Control	x	x	x	33.3 ± 8.8a
	half strength	x	x	x	0.0 ± 0.0b
	full strength	x	x	x	0.0 ± 0.0b
Gibberellic Acid (GA_3)	Control	x	x	x	33.3 ± 8.8a
	500 mg/L	x	x	x	23.3 ± 6.7a

[y] Means ± standard errors

[z] Means with different letters within a column are significantly different based upon Duncan's Multiple Range test at 5% level.

(x) Indicates treatment not used for *Scutellaria* species

Table 6. Percent viablity using tetrazolium on *S. floridana* seeds.

Seed Source	Date Collected	Storage	Mean ± SE
Bok Tower Gardens	May 2007	7° C	40.0 ± 7.7a[y]
Bok Tower Gardens	August 2007	25° C	26.7 ± 5.9a
ANF4	April 2012	4° C	0.0 ± 0.0a
ANF4[z]	April 2012	25° C	0.0 ± 0.0a

[y] Means with different letters within a column are significantly different based upon Duncan's Multiple Range test at 5% level.
[z] Imbibed for 4 months prior to tetrazolium test

Table 7. Monocot/dicot emergence over 64 days for December 2012-January 2013 soil collections.

Site	Monocot	Dicot	Combined
ANF5	3.8 ± 0.7a[y,z]	1.0 ± 0.4bc	4.8 ± 0.9a
ANF4	0.0 ± 0.0d	0.7 ± 0.3bc	0.7 ± 0.3c
SJBP3	0.5 ± 0.3cd	0.2 ± 0.2c	0.7 ± 0.3c
SJBP2ab	2.7 ± 0.7ab	1.2 ± 0.3b	3.8 ± 0.7ab
LB3	1.0 ± 0.3bcd	2.0 ± 0.4a	3.0 ± 0.4ab
LB6	2.0 ± 0.6bc	0.3 ± 0.2bc	2.3 ± 0.8bc
LB9	2.2 ± 0.7bc	0.3 ± 0.2bc	2.5 ± 0.8bc

[y] Means ± standard errors

[z] Means with different letters within a column are significantly different based upon Duncan's Multiple Range test at 5% level.

Table 8. Soil texture, moisture, and pH for sites where soil collected for seed bank studies with *Scutellaria floridana*.

Site	% Sand	% Silt	% Clay	Soil Texture	% Moisture	pH
Seed Bank Study 1						
SJBP2a	25	75	0	Silt Loam	32.7	4.4
SJBP2b	12	88	0	Silt	63.4	5.0
LB3	33	56	11	Silt Loam	16.8	4.4
Seed Bank Study 2						
ANF5	50	38	12	Loam	43.2	4.0
ANF4	50	40	10	Loam	50.8	4.2
SJBP3	41	59	0	Silt Loam	33.2	4.0
SJBP2ab	29	71	0	Silt Loam	31.2	4.0
LB3	38	50	12	Silt Loam	22.0	4.9
LB6	38	50	12	Silt Loam	21.0	4.7
LB9	50	38	12	Loam	18.0	4.4