

**Seed Germination and Growth Requirements
of Selected Wildflower Species**

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 14, 2010

Keywords: imbibition, stratification,
seed treatment, fungicide

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Abstract

The effect of imbibition, fungicidal pre-treatments, and stratification time length on seed germination of *Rudbeckia fulgida* Aiton (orange coneflower), *Penstemon laevigatus* Aiton (eastern smooth beardtongue), and *Lobelia cardinalis* L. (cardinal flower) was studied. Seeds were either imbibed or not imbibed (before stratification), treated with fungicide or not, (after stratification), and stratified at $5\pm 1^{\circ}\text{C}$ (41°F) 3 or 6 weeks, or not stratified, and then placed in germination chambers set at $30\pm 1^{\circ}\text{C}$. Each experiment was repeated for a total of three runs per species. Germination of *R. fulgida* was highest when seeds were imbibed and not stratified. Germination of *P. laevigatus* was higher when seeds were imbibed and stratified at 5°C (41°F) for 6 weeks. Germination for *L. cardinalis* was negligible, regardless of treatment.

Seeds of *Coreopsis tinctoria* Nutt. (golden tickseed), *Coreopsis verticillata* L. (whorled tickseed), *Echinacea purpurea* (L.) Moench (eastern purple coneflower), *Gaillardia pulchella* Foug. (firewheel), and *Rudbeckia hirta* L. (black-eyed Susan) were sown in three Alabama soil types at four sowing depths. For *R. hirta* and *C. tinctoria*, survival and subsequent growth were higher when seeds were surface sown than when sown below the surface. For *G. pulchella* and *E. purpurea*, survival and subsequent growth were highest when seeds were sown below the surface. Soil type influenced survival and subsequent growth of all species. There was higher survival and subsequent growth when seeds were sown in Wickham sandy loam and Marvyn loamy sand than when sown in Houston clay. Poorer germination in the Houston clay was likely due to the higher clay content of the soil, which retained water for a long period, causing seed and seedling rot.

Acknowledgements

The author extends deep-felt appreciation to Dr. Amy Wright for her generosity in time, patience, and expertise in guiding her student to successful completion of her Master of Science. Dr. Wright provided a fresh perspective and new insight into horticulture, making graduate studies a rich and rewarding experience. The author would also like to express gratitude to her other committee members, Dr. Beth Guertal and Dr. Raymond Kessler, for lightening her burdens and providing encouragement. She particularly enjoyed the friendship and support from faithful friends with special mention of Dr. Joe Eakes, Ann Fleener, Ashley Baker, Katie Werneth, Jessica Jones, Jane Hoehaver Farr, and Matt Wilson. Special thanks belong to her ever-loving family, the Bonds, Hansens, and Wilsons, who pushed her through the hard times and sacrificed their claim to her for two years. The utmost appreciation goes to her Heavenly Father who asked her to pursue higher education, and gave her the grace to do it.

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Chapter I

Literature Review

Wildflowers are ecologically important plants that provide sources of food and cover for insects and animals, and contributing to erosion control while adding visual appeal to the landscape (Billingsly and Grabowski, 1996; Flynn, 1997; Norcini et al., 1998). The Federal Highway Administration (FHWA) encourages integration of native plants, especially wildflowers, into rights-of-way along highways under the provisions of the Surface Transportation and Uniform Relocation Assistance Act of 1987 (STURAA). State highways are corridors through many natural areas of Alabama's diverse landscape, and wildflowers are a naturalizing enhancement to the public highway system. The responsibility for planting wildflower areas along highways rests with the Alabama Department of Transportation (Federal Highway Administration, 2008). Wildflowers are viewed by the Federal Highway Administration as an integral part in preventing soil erosion because of their fibrous root systems. Other benefits include lower maintenance costs, drought resistant foliage, and protection against invasive species (U.S. Forest Service, 2008; Federal Highway Administration, 2008).

Along with the wide diversity of wildflower species indigenous to Alabama, there are many animals and insects reliant on their pollen, nectar, seeds, forage, and cover (Flynn, 1997; Kessler and Carden, 2001; U.S. Forest Service, 2008). There is a reciprocal need in that wildflowers need pollinators to aid in the reproduction process.

Without pollinating insects, some wildflower populations could not persist (U.S. Forest Service, 2008).

Roadside planting programs have existed on a small scale since the 1920s, but interest in planting native wildflowers and forbs dramatically increased in 1965 when the Beautification Act was signed by President Lyndon B. Johnson, inspired by his wife, Claudia A. “Lady Bird” Johnson, to treat the “roadsides as our own backyards”. Progressively, native plants have become more prominent in federal and state parks, golf course secondary roughs (no mow zones), reclamation sites, commercial and residential landscapes, and around commercial signage (Aldrich, 2002; Hammond et al., 2007; Lindgren and Schaaf, 2005; Norcini et al., 2001).

Because they are able to grow in a variety of climates and soil types, wildflowers are an advantageous choice for commercial and residential settings because they may require less water and soil cultivation and relatively no pesticide or fertilizer as compared to typical landscape plants. Many species are fairly effortless to grow and can easily be incorporated in the urban landscape (Bratcher et al., 1993; Kessler and Carden, 2001). Because wildflowers require less maintenance, they are often utilized by Departments of Transportation along roadsides, by city governments for municipal open areas, and by the homeowner as bedding plants. Public awareness about the importance of wildflowers is increasing not only among gardeners, but also on local, state, and national levels (Aldrich, 2002). There is also increasing concern about the ecological impacts that non-native flowering plants may have on the native flowering plant populations such as their propensity to invade space and compete for nutrients. Both of these issues have created a demand for more natives (Norcini et al., 2004).

Several states, such as Florida and Texas, have been proactive in developing research programs to develop effective methods for incorporating native herbaceous plant material along their highways. The demand for Florida wildflower ecotypes is increasing, especially for roadsides, mine reclamation, and restoration (Allen and Meyer, 1990; Churchill et al., 1990; Hammond et al., 2007; Kabat et al., 2007; Norcini and Aldrich, 2003; Norcini et al., 2004; Norcini et al., 2006; Norcini et al., 1998).

Norcini et al. (2001) evaluated ecotypes of *Rudbeckia hirta* L. from two different sources, northern Florida and Texas, and planted them in low input landscapes in Florida. There was higher survival and growth among seeds from northern Florida. They also found that seeds from local, versus non-local, sources showed differences in phenotype, with increased survival and earlier flowering in local *Coreopsis lanceolata* and *Salvia lyrata*.

Although the Alabama Department of Transportation (ALDOT) has roadside areas planted with wildflowers, attempts to establish self-sustaining populations have failed and frequent seeding is required. Currently, ALDOT, golf courses, and homeowners have to rely on recommendations for wildflower establishment based on another state's climate and soils. Research is needed to evaluate native Alabama wildflower species for seed germination and landscape establishment needs.

The expanding market for wildflower species is a recent phenomenon, yet there is little information or formal evaluation of germination or survival of wildflowers from different regional seed sources and their performance in the landscape. Unsuccessful establishment resulting from low germination and limited subsequent growth may be largely due to the wildflower sources. Planting local seed provenance may promote

better establishment because they may be better suited to prevailing local site conditions (Bischoff et al., 2006). Seed provenance is known to affect germination, subsequent growth, and flowering of several wildflower species (Norcini et al. 2001). Also, incomplete knowledge of planting procedures such as sowing depth may inhibit successful establishment (Billingsly and Grabowski, 1996).

Alabama is one of the most biologically diverse states in the country, and its diversity includes a wide variety of wildflowers species. It was estimated that there were 3,000 flowering plants in Alabama (Flynn, 1997). Species that could be particularly suited to roadsides and landscapes because of their bright flower colors and showiness include *Rudbeckia hirta* L. (black-eyed Susan), *Rudbeckia fulgida* Aiton (orange coneflower), *Echinacea purpurea* (L.) Moench (eastern purple coneflower), *Gaillardia pulchella* Foug. (firewheel), *Coreopsis tinctoria* Nutt. (golden tickseed), *Coreopsis verticillata* L. (whorled tickseed), *Penstemon laevigatus* Aiton (eastern smooth beardtongue), and *Lobelia cardinalis* L. (cardinal flower).

Rudbeckia hirta, Asteraceae, is native to all of North America excluding desert and Arctic regions (Barker, 2004; Norcini and Aldrich, 2003). *R. hirta* is normally found in prairies, fields, or waste ground habitats (Barker, 2004) and is commonly used for cut flowers, background plantings, borders, and bedding plants (Still, 1994). *R. hirta* is considered an annual (dies after one growing season) that reseeds each year, or in milder climates, can be a perennial (more than one growing season) that is commonly propagated by division. Optimal seed germination occurs at soil temperature of 21°C (70°F). The inflorescence is a compound head (capitulum) 5-8 cm (2-3 in) in diameter, and the leaves are oblong or lanceolate and coarsely hairy. *R. hirta* flowers in October

and November growing to a height of 30-60cm (1-2 ft) (Still, 1994; Phillips, 1985; Armitage, 2001).

Another member of Asteraceae, *Rudbeckia fulgida*, is native to the eastern United States. It is a perennial species and considered among the best for meadows, gardens, and urban landscapes. It is often found growing in wet soils along river banks. It is similar to *R. hirta* except the leaves are more glabrous and a darker green, and is not susceptible to powdery mildew like *R. hirta*. The basal leaves are 3-veined and oblong to lanceolate supported by multi-branched stems. Inflorescences are approximately 6.5 cm (2.5 in) wide and have 12-14 yellow ray florets encircling a brown disc of florets appearing from midsummer into late fall. This species grows 45-75 cm (18-30 in) tall (Armitage, 1997; Fay et al., 1993; Fay et al., 1994; Still, 1994).

Echinacea purpurea, Asteraceae, is native to eastern North America. It prefers full sun and is drought tolerant. It is commonly used for cut flowers, borders, and naturalized areas. The roots are often harvested for medicinal uses. Seeds are not always true to type, and the seedlings can sometimes take up to two years before flowering. *E. purpurea* has woody rhizomes, pubescent stems, cauline leaves, and produces purple cone inflorescences in the summer. It grows to 60-122 cm (2-4 ft) high by 60 cm (2 ft) wide (NCRS, 2008b; Samfield et al., 1991; Still, 1994; Qu et al., 2004; Qu et al., 2005).

Gaillardia pulchella, also in Asteraceae, is native to the western states and has become naturalized in the eastern states, including Alabama, and is now widely known and cultivated (Hammond et al., 2007; Still, 1994; Norcini, 2005). It is considered an annual, but effectively reseeds. It is drought tolerant and will grow in most soils in full

sun. The coarsely serrate and gray-green leaves are 10-15 cm (4-6 in) long with alternate arrangement. *G. pulchella* produces a solitary inflorescence with ray florets colored in combinations of reds and yellows. This easy to grow plant flowers prolifically in the summer and fall and reaches a height of 30-61 cm (1-2 ft) (Armitage, 1997; Hammond et al., 2007; Norcini, 2005; Still, 1994.).

Coreopsis tinctoria, Asteraceae, is native to the eastern United States although it has spread to a wider range having escaped from cultivation in gardens. It can be sown directly from seed and germinates in 5-10 days under low light exposure. *C. tinctoria* prefers full sun and well-drained soils along with high temperatures. The leaves are opposite and divided into linear-lanceolate sections, contributing to fine textured foliage (Still, 1994). The inflorescence is radially symmetrical, 5 cm (2 in) in diameter, borne in loose panicles on thin peduncles. Inflorescence colors range from yellow to orange to crimson shades, flowering early summer through midsummer. It is a multi-stemmed plant with thin, wiry stems and reaches a height of 60-91 cm (2-3 ft) (Phillips, 1985; Still, 1994).

Coreopsis verticillata, Asteraceae, is native to the eastern part of North America ranging from Maryland to Florida and westward to Arkansas. It is common to find this species in full sun along roadsides, in open woodlands, and growing in dry soils due to its drought tolerance. The species has long, sessile, palmately-divided leaves that give the plant a fine texture. The single inflorescences are 4-5 cm (1.5-2 in) in diameter and are borne on a corymb clustered on slender peduncles. It grows to a height of 60-91cm (2-3 ft) (Armitage, 1997; Phillips, 1985; Still, 1994).

Penstemon laevigatus, Plantaginaceae, is native along the eastern United States from Pennsylvania to Florida. It is typically found in fields, roadsides, railways, mesic prairies, and woody areas and grows well in moderately dry soils. There has been limited study on this particular species of *Penstemon*. It is considered synonymous with *P. digitalis*, a species that has a white or pale pink inflorescence and flowers from May through July. Leaves are opposite. The showy flowers have a long corolla tube opening with five petals. There are five stamens from which the genus name is derived, yet one is sterile. This perennial reaches 60-120 cm (2-4 ft) in height (Armitage, 1997).

Lobelia cardinalis, Campanulaceae, is native to the eastern half of the United States and Canada extending from New Brunswick to Florida and west to Texas. It prefers wet soils and requires shade in the south. It was used by the Native Americans for medicinal purposes. The plant has 8-10cm (3-4 in) long, serrated leaves sessile to the stem. It has scarlet, pink, or white flowers 4cm (1.5 in) wide bracketed in racemes on a single 60 cm (2 ft) long inflorescence. Each flower has a corolla tube with three narrow lower lobes and exerted anthers. Flowering begins in August and continues for approximately 3 weeks. It grows to 60-120 cm (2-4 ft) (Armitage, 1997; NRCS, 2008a).

Though many companies are selling packaged wildflower seeds, availability is inconsistent, and provenance varies widely. Little research has been done on germination requirements for most wildflower species (Kaspar and McWilliams, 1982). Some wildflower seeds are easy to germinate and grow, while others are not. Species requiring seed treatments can be difficult and costly to grow because they require more time and labor to achieve acceptable germination rates. (Allen and Meyer, 1990; Fay et al., 1993; Fay et al., 1994; Pill et al., 2000; Lindgren and Schaaf, 2004). The high price

of packaged wildflower seeds presents another limitation to their use (Pill et al., 2000). The complex dormancy systems some species possess enable them to survive in natural environments by preventing seeds from germinating until there are favorable conditions for seedling survival, but make consistent propagation results difficult to obtain (Bratcher et al., 1993; Samfield et al., 1991). Germination requirements vary not only by species, but also seed age and seed source (Lindgren and Schaaf, 2004).

There is limited information on effective germination protocols for *R. fulgida*. Nau (1996) stipulates that germination occurs best after a moist stratification of 2 to 4 weeks at 5 to 7°C (40 to 45°F) whereas Fay et al. (1993) found that germination temperatures of 28 to 32°C (82 to 90°F) were the best with highest germination at 30°C (86°F). Further research was conducted with osmotic seed priming for seed lots with low vigor (germinates erratically and poor seedling emergence) (Hartmann et al., 2002). Seeds of *R. fulgida* primed in -1.3 MPa KNO₃ had twice the germination percentage and half the mean time to germination when compared to non-primed seeds (Fay et al., 1994).

Penstemon species were reported to need one or some combination of seed scarification, stratification, changing temperatures, growth hormones, or light for efficient germination (Lindgren and Schaaf, 2004). These authors researched the effects of seed stratification on *P. digitalis* Nutt., *P. angustifolius* Nutt. Ex. Pursh, *P. barbatus* (Cav.) Roth, *P. gracilis* Nutt., *P. grandiflorus* Nutt., *P. haydenii* S. Wats., and *P. strictus* Beneth. and found that for most of the selected species, 8 weeks cold stratification improved germination. However, stratification and light requirements appeared to be species specific. These results were supported by Allen and Meyer (1990) who found that 8 weeks stratification were necessary for *Penstemon spp.* (Bratcher et al., 1993).

Generally, *Penstemon* spp. seeds can germinate at 18 to 21°C (65 to 70°F) in 2-3 weeks, but some species might benefit from 8 weeks of stratification at 15°C (59°F) (Hartmann et al., 2002).

There are conflicting recommendations for meeting germination requirements of seeds of *L. cardinalis*. Still (1994) stipulated that seeds were easily germinated at 21°C (70°F) under light with no stratification. It was also found that seeds germinated best 4 years after harvesting probably because of the development of secondary dormancy (Muenscher, 1935; Still 1994). Other recommendations include up to 3 months of moist stratification (University of Texas at Austin, 2008).

Seed decay may occur during longer periods of stratification. In fact, Burgess et al. (2002) found that seeds of *Uniola paniculata* L. (sea oats) transferred to Petri dishes after 30 days of stratification exhibited fungal mycelium growth. It may be necessary for seeds to be treated with chemicals to ensure better emergence and reduce seed decay without negatively affecting early seedling growth. In later research, seed treatments of sea oats with surface disinfectants, fungicides, or a combination, significantly increased germination with no signs of damage to seedlings (Burgess et al., 2005).

For establishing wildflowers in the field, general recommendations include applying fertilizer low in nitrogen to sustain initial seedling growth while discouraging competition from weeds. A primary concern about successful establishment in the field is the need for seeds to make soil contact that is essential for the seed to have moisture (Aldrich, 2002; Kessler and Carden, 2001).

Three soils commonly found in central Alabama include Maryvn loamy sand (fine-loamy, kaolinitic typic Kanhapludult), Wickham sandy loam (fine-loamy, mixed,

semiactive, thermic typic Hapludult), and Houston clay (very fine, smectitic, thermic oxyaquic Hapludert). The Marvyn series is typically a deep, well-draining, permeable soil (NRSC, 2009a). The Houston clay comes from the Blackland Prairie soil area in Alabama. The area is known as the “Black Belt” because of the dark color of the soils. The soil is acidic and drains poorly. When wet, the soil swells and then cracks when it dries out (NRSC, 2009b). The Wickham sandy loam is typically a moderately permeable and deep well-draining soil formed from loamy sediments, commonly found on broad terraces along the Chattahoochee River (NRSC, 2009c). The properties to these common Alabama soils may affect the germination and establishment of wildflower seeds.

Because of Alabama’s diversity geologically and biologically, and the possibility that these attributes may affect wildflower establishment, it is important to research the impact of some of the different soil types on some of the diverse wildflower species. Public desire to integrate wildflowers for their aesthetic and ecological quality creates a need for more information on their germination and landscape establishment needs. Therefore, the objective of this research is to determine the germination and landscape establishment requirements of selected native Alabama wildflowers in order to enable ALDOT, growers, and home gardeners to incorporate attractive wildflowers in ecologically sustainable habitats in Alabama.

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Chapter II
Preliminary Studies to Determine Seed Stratification
Procedures for Three Wildflower Species

Additional index words. *Rudbeckia fulgida*, orange coneflower, *Penstemon laevigatus*, eastern smooth beardtongue, *Lobelia cardinalis*, cardinal flower, wildflower, stratification

Abstract

The effect of stratification temperature and duration; and fungicidal and sterilization treatments on seed germination of *Rudbeckia fulgida* Aiton, *Penstemon laevigatus* Aiton, and *Lobelia cardinalis* L. was studied. Seeds were stratified at either $5\pm 1^{\circ}\text{C}$ (41°F) or $10\pm 1^{\circ}\text{C}$ (50°F), for 3, 6, or 9 weeks, or not stratified, and then placed in germination chambers at $30\pm 1^{\circ}\text{C}$. Seeds were treated with: 1) bleach before stratification, 2) fungicide after stratification, or 3) a combination of both. Germination of seeds of *L. cardinalis* was negligible. Germination percentage of *R. fulgida* seeds was highest when they were not stratified, and treated with bleach or a combination of bleach and fungicide. At least 3 weeks of stratification and treatment with bleach, or a combination of bleach and fungicide improved germination percentage of *P. laevigatus*.

Introduction

Though many companies sell packaged wildflower seeds, availability is inconsistent and provenance varies widely. Little research has been done on germination requirements for most wildflower species (Kaspar and McWilliams, 1982). Some wildflower seeds are easily germinated and grown, while others are not. Species requiring seed treatments can be difficult and costly to grow because they require more time and labor to achieve acceptable germination rates (Allen and Meyer, 1990; Fay et al., 1993; Fay et al., 1994; Pill et al., 2000; Lindgren and Schaaf, 2004). The high price of packaged wildflower seeds presents another limitation to their use (Pill et al., 2000).

The complex seed dormancy systems some species possess enable them to survive ungerminated in natural environments until there are favorable conditions for seedling survival. Unfortunately, these mechanisms also make consistent propagation results difficult to obtain (Bratcher et al., 1993; Samfield et al., 1991). Germination requirements vary not only by species but also seed age and seed source (Lindgren and Schaaf, 2004).

There is limited information on effective germination protocols for *Rudbeckia fulgida* (orange coneflower). Nau (1996) stipulated that germination occurs best after a moist stratification of 2 to 4 weeks at 5 to 7°C (40 to 45°F), and Fay et al. (1993) found that germination temperatures of 28 to 32°C (82 to 90°F) were best, with highest germination at 30°C (86°F). Further research was conducted with osmotic seed priming for seed lots with low vigor. The vigor of a seed is its potential in germination and performance. Seeds in lots with low vigor germinated erratically and seedling emergence was poor (Hartmann et al., 2002). It was found that seeds of *R. fulgida*

primed in -1.3 MPa KNO₃ had twice the germination percentage and half the mean time to germination compared to non-primed seeds (Fay et al., 1994).

Penstemon species were reported to need one or some combination of seed scarification, stratification, changing temperatures, growth hormones, or light for efficient germination (Lindgren and Schaaf, 2004). In work with *P. digitalis* Nutt., *P. angustifolius* Nutt. Ex. Pursh, *P. barabatus* (Cav.) Roth, *P. gracilis* Nutt., *P. grandiflorus* Nutt., *P. haydenii* S. Wats., and *P. strictus* Beneth.), it was found that 8 weeks of cold stratification improved germination for most of the selected species. Stratification and light requirements appeared to be species specific (Lindgren and Schaaf, 2004). These results were supported by Allen and Meyer (1990) who found that 8 weeks of stratification was necessary for *Penstemon* spp. Generally, *Penstemon* spp. seeds can germinate at 18 to 21°C (65 to 70°F) in 2-3 weeks, but some species might benefit from 8 weeks of stratification at 15°C (59°F) (Hartmann et al., 2002).

There are conflicting recommendations for meeting germination requirements of seeds of *Lobelia cardinalis* (cardinal flower). Still (1994) stipulated that seed was easily germinated at 21°C (70°F) under light with no stratification. It was also found that seeds germinated best 4 years after harvesting, probably because of the development of secondary dormancy (Muenscher, 1935; Still, 1994). Other recommendations include up to 3 months of moist stratification (University of Texas at Austin, 2008).

Seeds decay may occur during longer periods of stratification. Seeds of *Uniola paniculata* L. (sea oats) transferred to Petri dishes after 30 days of stratification exhibited fungal mycelium growth (Burgess et al., 2002). In subsequent research, treatments of sea oats seed with surface disinfectants, fungicides, or a combination of

both, germination significantly increased with no signs of damage to seedlings (Burgess et al., 2005). Because there is limited formal research on effective germination procedures for *R. fulgida*, *Penstemon laevigatus* (eastern smooth beardtongue), and *L. cardinalis*, species indigenous to Alabama, it is necessary to determine the best protocols for successfully germinating seeds of these taxa for use in that state.

Therefore, the objective of this research was to determine the effect of stratification temperature and duration, and fungicidal and sterilization treatments on germination of *Rudbeckia fulgida*, *Penstemon laevigatus*, and *Lobelia cardinalis*.

Materials and Methods

On 28 Aug 2008, seeds of *Rudbeckia fulgida* (orange coneflower) (Everwilde Seed, Bloomer, Wis.) and *Penstemon laevigatus* (eastern smooth beardtongue) (Ernst Conservation Seeds, Meadville, Pa.) were placed in separate 1000 mL beakers (one species per beaker) filled with tap water. Air movement through the water in the beaker was created using a Tetra[®] Whisper In-Tank Filter (Blacksburg, Va.) without the filter and inserting the bubbling tube into the beaker of tap water. The seeds were allowed to imbibe for 10 hours at room temperature approximately 24°C (75°F). After imbibition, the beaker was drained and approximately 350 seeds of each taxa were removed from each beaker and laid on paper towels to air dry at room temperature for 3-4 min. while the remaining seeds remained in the beaker. A 15% bleach (6% sodium hypochlorite) solution was added to each beaker until seeds were submerged. The seeds and bleach solutions were stirred continuously for 10 min with a stirring rod. The seeds and bleach solution were poured through a cheesecloth-lined strainer and rinsed with tap water for approximately 2 min. Seeds treated with bleach were placed on paper towels to air dry

at room temperature for 3-5 min. Bleached and non-bleached seeds were kept separate. At least 100 seeds of one species were placed in each of 18 small plastic bags [16.5 cm x 8.2 cm (6.5 in x 3.25 in)]. Each bag was unsealed and placed into a larger plastic bag [17.8 cm x 20.3 cm (7 in x 8 in)] containing a moistened paper towel. Each exterior storage bag was sealed and placed on ice in an ice chest [9.5 L (10 qt)]. This was to keep the moist seeds cool during transport from Auburn, Ala. to the Seed Division Lab of the State Alabama Department of Agriculture and Industries, Montgomery, Ala. or to the U.S. Forestry Seed Lab, Dry Branch, Ga.

Upon arrival at each location, seeds were placed in one of two controlled environment chambers for stratification or into a controlled environment chamber for germination. The chambers used for stratification were set at $5\pm 1^{\circ}\text{C}$ (4°F) or $10\pm 1^{\circ}\text{C}$ (50°F). The chamber used for germination was set at $30\pm 1^{\circ}\text{C}$ (86°F). Seeds were stratified for 3, 6, or 9 weeks, or not stratified. Seeds were kept in the plastic bags (described above) during stratification.

Prior to transferring seeds to the germination chamber, seeds were placed in styrene jars [118 mL (4 oz), 89 mm (3.5 in)] (Parkway Plastics, Inc. Piscataway, N.J.). Each jar contained two pieces of 8.6 cm (3.4 in) Crocker Blue blotter circles, (Anchor Paper Company, St. Paul, Minn.) uniformly moistened with 6 mL (0.2 oz) of tap water or 6 mL (0.2 oz) of $100\text{ mg}\cdot\text{liter}^{-1}$ (100 ppm) Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, Calif.). Thus seeds were treated with bleach only (before stratification), fungicide only (after stratification but before germination), or a combination of both (hereafter referred to as seed treatment).

For each seed treatment, there were five jars with 10 seeds per jar resulting in a total of 15 jars per stratification temperature and duration combination per species.

Number of seeds germinated was recorded 3 times a week for three weeks, and seeds were removed from jars once germinated. Seeds were considered germinated when the radicle emerged was ≥ 1.0 mm (0.04 in). Blotter circles were remoistened with 2 mL (0.07 oz) of water or fungicide with a spray bottle as needed to keep them moist. Data were analyzed using PROC GLIMMIX in SAS version 9.1.3 (SAS Institute, Cary, NC). Temperature, length of stratification, and seed treatment were analyzed as a three-way factorial using the binomial probability distribution. Significance of main effects and interactions were determined at $\alpha = 0.05$. Though counts of the number of seeds germinated out of the total number of seeds sown were analyzed as a binomial proportion, data is presented as percent germination. Contrasts were used to determine trends and paired comparisons of simple effects of interactions.

The protocol described above was conducted with seeds of *R. fulgida* and *P. laevigatus* in Montgomery, Ala., and seeds of *P. laevigatus* and *L. cardinalis* in Dry Branch, Ga. With one exception, seeds of *L. cardinalis* were not imbibed prior to seed treatment.

Results

There was a higher germination percentage when seeds were not stratified than when stratified at 5°C or 10°C (Table 1, Fig. 1, 2). Within non-stratified seeds of *R. fulgida*, those treated with bleach or bleach and fungicide had higher germination percentages than those treated with fungicide only (Table 1, Fig. 1). When data were pooled over treatment, seeds of *R. fulgida* had a higher germination percentage at 5°C

than 10°C when stratified for 3 weeks (Table 1, Fig. 2). Additionally, seed germination decreased linearly as stratification length increased at both 5°C and 10°C.

When data were pooled over weeks, seeds stratified at 5°C and treated with bleach or bleach and fungicide had higher germination percentage than when treated with fungicide only (Table 1, Fig. 2). There were no differences among treatments when seeds were stratified at 10°C (Table 1, Fig. 2). Seeds treated with bleach only had higher germination percentage when stratified at 5°C than when stratified at 10°C (Table 1, Fig. 2).

There were no differences among seed treatments in the non-stratified seeds of *P. laevigatus* (Table 2, Fig.1). Furthermore, seeds stratified at 5°C and 10°C had higher germination percentage than non-stratified seeds (Table 2).

When data were pooled over temperature, germination percentage of seeds treated with fungicide increased linearly as stratification length increased (Table 2, Fig. 3). When stratified for 3 weeks, the highest germination percentages occurred when seeds were treated with either bleach or a combination of bleach and fungicide than when treated with fungicide only (Table 2, Fig. 3).

When data were pooled over weeks, seeds treated with only fungicide had a higher germination percentage when stratified at 5°C than when stratified at 10°C (Table 2). Within the 5°C stratification temperature, there were no differences among the seed treatments (Table 2). When stratified at 10°C, the highest germination percentage (45%) occurred when seeds were treated with bleach only (Table 2, Fig. 3).

In Dry Branch, Ga., there were no differences in germination percentage of *P. laevigatus* seeds among stratification temperatures and lengths or seed treatments (Fig.

4). Germination was <5% for *L. cardinalis*, therefore statistical analysis was not performed.

Discussion

For *R. fulgida* and *P. laevigatus*, most seeds germinated within 3-6 days of sowing (Fig.1-4). Highest germination percentages were 56% for *R. fulgida*, Montgomery, Ala.; 47% for *P. laevigatus*, Montgomery, Ala.; and 75% for *P. laevigatus*, Dry Branch, Ga. (Fig. 1-4).

In this study, non-stratified seeds of *R. fulgida* had a higher germination percentage than stratified seeds, indicating that stratification is not necessary for successful seed germination. The results are similar to those found by Fay et al. (1993) with acceptable germination percentage when not stratified and germinated at 30°C (86°F). Also, non-stratified seeds treated with bleach or a combination of bleach and fungicide germinated better than those treated with fungicide only, suggesting that the fungicide treatment was not needed. These results are in contrast to results found by Nau (1996) in which germination of *R. fulgida* occurred best when stratified for 2 to 4 weeks at 5 to 7°C (40 to 45°F). Bleach or a combination of bleach and fungicide increased the germination of both stratified and non-stratified seeds compared to seeds treated with fungicide only.

There was no difference among treatments for germination of *P. laevigatus* in Dry Branch, Ga. In Montgomery, Ala., seeds that were treated with bleach or bleach and fungicide germinated similarly when stratified for 3, 6, or 9 weeks. Germination percentage of seeds treated with fungicide only increased linearly as stratification length increased. These results are supported by previous studies in which 8 weeks cold

stratification improved germination of several selected *Penstemon* spp. (Allen and Meyer, 1990; Bratcher et al., 1993; Hartmann et al., 2002; Lindgren and Schaaf, 2004). The results of the current study suggest that seeds of *P. laevigatus* treated with bleach or a combination of bleach and fungicide, need no longer than 3 weeks cold stratification for good seed germination. Even though some species of *Penstemon* do not need stratification (Hartmann et al., 2002), *P. laevigatus* seeds require stratification for successful germination.

Although seeds of *P. laevigatus* used at both locations were from the same source, germination chambers in Alabama were older and seem to have higher relative humidity than the newer chambers in Georgia (although relative humidity data was not taken). Since the chambers were drier in Georgia, fungal growth may have been restricted excluding the need for bleach or fungicide treatments. Due to the high humidity of the chambers in Alabama, fungal growth occurred more readily and the bleach and fungicide treatments helped to counteract it. Even with sterilization and fungicide, gray mycelium fungal growth was prevalent among all treatments in both locations. Although the seed surface was disinfested, fungus may have originated from within the seed coat. Compared to preliminary experiments where no bleach or fungicide treatments were used, there was a marked improvement in germination percentage in this experiment when seeds were treated with fungicide and bleach. This conclusion agrees with work done on seeds of sea oats in which surface disinfestants improved germination percentage (Burgess et al., 2002, 2003).

Overall, for *R. fulgida*, results suggest that stratification reduced seedling emergence, and seeds that were not stratified and treated with bleach or a combination

of bleach and fungicide had the best germination. For *P. laevigatus*, stratification of 3 weeks is necessary for seedling emergence, and seed treatments of bleach improve germination.

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Table 1. Effect of stratification temperature and duration, and fungicidal and sterilization treatments on germination percentage of *R. fulgida* in Montgomery, Ala. Seeds were stratified at either 5±1°C (41°F) or 10±1°C (50°C), for 0, 3, 6, or 9 weeks. Seeds were treated with either bleach, fungicide, or a combination of both and germinated at 30±1°C.

Treatment	Germination percentage			Significance
	No stratification			
Fungicide	20 b ^z			
Bleach+Fungicide	52 a			
Bleach	56 a			

Temperature (°C)	Germination percentage			Significance
	Weeks			
No stratification	42 a			
5	20 b			
10	16 b			

Temperature (°C)	Germination percentage			Significance
	Weeks			
5	39 a	13	6	L ^{***y}
10	23 b	17	8	L ^{**}

Treatment	Germination percentage	
	Temperature (°C)	
	5 b	10
Fungicide	24 b	26
Bleach+Fungicide	40 a	26
Bleach	52 aA	18 B

^z Differences among percentages within columns denoted by letters (lower case) and within rows (upper case) as determined using paired contrasts at $\alpha = 0.05$.

^y Linear orthogonal contrast at $\alpha = 0.01(**)$ or $0.001(***)$.

Table 2. Effect of stratification temperature and duration, and fungicidal and sterilization treatments on germination percentage of *Penstemon laevigatus* in Montgomery, Ala. Seeds were stratified at either 5±1°C (41°F) or 10±1°C (50°C), for 0, 3, 6, or 9 weeks. Seeds were treated with either bleach, fungicide, or a combination of both and germinated at 30±1°C.

Treatment	Germination percentage			Significance
	No stratification			
Fungicide	2 ns ^z			
Bleach+Fungicide	12			
Bleach	12			

Temperature (°C)	Germination percentage		
	No stratification		
No stratification	9 b		
5	40 a		
10	35 a		

Treatment	Germination percentage			Significance
	Weeks			
	3	6	9	
Fungicide	22 b	38	40	L ^{**y}
Bleach+Fungicide	43 a	34	33	NS
Bleach	47 a	44	34	NS

Treatment	Germination percentage	
	Temperature (°C)	
	5	10
Fungicide	39 A	27 bB
Bleach+Fungicide	41	33 b
Bleach	39	45 a

^z Differences among percentages within columns denoted by letters (lowercase), within rows (upper case), and NS=not significant, as determined using paired contrasts at $\alpha = 0.05$.

^yLinear orthogonal contrast at $\alpha = 0.01(**)$ or $0.001(***)$.

Figure 1. Germination percentage of unstratified seeds of (A) *Rudbeckia fulgida* in Montgomery, Ala., (B) *Penstemon laevigatus* in Montgomery, Ala., and (C) *Penstemon laevigatus* in Dry Branch, Ga. treated with fungicide, bleach, or a combination of both. Seeds were germinated at 30°C (86°C).

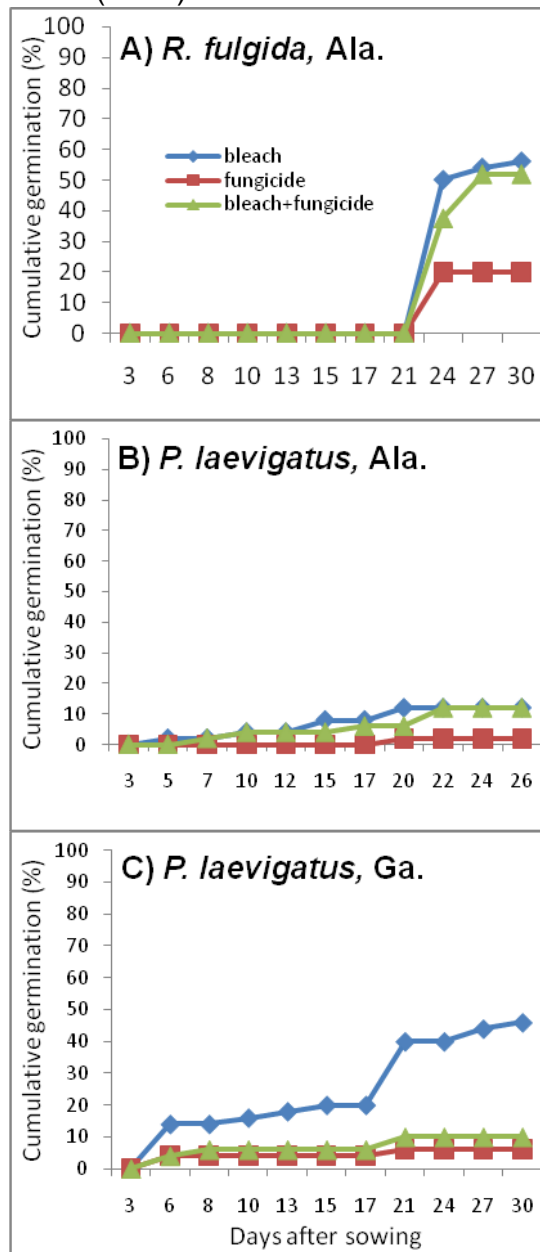


Figure 2. Germination percentage of *Rudbeckia fulgida* in Montgomery, Ala. treated with fungicide, bleach, or a combination of both at (A) 5°C for 3 weeks, (B) 5°C for 6 weeks, (C) 5°C for 9 weeks, (D) 10°C for 3 weeks, (E) 10°C for 6 weeks, (F) 10°C for 9 weeks and seeds were germinated at 30°C.

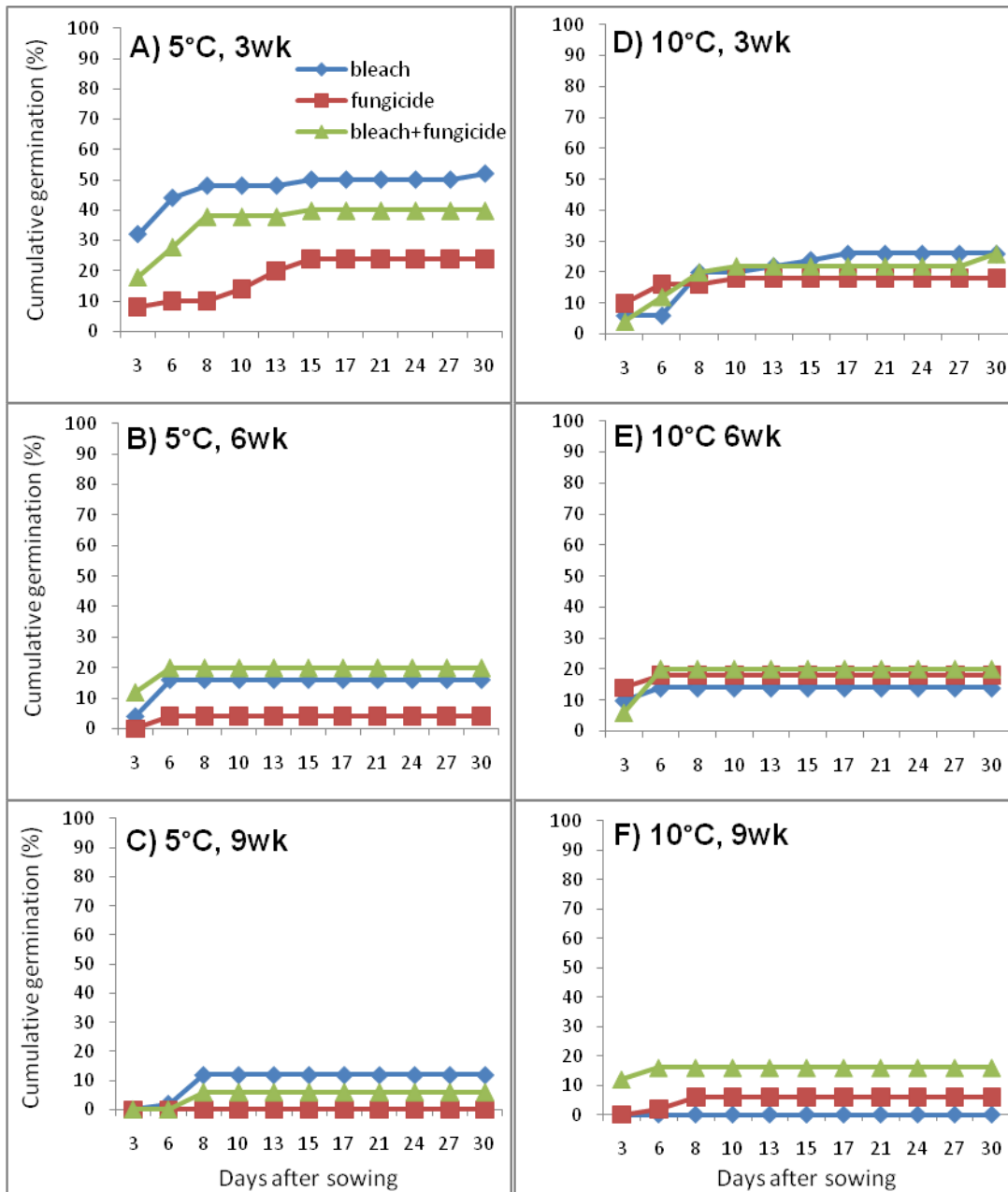


Figure 3. Germination percentage of *Penstemon laevigatus* in Montgomery, Ala. treated with fungicide, bleach, or a combination of both at (A) 5°C for 3 weeks, (B) 5°C for 6 weeks, (C) 5°C for 9 weeks, (D) 10°C for 3 weeks, (E) 10°C for 6 weeks, (F) 10°C for 9 weeks and seeds were germinated at 30°C.

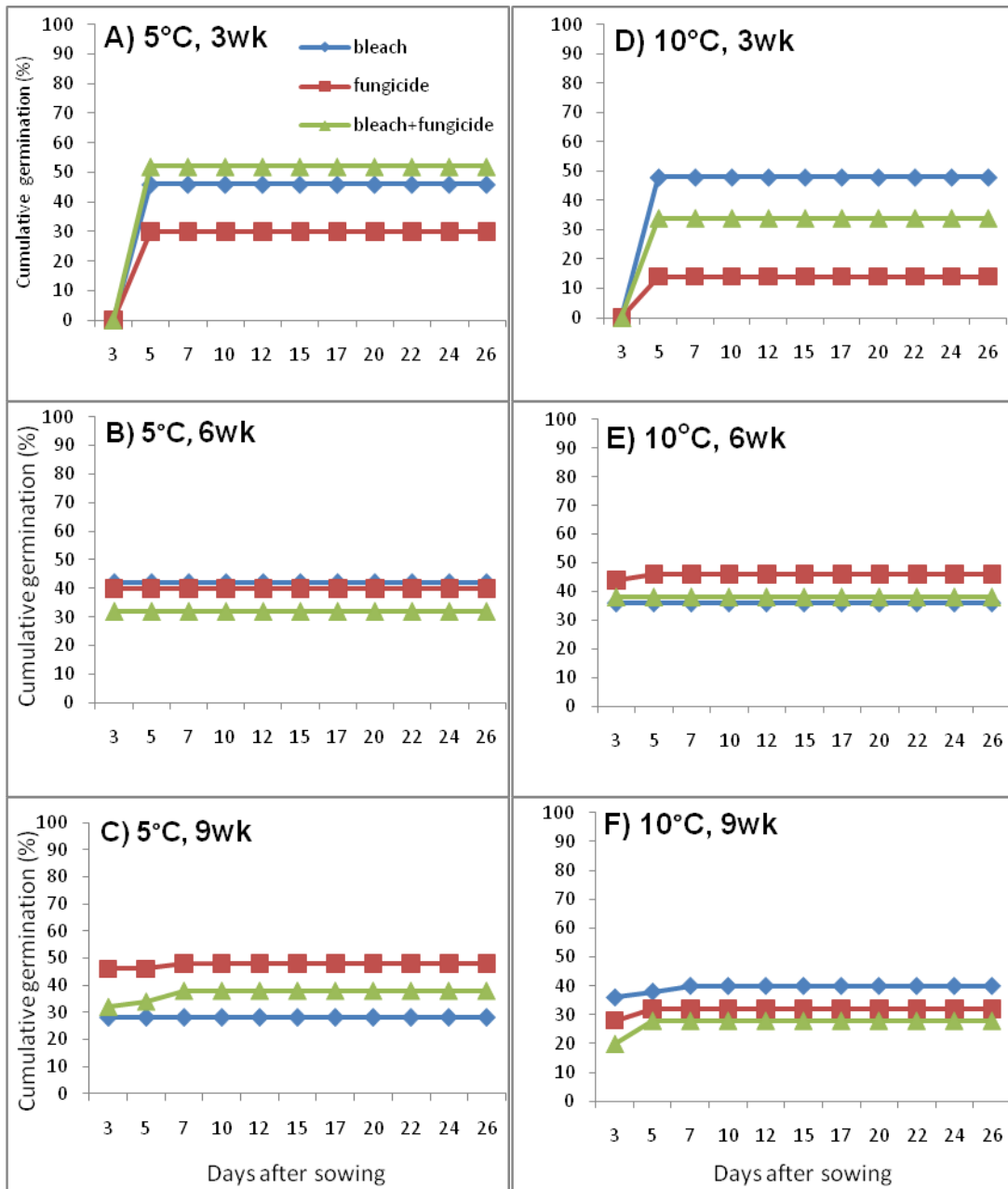
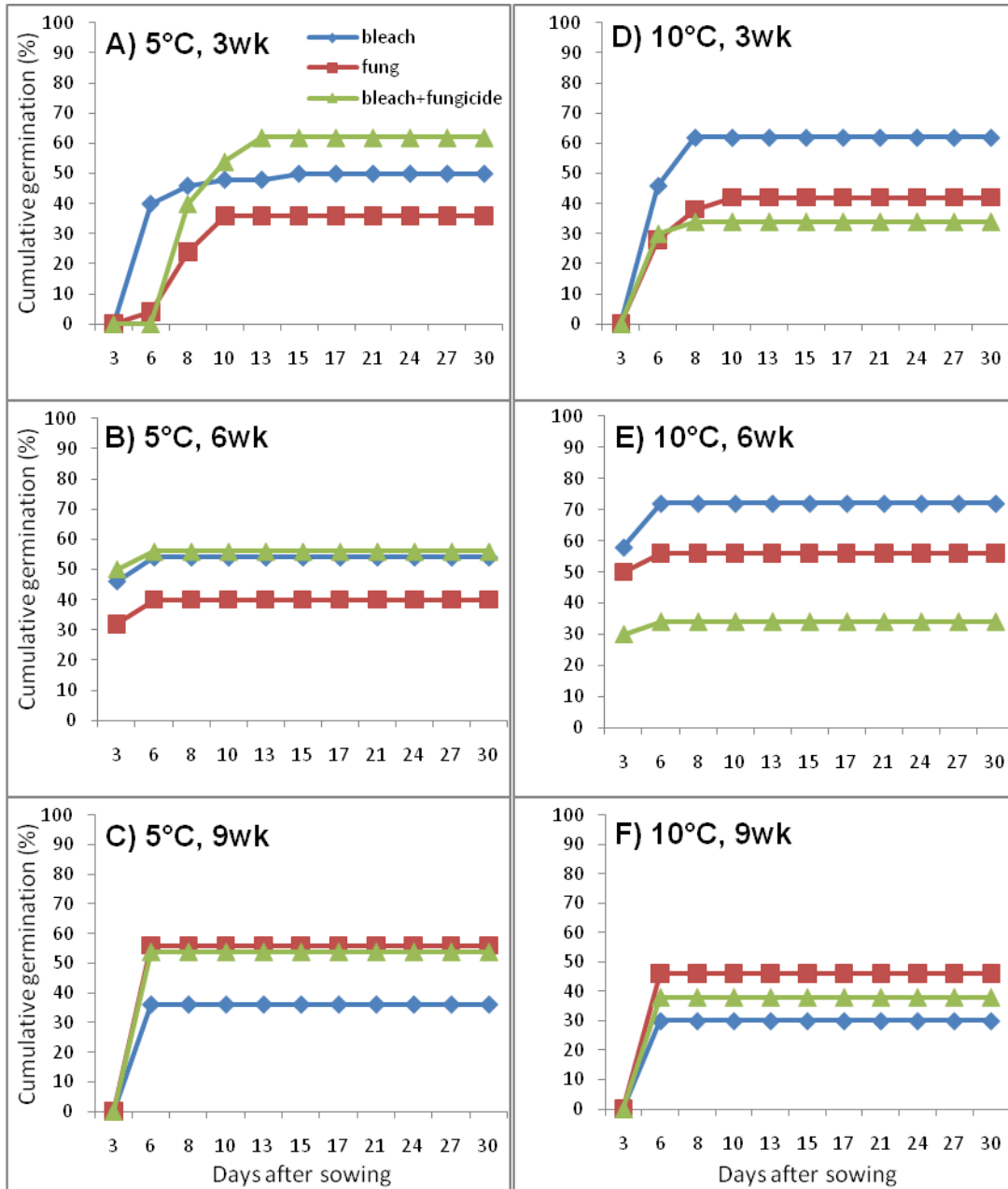


Figure 4. Germination percentage of *Penstemon laevigatus* in Dry Branch, Ga. treated with fungicide, bleach, or a combination of both at (A) 5°C for 3 weeks, (B) 5°C for 6 weeks, (C) 5°C for 9 weeks, (D) 10°C for 3 weeks, (E) 10°C for 6 weeks, (F) 10°C for 9 weeks. Seeds were germinated at 30°C.



Chapter III
Effect of Imbibition and Fungicidal Pre-Treatments
and Stratification Length on Seed Germination
of Three Wildflower Species

Additional index words. *Rudbeckia fulgida*, orange coneflower, *Penstemon laevigatus*, eastern smooth beardtongue, *Lobelia cardinalis*, cardinal flower

Abstract

The effect of imbibition and fungicidal pre-treatments, and stratification length on seed germination of *Rudbeckia fulgida* Aiton (orange coneflower), *Penstemon laevigatus* Aiton (eastern smooth beardtongue), and *Lobelia cardinalis* L. (cardinal flower) was studied. Seeds were either imbibed or not imbibed (before stratification), and treated with fungicide or not with fungicide (after stratification), and stratified at $5\pm 1^{\circ}\text{C}$ (41°F) for 3 or 6 weeks, or not stratified, and then placed in germination chambers set at $30\pm 1^{\circ}\text{C}$. The experiment was repeated three times with all species. Germination of *R. fulgida* was higher when seeds were imbibed and not stratified. Germination of *P. laevigatus* was higher when seeds were imbibed and stratified at 5°C (41°F) for 6 weeks. Germination for *L. cardinalis* was negligible.

Introduction

Wildflower seed germination requirements vary by species (Lindgren and Schaaf, 2004), and research on germination requirements for most wildflower species is limited (Kaspar and McWilliams, 1982). The complex seed dormancy systems that some species possess enable them to survive in natural environments by until there are favorable conditions for seedling survival. Unfortunately, these mechanisms also make consistent propagation results difficult to obtain and can present limitations to municipalities, state departments of transportation, and individuals wishing to incorporate wildflowers into the urban or home landscape (Bratcher et al., 1993; Samfield et al., 1991). *Rudbeckia fulgida* (orange coneflower), *Penstemon laevigatus* (eastern smooth beardtongue), and *Lobelia cardinalis* (cardinal flower) are attractive wildflower species for which there are incomplete or conflicting propagation recommendations.

There is limited information on effective germination protocols for *Rudbeckia fulgida*. Nau (1996) stipulated that germination occurs best after a moist stratification of 2 to 4 weeks at 5 to 7°C (40 to 45°F), and Fay et al. (1993) found that germination temperatures of 28 to 32°C (82 to 90°F) were the best with highest germination at 30°C (86°F). Further research was conducted with osmotic seed priming of seed lots with low vigor. Low vigor generally means inconsistent germination and poor seedling emergence (Hartmann et al., 2002). They found that seeds of *R. fulgida* primed in -1.3 MPa KNO₃ had twice the germination percentage and half the mean time to germination when compared to non-primed seeds (Fay et al., 1994). In spite of these recommendations for stratification temperatures and lengths, germination temperatures,

and seed priming, no information exists on the effect of imbibition and fungicidal pre-treatments on seed germination.

Penstemon species are reported to need one or some combination of seed scarification, stratification, changing temperatures, growth hormones, or light for satisfactory germination (Lindgren and Schaaf, 2004). In work with *P. digitalis* Nutt., *P. angustifolius* Nutt. Ex. Pursh, *P. barabatus* (Cav.) Roth, *P. gracilis* Nutt., *P. grandiflorus* Nutt., *P. haydenii* S. Wats., and *P. strictus* Beneth.), it was found that 8 weeks of cold stratification at $1\pm 3^{\circ}\text{C}$ improved germination for most of the selected species. Stratification and light requirements appear to be species specific. These results are supported by Allen and Meyer (1990) who found that 8 weeks stratification at 5°C was necessary for *Penstemon* spp. Generally, *Penstemon* spp. seeds germinate at 18 to 21°C (65 to 70°F) in 2-3 weeks, but some species might benefit from 8 weeks of stratification at 15°C (59°F) (Hartmann et al., 2002). Specific stratification and germination requirements have not been published for *Penstemon laevigatus*.

There are conflicting recommendations regarding germination requirements for seeds of *Lobelia cardinalis* (cardinal flower). Still (1994) stipulated that seed is easily germinated at 21°C (70°F) under light with no stratification. Seeds also germinated best 4 years after harvesting probably because of the development of secondary dormancy (Muenscher, 1935; Still, 1994). Other recommendations include up to 3 months of moist stratification (University of Texas at Austin, 2008).

Seed decay may occur during longer periods of stratification. Seeds of *Uniola paniculata* L. (sea oats) transferred to Petri dishes after 30 days of stratification exhibited fungal mycelium growth (Burgess et al., 2002). In subsequent research,

treatments of sea oats seed with surface disinfectants, fungicides, or a combination of both, significantly increased germination with no signs of damage to seedlings (Burgess et al., 2005). Building on past research with *R. fulgida*, *L. cardinalis*, and species related to *P. laevigatus*, examining the effect of imbibition and fungicidal pre-treatments may improve seed germination of these species. Therefore, the objective of this research is to determine the effect of stratification length and imbibition and fungicidal pre-treatments on germination of seeds of *Rudbeckia fulgida*, *Penstemon laevigatus*, and *Lobelia cardinalis*.

Materials and Methods

Seeds of *Rudbeckia fulgida* (orange coneflower) (Everwilde Seed, Bloomer, Wis.), *Penstemon laevigatus* (eastern smooth beardtongue) (Ernst Conservation Seeds, Meadville, Pa.), and *Lobelia cardinalis* L. (cardinal flower) (Native American Seeds, Junction, Tx.) were separated in two groups: seeds to be imbibed and seeds that were not. Seeds to be imbibed were placed in separate 1000 ml beakers (one species per beaker) filled with tap water. Air movement through the water in the beaker was created using a Tetra[®] Whisper In-Tank Filter (Blacksburg, Va.) without the filter and inserting the bubbling tube into the beaker of tap water. The seeds were allowed to imbibe for 10 hours at room temperature. After imbibition, the beakers were drained and seeds of each taxa were removed from each beaker and laid on paper towels to air dry at room temperature for 3-4 min. For seeds to be stratified, at least 200 seeds of one species were placed in each of 16 small plastic bags [16.5 cm x 8.2 cm (6.5 in x 3.25 in)]. Each bag was unsealed and placed into a larger plastic bag [17.8 cm x 20.3 cm (7 in x 8 in)] that contained a moistened paper towel. Seeds were placed in one of two controlled

environment chambers for stratification or into a controlled environment chamber for germination. The chambers used for stratification were set at $5\pm 1^{\circ}\text{C}$ (40°F) (Adaptis A1000, Conviron, Winnipeg, Manitoba, Can.) (Plant Science Research Center, Auburn University, Auburn, Ala.) . The chamber used for germination was set at $30\pm 1^{\circ}\text{C}$ (86°F) (All-Stainless Steel Germinator, D7440/B, Seedburo Equipment Company Des Plaines, Ill.) (Auburn University, Auburn, Ala.). Seeds were stratified for 3 or 6 weeks, or not stratified. Seeds were kept in the plastic bags (described above) during stratification.

Prior to transferring seeds to the germination chamber, seeds were placed in styrene jars [118 mL (4 oz), 89 mm (3.5 in)] (Parkway Plastics, Inc. Piscataway, N.J.). Each jar contained two pieces of 8.6 cm (3.4 in) Crocker Blue blotter circles, (Anchor Paper Company, St. Paul, Minn.) uniformly moistened with 6 mL (0.2 oz) of tap water or 6 mL (0.2 oz) of $100\text{ mg}\cdot\text{liter}^{-1}$ (100 ppm) Blocker 4F Flowable Fungicide (38.3% penta chloro nitro benzene, Amvac Chemical Corporation, Los Angeles, Calif.). Thus seeds were imbibed or not imbibed (before stratification), and treated with or without fungicide (after stratification but before germination) for a total of four seed pre-treatments. For each seed pre-treatment, there were six jars with 10 seeds per jar resulting in a total of 24 jars per stratification temperature and duration combination per species.

Number of seeds germinated was recorded 3 times a week for 3 weeks, and seeds were removed from jars once germinated. Seeds were considered germinated when the radicle emerged $\geq 1.0\text{ mm}$ (0.04 in). Blotter circles were remoistened with 2 mL (0.07 oz) of water or fungicide with a spray bottle as needed to keep them moist. Data were analyzed using PROC GENMOD in SAS version 9.1.3 (SAS Institute, Cary, NC) with the binomial probability distribution. Significance of main effects and interactions for

germination percentage were determined at $\alpha = 0.05$. Contrasts were used to determine trends and paired comparisons of simple effects of interactions. Though counts of the number of seeds germinated out of the total number of seeds sown were analyzed as a binomial proportion, data is presented as percent germination.

The experiment was repeated three times for all species. The experiment was conducted for *R. fulgida* beginning on 23 July 2009, 13 August 2009, and 14 October 2009; for *P. laevigatus* on 2 April 2009, 12 June 2009, and 13 August 2009. In the third run for *P. laevigatus* and second run of *R. fulgida*, after seeds were stratified for 6 weeks, they were transferred to a different chamber (Adaptis A1000, Conviron, Winnipeg, Manitoba, Can.) (Plant Science Research Center, Auburn University, Auburn, Ala.), previously set at $5\pm 1^{\circ}\text{C}$ (40°F), but reset to $30\pm 1^{\circ}\text{C}$ (86°F) for germination. The experiment was conducted for *L. cardinalis* 23 July 2009 and 13 August 2009.

Results

Rudbeckia fulgida

In Jun. 2009, imbibed seeds had a higher germination percentage than seeds that were not imbibed, and seeds that were not treated with fungicide had a higher germination percentage than those treated with fungicide (Table 1, Fig.1). There were no differences between non-stratified and stratified seeds.

In Aug. 2009, at all stratification time, imbibed seeds had a higher germination percentage than seeds that were not imbibed (Table 1, Fig. 1). As stratification length increased, germination percentage fluctuated quadratically. There were no differences between seeds treated with or without fungicide.

In Oct. 2009, all seeds had higher germination percentages when imbibed than when not imbibed (Table 1, Fig.1). Imbibed, but not stratified seeds had a higher germination percentage than imbibed seeds stratified for 3 weeks. Among unstratified seeds, there were no differences when seeds were treated with or without fungicide. However, among seeds stratified for 3 weeks, those treated with fungicide had a higher germination percentage than those not treated with fungicide. While the seeds stratifying for 6 weeks were in the chamber, the temperatures were mistakenly reset to 23°C (73°F) for more than 24 hours, after seeds had only stratified for 4 weeks. At that point, the experiment was terminated because of the interrupted stratification and, therefore, data is not available for seeds stratified for 6 weeks.

Penstemon laevigatus

In Apr. 2009, the stratification weeks by imbibition by fungicide interaction was significant (Table 2). Within unstratified seeds, there was no difference when seeds were treated with or without fungicide, either for seeds imbibed or not imbibed (Table 2, Fig. 2-3). When stratified for 3 weeks, seeds imbibed and pre-treated with fungicide had a higher germination percentage than seeds not imbibed and pre-treated with fungicide (Table 2, Fig. 2-3). When stratified for 6 weeks, imbibed seeds had higher germination percentages than seeds not imbibed. For imbibed seeds pre-treated with fungicide, germination increased quadratically as stratification length increased (Table 2, Fig. 2-3). Seeds not imbibed, but treated with fungicide, had a higher germination percentage when seeds were stratified for 3 weeks (Table 2, Fig. 2-3). For imbibed seeds receiving pre-treatment, germination increased linearly as stratification length

increased (Table 2, Fig. 2-3). For seeds not imbibed and receiving pre-treatment, there was germination only when stratified for 3 weeks.

In Jun. 2009, for unstratified seeds, there were no differences among seeds that were or were not imbibed (Table 2, Fig. 3). However, when stratified for 3 or 6 weeks, imbibed seeds had a higher germination percentage than seeds that were not imbibed. There were no differences among seeds treated with or without fungicide. For imbibed seeds, germination percentage increased quadratically as stratification length increased, but there were no differences among stratification weeks for seeds not imbibed (Table 2, Fig. 3).

In Aug. 2009 once again, there were no differences among unstratified seeds that were or were not imbibed (Table 2, Fig. 3). However, when stratified for 3 or 6 weeks, imbibed seeds had a higher germination percentage than seeds that were not imbibed (Table 2, Fig. 3). There were no differences among seeds treated with or without fungicide (Table 2, Fig. 3). For imbibed seeds, germination increased quadratically as stratification length increased, but there were no differences among stratification weeks for seeds not imbibed (Table 2, Fig. 3).

Lobelia cardinalis

Germination was <5% for *L. cardinalis*, therefore statistical analysis was not performed.

Discussion

Results varied across runs for *R. fulgida*. In the first run, there were no differences in germination percentages among stratification time. In the second run, germination percentage was highest when seeds were stratified for 6 weeks although there was acceptable germination when seeds were not stratified. In the third run, seeds

not stratified had the highest germination percentage compared to seeds stratified for 3 weeks. The results are similar to those found by Fay et al. (1993) with acceptable germination percent when seeds were not stratified and germinated at 30°C (86°F). Additionally, imbibing improved germination in all runs. For imbibed seeds, there was not a substantial difference between treating or not treating seeds with fungicide. Therefore, it is not necessary to stratify or treat seeds of *R. fulgida* with fungicide before sowing. However, imbibing seeds does improve germination.

Across all three runs of *P. laevigatus*, imbibed seeds stratified for 3-6 weeks had higher germination percentages than seeds that were not stratified and not imbibed. These results are supported by previous studies in which cold stratification improved germination of several selected *Penstemon* spp. (Allen and Meyer, 1990; Bratcher et al., 1993; Hartmann et al., 2002; Lindgren and Schaaf, 2004). Even though some species of *Penstemon* do not need stratification (Hartmann et al., 2002), seeds of *P. laevigatus* needed at least 3-6 weeks cold stratification and imbibing for good germination.

Even with fungicide, gray mycelium fungal growth was present among all treatments for both species. Even when fungicide was applied, small colonies of fungus were visible (not quantified). Seeds germinated quickly within the first week, and the fungal growth did not appear to deter seedling emergence. Ungerminated seeds and those that appeared rotted had more fungal growth. Overall, for both *R. fulgida* and *P. laevigatus*, fungicide was not a contributing factor to improved germination percent, and results suggest that treating seeds with fungicide may be unnecessary. Additionally, if seeds are not imbibed, then fungicide applications appears to be detrimental. In

conclusion, seeds for *R. fulgida* do not require stratification, and seeds of *P. laevigatus* germinate best when stratified for 3-6 weeks. For both taxa, imbibing greatly improves germination percent.

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Table 1. Effect of imbibition and fungicidal pre-treatments and stratification length on germination percentage of *Rudbeckia fulgida* seeds. Seeds were either imbibed or not imbibed, stratified at 5±1°C (41°F) for 0, 3, or 6 week, and then treated with or without fungicide and germinated at 30±1°C.

June 2009 ^z								
Imb. ^y	Germ ^x .%	Fung. ^w	Germ.%				Sig. ^v	P-Value
Imb.	19a ^u	Fung.	7b				wks	NS
No Imb.	2b	None	10a				imb.	<.0001
							fung.	0.0108
August 2009 ^t								
	Stratification (weeks)							
Imb.	0	3	6	Sig. ^s	Fung.	Germ.%	Sig.	P-Value
Imb.	32a	20a	73a	Q***	Fung.	71 NS	wks	<.0001
No Imb.	16b	8b	8b	NS	None	86	imb.	<.0001
							fung.	0.0300
							imb*wks	<.0001
October 2009								
	Stratification (weeks)		Fung.	Stratification (weeks)				
Imb.	0	3	Fung.	0	3		Sig.	P-Value
Imb.	80aA	51aB	Fung.	58	56a		wks	0.0001
No Imb.	38b	35b	None	60A	30bB		imb.	<.0001
							fung.	0.0178
							imb*wks	0.0029
							fung*wks	0.0021

^z All data for Jun. 2009 are percentage of seeds germinated out of 360 seeds sown per treatment.

^y Imbibition.

^x Germination.

^w Fungicide.

^v Significance.

^u Differences among percentages within columns denoted by letters (lower case), within rows (upper case), and NS = not significant, as determined using paired contrasts at $\alpha = 0.05$.

^t All data for Aug. and Oct. 2009 are percentage of seed germinated out of 120 seeds sown per treatment.

^s Not significant (NS) or quadratic (Q) trend at $\alpha = 0.001$ (***).

Table 2. Effect of imbibition and fungicidal pre-treatments, and stratification length on germination percent of *Penstemon laevigatus* seeds. Seeds were either imbibed or not imbibed, stratified at 5±1°C (41°F) for 0, 3, or 6 week, and then treated with or without fungicide and germinated at 30±1°C.

April 2009						
Weeks	Imbibed		Not imbibed		Sig. ^y	P-Value
	Fung. ^z	No fung.	Fung.	No fung.		
0 ^x	0 ^w	3	3b	0b	Wks	<.0001
3 ^v	16A	12	4aB	13a	imb.	NS
6	21A	28A	0bB	0bB	fung.	NS
Sig. ^u	Q**	L***	NS	Q***	wks*imb.*fung.	0.0087

June 2009						
Imb. ^t	Stratification (weeks)			Sig.	Sig.	P-Value
	0	3	6			
Imb.	1	19 a	38a	Q**	wks	0.0007
No Imb.	2	2b	3b	NS	imb.	NS
					imb.*wks	0.0110

July 2009						
Imb.	Stratification (weeks)			Sig.	Sig.	P-Value
	0	3	6			
Imb.	1	18a	45a	Q*	wks	0.0014
No Imb.	2	2b	3b	NS	imb.	NS
					imb.*wks	0.0029

^z Fungicide.

^ySignificance.

^x All non-stratified data for Apr. 2009 are number of seed percentages germinated out of 60 seeds sown per treatment

^vAll 3 and 6 weeks data for Apr. 2009 and all data for Jun. and Jul. 2009 are percentage of seed germinated out of 120 seeds sown per treatment.

^wDifferences among percentages within columns denoted by letters (lower case) and within rows (upper case) as determined using paired contrasts at $\alpha = 0.05$.

^uNot significant (NS) or significant linear (L) or quadratic (Q) trend at $\alpha = 0.05$ (*), 0.01(**), or 0.001(***)

^tImbibition.

Figure 1. Effect of imbibition and fungicidal pre-treatments, and stratification length on germination percent of seeds of *Rudbeckia fulgida*. Seeds were either imbibed or not imbibed, stratified at $5\pm 1^\circ\text{C}$ (41°F) for 0, 3, or 6 week, and then treated with or without fungicide and germinated at $30\pm 1^\circ\text{C}$ in three runs beginning 23 July 2009, 13 August 2009 and 14 October 2009, and germinated at 30°C .

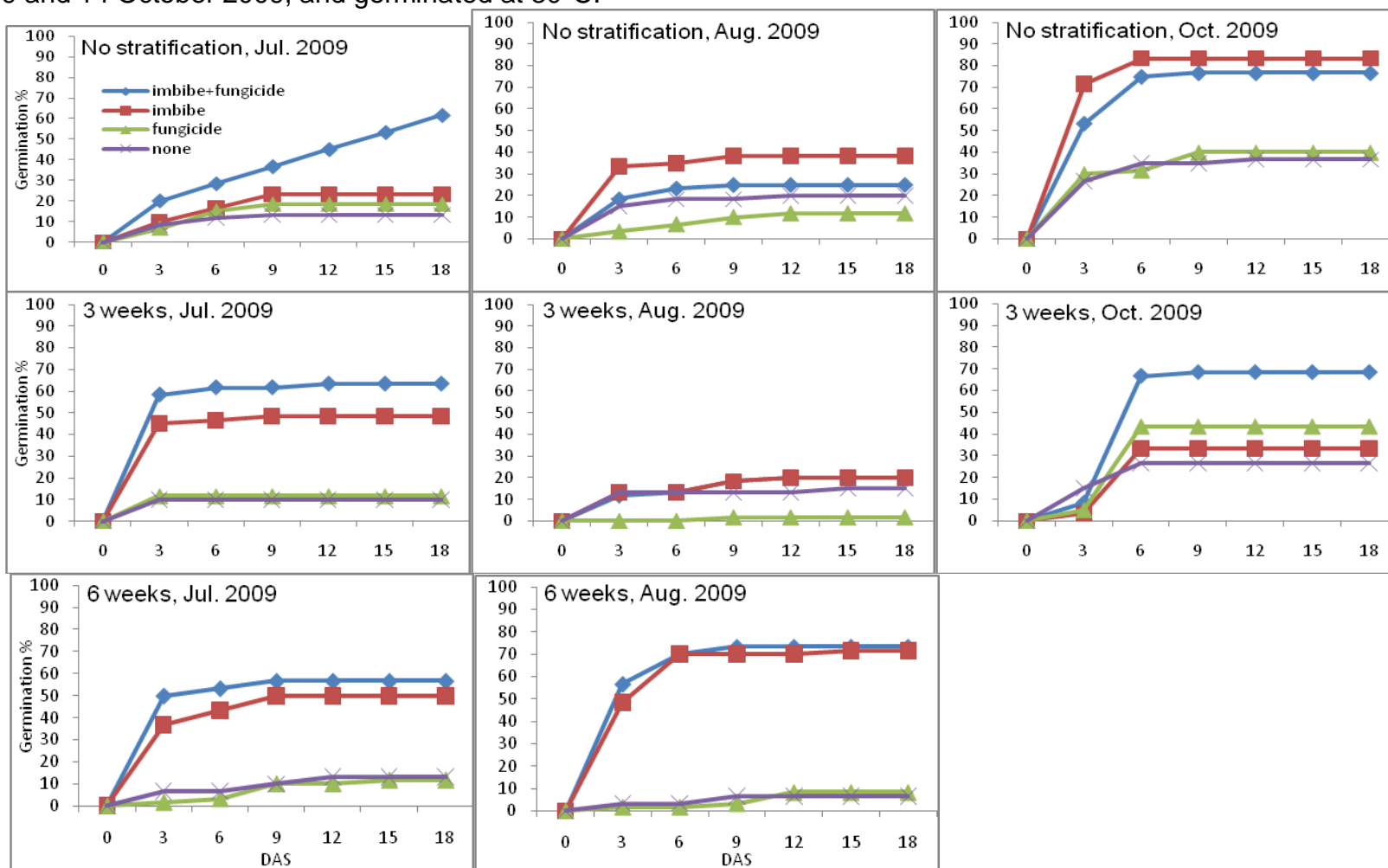


Figure 2. Effect of imbibition and fungicidal pre-treatments, and stratification length on germination percent of seeds of *Penstemon laevigatus*. Seeds were either imbibed or not imbibed, stratified 5±1°C (41°F) or 15°C for 0, 3, or 6 week, and then treated with or without fungicide and germinated at 30±1°C beginning 2 April 2009.

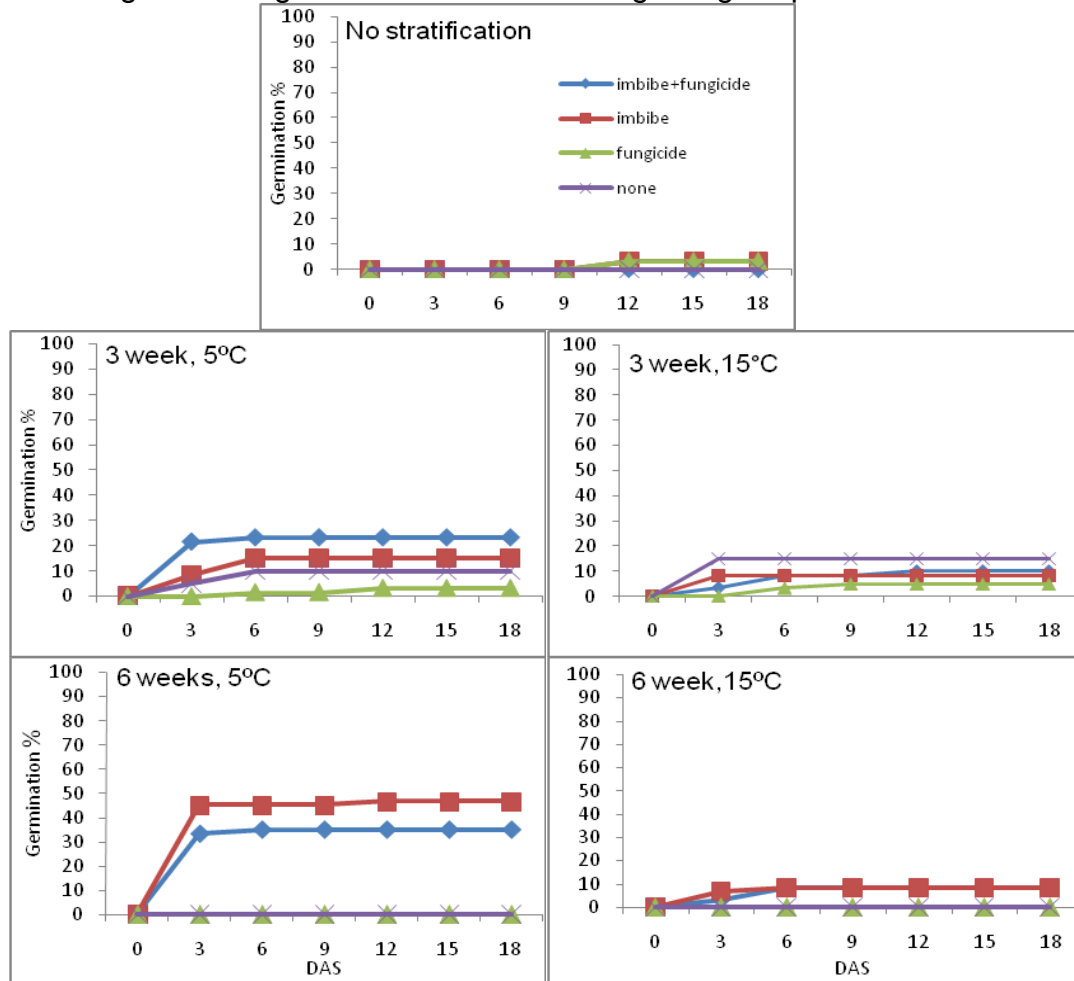
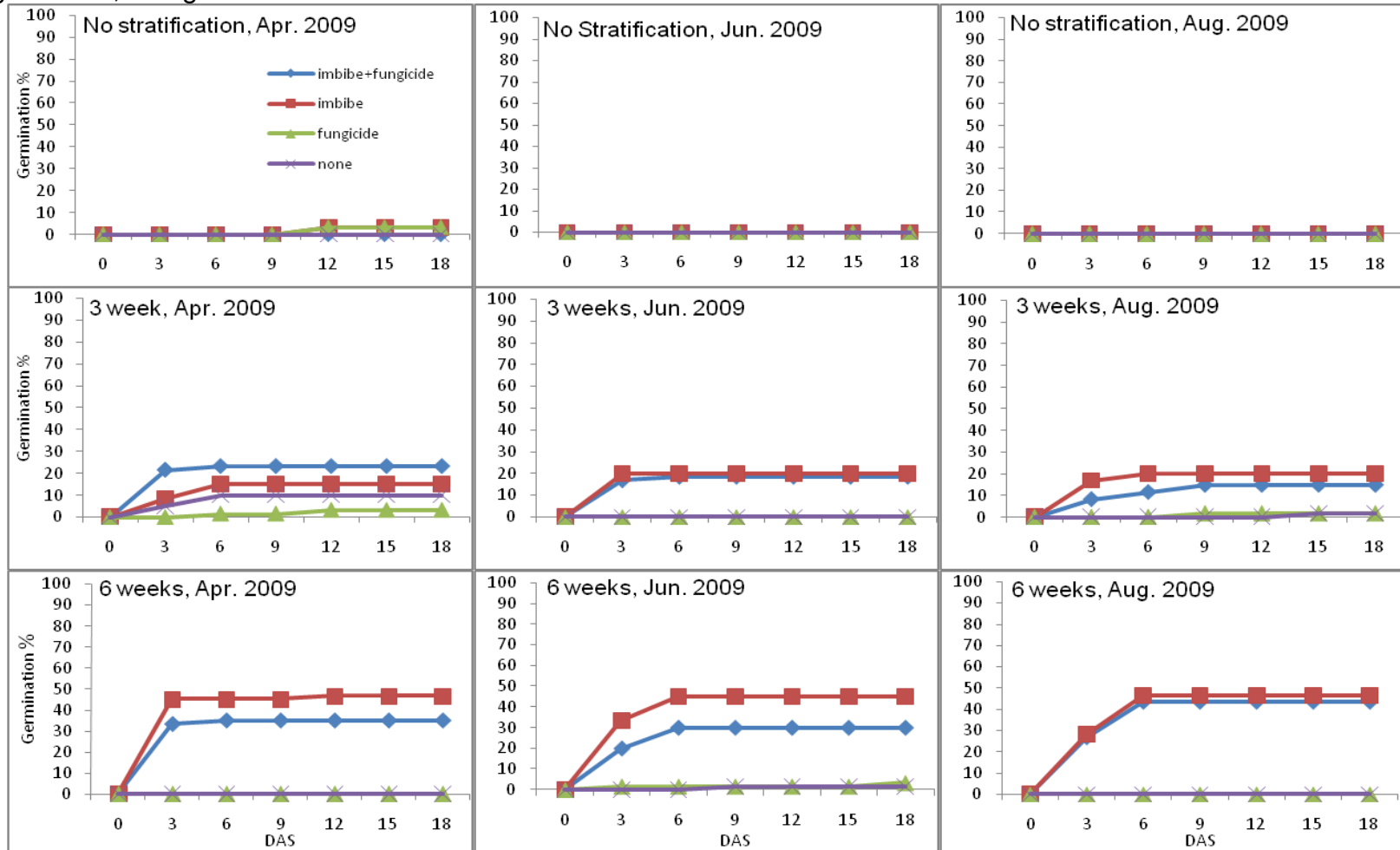


Figure 3. Effect of imbibition and fungicidal pre-treatments, and stratification length on germination percent of seeds of *Penstemon laevigatus*. Seeds were either imbibed or not imbibed, stratified at $5\pm 1^{\circ}\text{C}$ (41°F) for 0, 3, or 6 week, and then treated with or without fungicide and germinated at $30\pm 1^{\circ}\text{C}$ in three runs beginning 2 April 2009, 12 June 2009 and 13 August 2009, and germinated at 30°C .



Chapter IV
**Effect of Sowing Depth and Soil Type on Seed
Germination, Seedling Survival, and Growth
of Selected Wildflower Species**

Index Words: *Coreopsis tinctoria*, golden tickseed, *Coreopsis verticillata*, whorled tickseed, *Echinacea purpurea*, eastern purple coneflower, *Gaillardia pulchella*, firewheel, *Rudbeckia hirta*, black-eyed Susan, native plants

Abstract

Seeds of *Coreopsis tinctoria* Nutt. (golden tickseed), *Coreopsis verticillata* L. (whorled tickseed), *Echinacea purpurea* (L.) Moench (eastern purple coneflower), *Gaillardia pulchella* Foug. (firewheel), and *Rudbeckia hirta* L. (black-eyed Susan) were sown in three Alabama soil types at four sowing depths. Survival and subsequent growth of *R. hirta* and *C. tinctoria* were higher when seeds were surface sown than when sown below the surface. Survival and subsequent growth of *G. pulchella* and *E. purpurea* were higher when seeds were sown below the surface rather than above. Soil type influenced survival and growth of all species. There was higher survival and growth when seeds were sown in Wickham sandy loam and Marvyn loamy sand than when sown in Houston clay. Although pH was similar among all soil types, the water holding capacity of the Houston clay was high, resulting in rotted and non-germinating seed.

Introduction

The Federal Highway Administration (2008) encourages integration of native plants, especially wildflowers, into right-of-ways along highways under the provisions of the Surface Transportation and Uniform Relocation Assistance Act of 1987. Many species of wildflowers like *Rudbeckia spp.*, *Gaillardia spp.*, *Echinacea spp.*, and *Coreopsis spp.* are simple to grow and easily incorporated into the urban landscape (Bratcher et al., 1993; Kessler and Carden, 2001). Because wildflowers are low maintenance plants, they are often utilized by the U.S. Department of Transportation along roadsides, by city governments in municipal open areas, and by homeowners as bedding plants. Florida and Texas have research programs to develop methods for establishing native herbaceous plant material along their highways. Although the Alabama Department of Transportation (ALDOT) has roadside areas planted with wildflowers, attempts to establish self-sustaining populations have failed, and frequent re-seeding is required. Currently, ALDOT, golf courses, and homeowners often have to rely on recommendations for wildflower establishment that are based on another state's climate and soils. Research is needed to evaluate wildflower species for seed germination and landscape establishment in Alabama.

The primary concern about successful landscape establishment of wildflowers is the need for seed-to-soil contact that is essential for the seed to have adequate moisture for germination (Aldrich, 2002; Kessler and Carden, 2001). Unsuccessful establishment resulting from low germination and limited subsequent seedling growth may also be due to the wildflower seed sources. Also, an incomplete knowledge of

planting procedures, such as sowing depth, greatly inhibits successful establishment (Billingsly and Grabowski, 1996).

Three soils that are commonly found in central Alabama include Maryvn loamy sand (fine-loamy, kaolinitic typic Kanhapludult), Wickham sandy loam (fine-loamy, mixed, semiactive, thermic typic Hapludult), and Houston clay (very fine, smectitic, thermic oxyaquic Hapludert). Marvyn loamy sand is typically a deep, well-draining, permeable soil found in east central Alabama (NRSC, 2009a). Houston clay comes from the Blackland Prairie soil area in central Alabama. The area is known as the “Black Belt” because of the dark color of the soils. The soil is acidic, drains poorly, swell when wet, and cracks when it dries out (NRSC, 2009b). Wickham sandy loam is typically a moderately permeable, deep, well-draining soil formed from loamy sediments and commonly found on broad terraces along the Chattahoochee River in central Alabama (NRSC, 2009c). Physical properties of these Alabama soils may affect wildflower seed germination, subsequent seedling establishment, and plant growth. Therefore, the objective of this experiment was to determine survival and subsequent growth of five wildflower species sown at four sowing depths in three Alabama soil types.

Materials and Methods

Seeds of several wildflower species were sown in three Alabama soil types at four depths over three runs. Plastic 3.8 L (1 gal) containers (Cassco, Montgomery, Ala.) were lined along the bottom with brown paper towels (to prevent soil from escaping containers through drainage holes) and then filled with Houston clay [very fine, smectitic, thermic oxyaquic Hapludert (E.V. Smith Experiment Station, Macon Co.)], Maryvn loamy sand [fine-loamy, kaolinitic typic Kanhapludult (Old Cotton Rotation, Lee

Co.], or Wickham sandy loam [fine-loamy, mixed, semiactive, thermic typic Hapludult (Plant Breeding Unit, Elmore Co.)]. Soils were analyzed using a routine soil test at Auburn University Soil Testing Lab for pH, phosphorus, potassium, magnesium, and calcium. Results are presented in table 7. Twenty seeds of one species were sown in each container at one of four planting depths: 0 cm (surface), 0.3 cm (0.125 in.), 0.6 cm (0.25 in.), and 1.3 cm (0.5 in.). Seeds were distributed evenly on the soil surface, and then they were covered with soil to achieve the correct depth. Treatments were arranged as a 3 soil type x 4 sowing depth factorial arrangement as listed in the treatments. There were three single-container replications per treatment in the first run, and five single-container replications in subsequent runs. Treatments were arranged in a completely randomized design on raised benches in greenhouses at the Plant Science Research Center at Auburn University in Auburn, Ala. Day time greenhouse temperature average was 29°C (84°F) and relative humidity (RH) was 77%. Night time temperature average was 24°C (76°F) and RH 90%. Day/night temperature average was 26°C (79°F) and RH was 83%. The containers were misted by hand one to two times a day as needed. Germination was recorded every other day for 2 weeks and then weekly until experiment termination. Seeds were considered germinated when one or two cotyledons were visible and were >2 mm in length. At experiment termination shoots (SDW) and roots (RDW) were separated, and soil was rinsed from roots. Shoots and roots were dried separately at 66°C (150°F) for 48 hours, and shoot (SDW) and root (RDW) dry weight per container were determined. Total leaf area (LA) per container was measured using a LI-3100C Area Meter (LI-COR, Lincoln, Nebr.). Data were analyzed using PROC MIXED in SAS version 9.1.3 (SAS Institute, Cary, NC).

Significance of main effects and interactions for percent survival, LA, SDW, and RDW were determined at $\alpha = 0.05$. Contrasts were used to determine trends and paired comparisons of simple effects of interactions. In the first run, seeds of *Coreopsis tinctoria* (golden tickseed) and *Rudbeckia hirta* (black-eyed Susan) (Native American Seed Farm, Junction, Tx.) were sown on 5 August 2008, and seeds of *Gaillardia pulchella* (firewheel) (Everwilde Farms, Bloomer, Wis.), were sown on 12 August 2008. Plants were harvested 14 October 2008.

In the second run, seeds of *C. tinctoria* (Native American Seed Farm, Junction, Tx.), seeds from two sources of *R. hirta* (Native American Seed Farm, Junction, Tx.; Ernst Seeds, Meadville, Pa., but collected from N.C.), seeds of *G. pulchella* (Everwilde Farms, Bloomer, Wis.), seeds of *Coreopsis verticillata* (whorled tickseed) (Ernst Seed, Meadville, Pa.), and seeds from two sources of *Echinacea purpurea* (eastern purple coneflower) (Native American Seed Farm, Junction, Tx.; Everwilde Seed, Bloomer, Wis.) were sown on 1 June 2009, and harvested 31 July 2009.

In the third run, seeds of *C. tinctoria*, *R. hirta* (same two sources as in run 2), *G. pulchella*, and *E. purpurea* (same two sources as in run 2) were sown on 20 August 2009 and harvested 12 October 2009. Marvyn loamy sand was amended with 0.6 g (0.2 oz) per pot [$80 \text{ kg}\cdot\text{ha}^{-1}$ (70 lbs/A)] P_2O_5 (Peafowl Fertilizers, Piedmont Fertilizer, Inc., Opelika, Ala.), which was incorporated in the top inch of soil before sowing. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Results

Coreopsis tinctoria

In Aug. 2008 and 2009 and percent survival increased quadratically and linearly, respectively, with decreasing sowing depth (Table 1, Fig. 1). In Jun. 2009, percent survival increased linearly with decreasing sowing depth when seeds were sown in Marvyn loamy sand or Wickham sandy loam (Table 1, Fig.1). There was no difference in percent survival among sowing depths when seeds were sown in Houston clay. There was higher percent survival when seeds were sown in Marvyn loamy sand. When sown on the soil surface or at 0.3 cm (0.125 in), there was higher percent survival when seeds were sown in Marvyn loamy sand than in other soils.

In Aug. 2008, LA and SDW increased linearly with decreasing sowing depth when seeds were sown in Houston clay or Wickham sandy loam (Table 1, Fig. 1). When seeds were sown on the soil surface and at 0.6 cm (0.25 in), LA was higher when sown in Wickham sandy loam. When seeds were sown on the soil surface or at 0.6 cm (0.125 in), SDW was higher when sown in either Houston clay or Wickham sandy loam.

In Jun. and Aug. 2009, LA increased quadratically with decreasing sowing depth, and SDW increased linearly with decreasing sowing depth when seeds were sown in Marvyn loamy sand (Table 1, Fig. 1). There were no differences in LA and SDW among sowing depths for seeds sown in Houston clay or Wickham loamy sand. When seeds were sown on the soil surface or just below the soil surface, LA and SDW were higher when sown in Marvyn loamy sand

In Jun. 2009, RDW increased linearly with decreasing sowing depth in Marvyn loamy sand (Table 1, Fig. 1). When seeds were sown on the soil surface or just below the soil surface, RDW was higher when sown in Marvyn loamy sand. In Aug. 2009,

RDW was higher when seeds were sown in Marvyn loamy sand than when sown in other soils.

***Rudbeckia hirta* (Northern Texas)**

In Aug. 2008, percent survival increased linearly with decreasing sowing depth (Table 2, Fig. 2). In Jun. 2009, percent survival increased linearly with decreasing sowing depth when seeds were sown in Marvyn loamy sand or Wickham sandy loam. When seeds were sown on the soil surface or just below, percent survival was higher when seeds were sown in Marvyn loamy sand. In Aug. 2009, percent survival increased linearly with decreasing sowing depth and was higher when seeds were sown in Wickham sandy loam.

In Aug. 2008 and 2009, LA and SDW were highest when seeds were sown in Wickham sandy loam (Table 2, Fig. 2). In Jun. 2009, LA and SDW were highest when seeds were sown in Marvyn loamy sand. In Aug. 2008 and 2009, LA increased linearly with decreasing sowing depth. In Aug. 2009, SDW increased linearly with decreasing sowing depth.

In Aug. 2008, RDW was highest when seeds were sown in Marvyn loamy sand, and in Jun. 2009, RDW was highest when seeds were sown in Wickham sandy loam (Table 2, Fig. 2). In Aug. 2009, RDW increased linearly with decreasing sowing depth and was higher when seeds were sown in Wickham sandy loam.

***Rudbeckia hirta* (North Carolina)**

In Jun. 2009, percent survival increased linearly with increasing sowing depth for seeds sown in Marvyn loamy sand and increased quadratically with increasing sowing depth for seeds sown in Wickham sandy loam (Table 2, Fig. 2). When seeds were sown

below the soil surface, percent survival was highest in Marvyn loamy sand and lowest in Houston clay. In Aug. 2009, percent survival was highest when seeds were sown in Wickham sandy loam.

In Jun. 2009, LA, SDW, and RDW were highest when seeds were sown in Marvyn loamy sand, and lowest in Houston clay (Table 3, Fig. 3). LA increased quadratically with increasing sowing depth. In Aug. 2009, LA and SDW increased quadratically with increasing sowing depth when seeds were sown in Marvyn loamy sand. When seeds were sown at 0.3 and 0.6 cm (0.125 in and 0.25 in), LA was highest when sown in Marvyn loamy sand. When seeds were sown at 0.3 and 1.3 cm (0.125 and 0.5 in), SDW was higher when sown in Marvyn loamy sand. In Aug. 2009, RDW increased quadratically with increasing sowing depth with highest RDW occurring when seeds were sown at 0.3 and 0.6 cm (0.125 and 0.25 in) in Wickham sandy loam. When seeds were sown at 0.3 cm (0.125 in), RDW was higher when sown in Marvyn loamy sand

Gaillardia pulchella

In Aug. 2008, percent survival increased quadratically with increasing sowing depth (Table 4, Fig. 4). In Jun. 2009, percent survival was higher when seeds were sown in Marvyn loamy sand, whereas in Aug. 2009, percent survival was higher when seeds were sown in Wickham sandy loam. In both Jun. and Aug. 2009, percent survival increased quadratically with increasing sowing.

In Aug. 2008 and Jun. 2009, LA was higher when seeds were sown in Houston clay and Wickham sandy loam (Table 4, Fig. 4). SDW was higher when seeds were sown Wickham sandy loam, and RDW was highest when sown in Marvyn loamy sand.

In Jun. 2009, LA was highest when seeds were sown in Marvyn loamy sand, SDW was highest when sown in either Marvyn loamy sand or Wickham sandy loam, and RDW was highest when sown in Wickham sandy loam.

In Aug. 2009, LA and SDW increased linearly with increasing sowing depth when seeds were sown in Marvyn loamy sand (Table 4, Fig. 4). LA was highest when seeds were sown in Marvyn loamy sand than in the other soils. RDW was not significant.

Coreopsis verticillata

In all runs, survival and subsequent growth were insufficient ($n < 3$) for statistical analysis to be performed.

***Echinacea purpurea* (North Texas)**

In Jun. 2009, percent survival increased quadratically with increasing sowing depth (Table 5, Fig. 5). In Aug. 2009, percent survival was higher when seeds were sown in either Houston clay or Wickham sandy loam.

In Jun. and Aug. 2009, LA increased quadratically with increasing sowing depth when seeds were sown in Marvyn loamy sand or Wickham sandy loam (Table 5, Fig. 5). In Jun. 2009, when seeds were sown at 0.3, 0.6, and 1.3 cm (0.125, 0.25, and 0.5 in), LA was highest when sown in Marvyn loamy sand than in other soils at those depths. In Aug. 2009, LA was highest in Marvyn loamy sand and Wickham sandy loam when sown at 0.3 cm (0.125 in), and highest when sown at 0.6 cm (0.25 in) in Wickham sandy loam.

In Jun. 2009, SDW and RDW increased linearly with increasing sowing depth, and were highest when seeds were sown at 0.3, 0.6, and 1.3 cm (0.125, 0.25, and 0.5 in) in Marvyn loamy sand. SDW increased quadratically with increasing sowing depth

when seeds were sown in Wickham sandy loam. In Aug. 2009, SDW and RDW were highest when seeds were sown in Wickham sandy loam.

***Echinacea purpurea* (Wisconsin)**

In Jun. 2009, percent survival was highest when seeds were sown in Marvyn loamy sand (Table 6, Fig. 6). Percent survival increased quadratically with increasing sowing depth. In Aug. 2009, percent survival increased quadratically with increasing sowing depth when seeds were sown in Marvyn loamy sand, and increased linearly with increasing sowing depth when sown in Wickham sandy loam. Percent survival was higher when seeds were sown on the soil surface in Houston clay, and at 0.6 and 1.3 cm (0.25 and 0.5 in) in either Houston clay or Wickham sandy loam.

In Jun. 2009, LA and SDW increased quadratically with increasing sowing depth, and were highest when seeds were sown in Marvyn loamy sand (Table 6, Fig. 6). In Aug. 2009, LA and SDW were highest when seeds were sown in Wickham sandy soil.

In Jun. 2009, RDW was highest when seeds were sown in Marvyn loamy sand, and in Aug. 2009, RDW was highest when seeds were sown in Wickham sandy loam (Table 6, Fig. 6). In Jun. 2009, RDW increased quadratically with increasing sowing depth.

Discussion

For most species in all runs, the most germination for all species occurred within two weeks after sowing. For *C. tinctoria* and *R. hirta* (North Texas) in all three runs, seedling survival, LA, SDW, and RDW were generally highest when seeds were surface sown, with values decreasing with increasing sowing depth. In most cases there was little or no germination or subsequent growth at the deepest sowing depth. This was

likely due to these seeds being very small (0.1 to 0.2 mm) and seedlings being unable to penetrate to the soil surface in a natural habitat. For seedlings of *R. hirta* (North Carolina), *G. pulchella*, and both sources of *E. purpurea*, survival, LA, SDW, and RDW were highest when sown below the soil surface. The seeds for *G. pulchella* and *E. purpurea* were larger (0.4 to 0.5 mm) than seeds of *C. tinctoria* and *R. hirta*. Larger seeds need to be buried so that they will have better seed to soil contact (Aldrich, 2002; Kessler and Carden, 2001). In this study, the soil was bare and weeds were removed by hand continually, conditions not commonly found along roadsides. In situations where there is vegetation cover, it may be more difficult for smaller seeds to have good seed-to-soil contact, while larger seeds may be able to sink to the soil level and benefit from the vegetation cover. Also, vegetation competes with wildflower seeds for moisture and nutrients.

In general, seedling survival tended to be highest when the seeds were sown in the Wickham sandy loam followed by Marvyn loamy sand. LA, SDW, and RDW also tended to be highest in Marvyn loamy sand. For both sources of *R. hirta*, there was little to no survival in the Houston clay. Initially there was high germination, but seedlings died soon afterwards. All species, excluding *G. pulchella*, had low germination in Houston clay. The pH of all the soils was similar (Table 7) so the differences are most likely due to the physical properties of the soils such as particle size.

Particle size of sand is 2.0-0.05 mm, silt is 0.05-0.002 mm, and clay is smaller than 0.002 mm (Brady and Weil, 2002). Clay is a soil containing 40% or more clay, less than 45% sand, and less than 40% silt. Sandy loam is a soil containing 20% or less clay, with a percentage of silt plus twice the percentage of clay exceeding 30%, and

52% or more sand. Loamy sand is a soil containing 70 to 85% sand, and the percentage of silt plus twice the percentage of clay does not exceed 30% (Agriculture and Agri-Food Canada, 2009). Houston clay tends to hold water more tightly than to the other soils, due to its higher clay content. The high moisture encourages quick seedling emergence, but it can also cause the seedlings to subsequently rot and die. The Marvyn loamy sand has a coarser texture and dries more quickly than the other two soil types. Seeds are slower to germinate, but the moderate moisture content of the soil prevents seedlings from rotting following germination, and allows for more subsequent growth. The Wickham sandy loam has a fine texture and dries more slowly than Marvyn loamy sand, but more quickly than Houston clay, allowing for good seed-to-soil contact that is essential for high seed germination.

Seeds of *R. hirta* from North Carolina had the highest germination percent survival when sown below the surface, while seeds from north Texas grew best when sown on the soil surface. Although not compared statistically, survival appeared to be higher and subsequent growth more vigorous among seedlings from North Carolina than among those from North Texas. It may be that because North Carolina is in a closer provenance to Alabama than Texas, a similar climate along with similar soils may result in better performance of the North Carolina seeds in Alabama. Seeds of *E. purpurea* from North Texas had higher germination and subsequent growth than seeds from Wisconsin. A similar provenance may again be a factor. In previous studies, it was found that two ecotypes of *Rudbeckia hirta* L. (black-eyed Susan) from northern Florida and Texas, planted in low input landscapes in Florida showed higher survival and growth among seedlings from northern Florida (Hammond et al., 2007; Norcini et al.,

2001; Norcini and Aldrich, 2003). Another study found that seedlings from local, versus non-local, sources showed differences in phenotype with increased survival and earlier flowering in *Coreopsis lanceolata* L. (lanceleaf tickseed) and *Salvia lyrata* L. (lyreleaf sage) (Norcini et al., 2001). However, previous studies have considered the effect of seed source on growth, flowering, and survival of wildflowers and found that there is great variability in performance even when seeds come from a similar climate (Norcini et al., 2001; Norcini and Aldrich, 2003).

Although not compared statistically, of all the species, *G. pulchella* appeared to have the best survival and subsequent growth, suggesting it may be the most vigorous of the species evaluated and would certainly make a good choice for roadside plantings. Although survival tended to range from low to moderate in most species, results indicate that wildflowers can be established in various soil types throughout the state, but care must be taken to ensure proper sowing depth for each species. The somewhat low survival would also need to be taken into account when planning sowing rates, however, seeds are small and inexpensive, and this should not present a barrier to their use. Regardless, all species of wildflowers evaluated in this work can be a valuable, low-maintenance component of a variety of landscapes including roadside, municipal, and residential. Development of local ecotypes and evaluation of performance in different soil types will contribute to their increased use and appreciation.

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Table 1. The effect of sowing depth and/or soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on final seedling % survival, leaf area, shoot dry weight, and root dry weight (RDW) of *Coreopsis tinctoria* in three runs: 5 Aug. - 4 Oct. 2008, 1 Jun. - 10 Aug. 2009, and 20 Aug. - 10 Oct. 2009. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Depth (cm)	% Survival		Soil type	% Survival				Sign.
	5 Aug. 08	20 Aug. 09		Depth (cm)				
0	44	17		0	0.3	0.6	1.3	
0.3	9	10		5 Aug. 08				
0.6	9	3	Houston	2b ^y	3b	0	0	NS
1.3	2	2	Marv	37a	31a	5	1	L ^{***}
Sign. ^z	Q ^{***}	L ^{**}	Wickham	0b	13b	3	4	L [*]

Soil type	Leaf area (cm ²)					Shoot dry weight (g)				
	Depth (cm)					Depth (cm)				
	0	0.3	0.6	1.3	Sign.	0	0.3	0.6	1.3	Sign.
	5 Aug. 08					5 Aug. 08				
Houston	211.7b	47.9	41.9b	0	L ^{**}	0.55a	0.10	0.08a	0	L [*]
Marvyn	31.9c	108.5	0.0b	0.0	NS	0.09b	0.30	0.00b	0.00	NS
Wickham	397a	51.9	215.4a	0.7	L ^{***}	1.19a	0.04	0.66a	0.01	L ^{***}
	1 Jun. 09					1 Jun. 09				
Houston	0.0b	0.4b	0.0b	0	NS	0.00b	0.00b	0.00	0.00	NS
Marvyn	281.2a	326a	71.8a	0.0	Q ^{**}	1.54a	1.22a	0.33	0.00	L ^{***}
Wickham	0.0b	1.0b	0.0b	2.0	NS	0.00b	0.00b	0.00	0.00	NS
	20 Aug. 09					20 Aug. 09				
Houston	0.4b	0.0	0.7	16.0	NS	0.00b	0.00	0.00	0.02	NS
Marvyn	239.8a	96.7	0	0	Q [*]	0.61a	0.23	0.00	0.00	L ^{***}
Wickham	53.3b	90.5	10.1	0	NS	0.00b	0.00	0.00	0.00	NS

	Root dry weight (g)					Soil type	Root dry weight (g)
	1 Jun. 09						20 Aug. 09
Houston	0.000b	0.002b	0.000	0.000	NS	Houston	0.002b
Marvyn	1.864a	1.616a	0.420	0.000	L***	Marvyn	0.056a
Wickham	0.000b	0.006b	0.000	0.002	NS	Wickham	0.002b

^z Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***)).

^yDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

Table 2. The effect of sowing depth and soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam), on final seedling % survival, leaf area, shoot dry weight, and root dry weight of *Rudbeckia hirta* (North Texas) in three runs: 5 Aug. - 4 Oct. 2008, 1 Jun. - 10 Aug. 2009, and 20 Aug. - 10 Oct. 2009. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Depth (cm)	% Survival		Soil type	% Survival				Sign.
	5 Aug. 08			Depth (cm)				
0	43			0	0.3	0.6	1.3	
0.3	16			1 Jun. 09				
0.6	6		Houston	9c ^y	5b	0	7	NS
1.3	4		Marvyn	52a	30a	10	5	L***
Sign. ^z	L***		Wickham	29b	15b	2	2	L***
				20 Aug. 09				
			Houston	6b	0b	3	2	NS
			Marvyn	7b	4b	0	0	NS
			Wickham	32a	17a	5	2	L***

Soil type	Leaf area (cm ²)			Depth (cm)	Leaf area (cm ²)	
	5 Aug. 08	1 Jun. 09	20 Aug. 09		5 Aug. 08	20 Aug. 09
Houston	42.4b	5.7b	1.2b	0	227.8	62.6
Marvyn	43.7b	96a	17.6b	0.3	102.5	32.6
Wickham	237.9a	9.8b	66.1a	0.6	73.9	12.1
				1.3	27.6	5.9
				Sign.	L**	L***

Soil type	Shoot dry weight (g)		
	5 Aug. 08	1 Jun. 09	20 Aug. 09
Houston	0.13b	0.02b	0.00b
Marvyn	0.15b	0.39a	0.05b
Wickham	1.01a	0.05b	0.17a

Depth (cm)	Shoot dry weight (g)
	20 Aug. 09
0	0.16
0.3	0.10
0.6	0.04
1.3	0.00
Sign.	L***

Soil type	Root dry weight (g)	
	5 Aug. 08	1 Jun. 09
Houston	0.016b	0.004b
Marvyn	0.083b	0.201a
Wickham	1.258a	0.029b

Soil type	Root dry weight (g)				
	Depth (cm)				
	0	0.3	0.6	1.3	Sign.
	20 Aug. 09				
Houston	0.002b	0.000	0.004	0.002	NS
Marvyn	0.018b	0.010	0.000	0.000	NS
Wickham	0.120a	0.048	0.028	0.002	L***

^z Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***).

^yDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

Soil type	Root dry weight (g)
	1 Jun. 09
Houston	0.010c
Marvyn	1.045a
Wickham	0.459b

Soil type	Root dry weight (g)				Sign.
	Depth (cm)				
	0	0.3	0.6	1.3	
	20 Aug. 09				
Houston	0.016ns	0.015b	0.004b	0.002	NS
Marv	0.126	0.902a	0.526b	0.178	NS
Wickham	0.192	1.494a	3.166a	0.638	Q***

^zDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

^y Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***)).

Table 4. The effect of sowing depth and/or soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on final seedling % survival, leaf area, shoot dry weight, and root dry weight of *Gaillardia pulchella* in three runs: 5 Aug. - 4 Oct. 2008, 1 Jun. - 10 Aug. 2009, and 20 Aug. - 10 Oct. 2009. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Depth (cm)	% Survival		Soil type	% Survival		Depth(cm)	% Survival	
	5 Aug. 09			1 Jun. 09	20 Aug. 09		1 Jun. 09	20 Aug. 09
0	56		Houston	48b ^y	51b	0	40	43
0.3	82		Marvyn	70a	24c	0.3	67	55
0.6	73		Wickham	47b	81a	0.6	61	56
1.3	63					1.3	51	53
Sign. ^z	Q***					Sign.	Q**	Q*

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Soil type	Leaf area (cm ²)	
	5 Aug. 08	1Jun. 09
Houston	155.5a	207.8b
Marvyn	190.9b	255.1a
Wickham	529.6a	86.0c

Soil type	Leaf area (cm ²)				
	Depth (cm)				
	0	0.3	0.6	1.3	Sign.
	20 Aug. 09				
Houston	145.9b	137.9b	63.4b	118.9b	NS
Marvyn	412.8a	488.1a	830.1a	767.6a	L***
Wickham	158.1b	194.3b	240.4b	209.3b	NS

Soil type	Shoot dry weight (g)	
	5 Aug. 08	1Jun. 09
Houston	0.41b	0.74a
Marvyn	0.62b	1.00a
Wickham	1.67a	0.23b

Soil type	Shoot dry weight (g)				
	Depth (cm)				
	0	0.3	0.6	1.3	Sign.
	20 Aug. 09				
Houston	0.38	0.39	0.15	0.30	NS
Marvyn	1.26	1.80	2.70	2.30	L***
Wickham	0.53	0.56	0.76	0.80	NS

Soil type	Root dry weight (g)	
	1 Jun. 09	20 Aug. 09
Houston	0.637b	0.180c
Marvyn	1.149a	0.414b
Wickham	0.310b	0.698a

^zDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

^y Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***)).

Table 5. The effect of sowing depth and soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on final seedling % survival, leaf area, shoot dry weight, and root dry weight of *Echinacea purpurea* (North Texas) in two runs: 1 Jun. - 10 Aug. 2009, and 20 Aug. - 10 Oct. 2009. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Depth (cm)	Survival %		Soil type	Survival %	
	1 Jun. 09			20 Aug. 09	
0	15		Houston	49a ^y	
0.3	35		Marvyn	12b	
0.6	48		Wickham	54a	
1.3	37				
Sign. ^z	Q**				

Soil type	Leaf area (cm ²)				Sign.	Soil type	Leaf area (cm ²)				Sign.
	Depth (cm)						Depth (cm)				
	0	0.3	0.6	1.3			0	0.3	0.6	1.3	
	1 Jun. 09					20 Aug. 09					
Houston	18	34.7b	40.6b	27.6b	NS	Houston	25.4	22.6b	23.3b	26.1	NS
Marvyn	14.2	156.4a	202.2a	235.1a	Q**	Marvyn	16.0	100.7a	32.6b	9.8	Q**
Wickham	9.8	65.1b	68.6b	35.7b	Q*	Wickham	41.0	94.7a	109.8a	57.0	Q**

Soil type	Shoot dry weight (g)				Sign.	Soil type	Shoot dry weight (g)
	Depth (cm)						20 Aug. 09
	0	0.3	0.6	1.3			
	1 Jun. 09						
Houston	0.08	0.18b	0.22b	0.13b	NS	Houston	0.08c
Marvyn	0.06	0.57a	0.97a	1.30a	L***	Marvyn	0.14b
Wickham	0.06	0.34b	0.35b	0.19b	Q**	Wickham	0.36a

Soil type	Root dry weight (g)				Sign.	Soil type	Root dry weight (g)
	Depth (cm)						20 Aug. 09
	0	0.3	0.6	1.3			
	1 Jun. 09						
Houston	0.038	0.086b	0.146b	0.086b	NS	Houston	0.140b
Marvyn	0.028	0.448a	0.714a	0.838a	L***	Marvyn	0.034b
Wickham	0.032	0.444a	0.305b	0.340b	NS	Wickham	0.316a

^z Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***) .

^yDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

Table 6. The effect of sowing depth and/or soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on final seedling % survival, leaf area, shoot dry weight, and root dry weight of *Echinacea purpurea* (Wisconsin) in two runs: 1 Jun. - 10 Aug. 2009, and 20 Aug. - 10 Oct. 2009. For each run, interactions are presented when significant ($\alpha = 0.05$), otherwise significant main effects ($\alpha = 0.05$) are presented.

Soil type	% Survival		% Survival		Soil type	% Survival				Sign. ^y
	1 Jun. 09	Depth (cm)	1 Jun. 09	Depth (cm)		0	0.3	0.6	1.3	
Houston	5b ^z	0	3		Houston	17a	11	22a	13a	NS
Marvyn	13a	0.3	7		Marvyn	0b	16	3b	0b	Q*
Wickham	7b	0.6	13		Wickham	2b	9	18a	16a	L**
		1.3	10							
		Sign.	Q*							

Soil type	Leaf area (cm ²)				Sign.	Soil type	Leaf area (cm ²)
	Depth (cm)						20 Aug. 09
	0	0.3	0.6	1.3		Houston	3.2b
	1 Jun. 09					Marvyn	6.7b
Houston	2.8	9.3b	3.1b	1.2b	NS	Wickham	23.6a
Marvyn	38.5	139.7a	192.8a	105.4a	Q***		
Wickham	1.2	0.4b	15.9b	9b	NS		

Soil type	Shoot dry weight (g)				Sign.	Soil type	Shoot dry weight (g)
	Depth (cm)						20 Aug. 09
	0	0.3	0.6	1.3		Houston	0.01b
	1 Jun. 09					Marvyn	0.02b
Houston	0.03	0.03b	0.03b	0.00b	NS	Wickham	0.08a
Marvyn	0.18	0.61a	1.02a	0.36a	Q***		
Wickham	0.01	0.00b	0.04b	0.05b	NS		

Soil type	Root dry weight (g)		Depth (cm)	Root dry weight (g)
	1 Jun. 09	20 Aug. 09		1 Jun. 09
Houston	0.014b	0.008b	0	0.028
Marvyn	0.350a	0.007b	0.3	0.145
Wickham	0.045b	0.075a	0.6	0.223
			1.3	0.149
			Sign.	Q*

^zDifferences among least square means (in columns) using paired contrast at $\alpha = 0.05$.

^y Sign.= significance; Not significant (NS) or linear (L) or quadratic (Q) trends at $\alpha = 0.05$ (*), 0.01 (**), or 0.001(***)).

Table 7. Soil analysis of three soils: Houston clay (Macon Co.), Marvyn loamy sand (Lee Co.), and Wickham (Elmore Co.) in Alabama. Soils were collected at three different times: 4 Aug 2008 (run 1), 28 May 2009 (run 2), and 19 Aug 2009 (run 3).

Soil series	Run	pH	P	K	Mg	Ca
		kg·ha ⁻¹				
Houston						
	1	7.6	124	277	248	11199
	2	7.3	232	131	250	1236
	3	7.5	121	213	348	11199
Marvyn						
	1	6.4	83	76	75	632
	2	6.9	226	129	230	1233
	3	7.0	26	103	168	2265
Wickham						
	1	6.3	193	174	249	1159
	2	7.6	84	222	327	11199
	3	7.3	251	195	269	1596

Figure 1. The effect of sowing depth (0 cm, 0.3 cm, 0.6 cm, or 1.3 cm) and soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on cumulative germination percentage within days after sowing (DAS) of *Coreopsis tinctoria* in three runs: 5 Aug.- 4 Oct. 2008, 1 Jun.-10 Aug. 2009, and 20 Aug.-10 Oct. 2009.

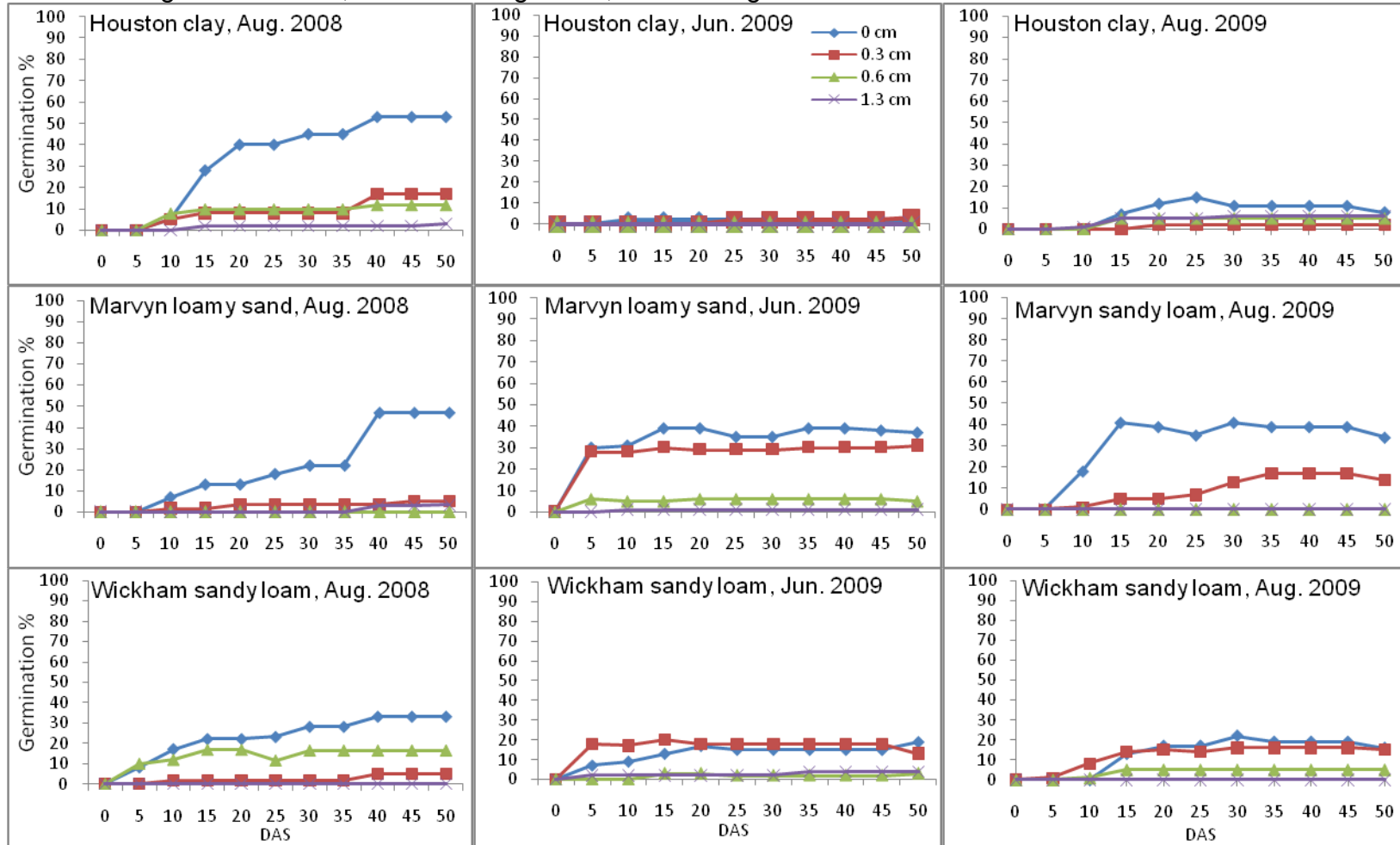


Figure 2. The effect of sowing depth (0 cm, 0.3 cm, 0.6 cm, or 1.3 cm) and soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on cumulative germination percentage within days after sowing (DAS) of *Rudbeckia hirta* (North Texas) in three runs: 5 Aug.- 4 Oct. 2008, 1 Jun.-10 Aug. 2009, and 20 Aug.-10 Oct. 2009.

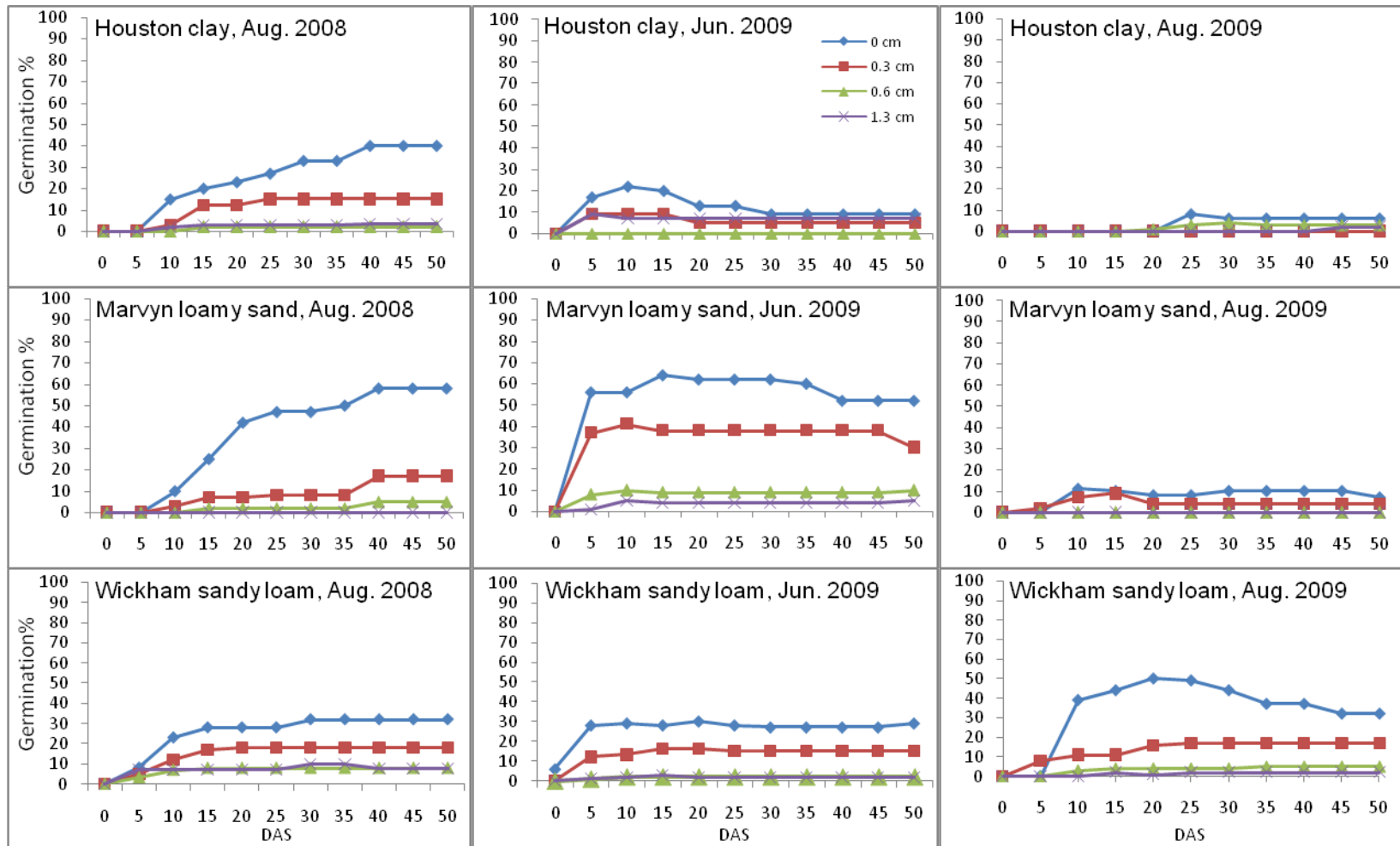


Figure 3. The effect of sowing depth (0 cm, 0.3 cm, 0.6 cm, or 1.3 cm) and soil type, (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on cumulative germination percentage within days after sowing (DAS) of *Rudbeckia hirta* (North Carolina) in 2 runs: 1 Jun.-10 Aug. 2009, and 20 Aug.-10 Oct. 2009.

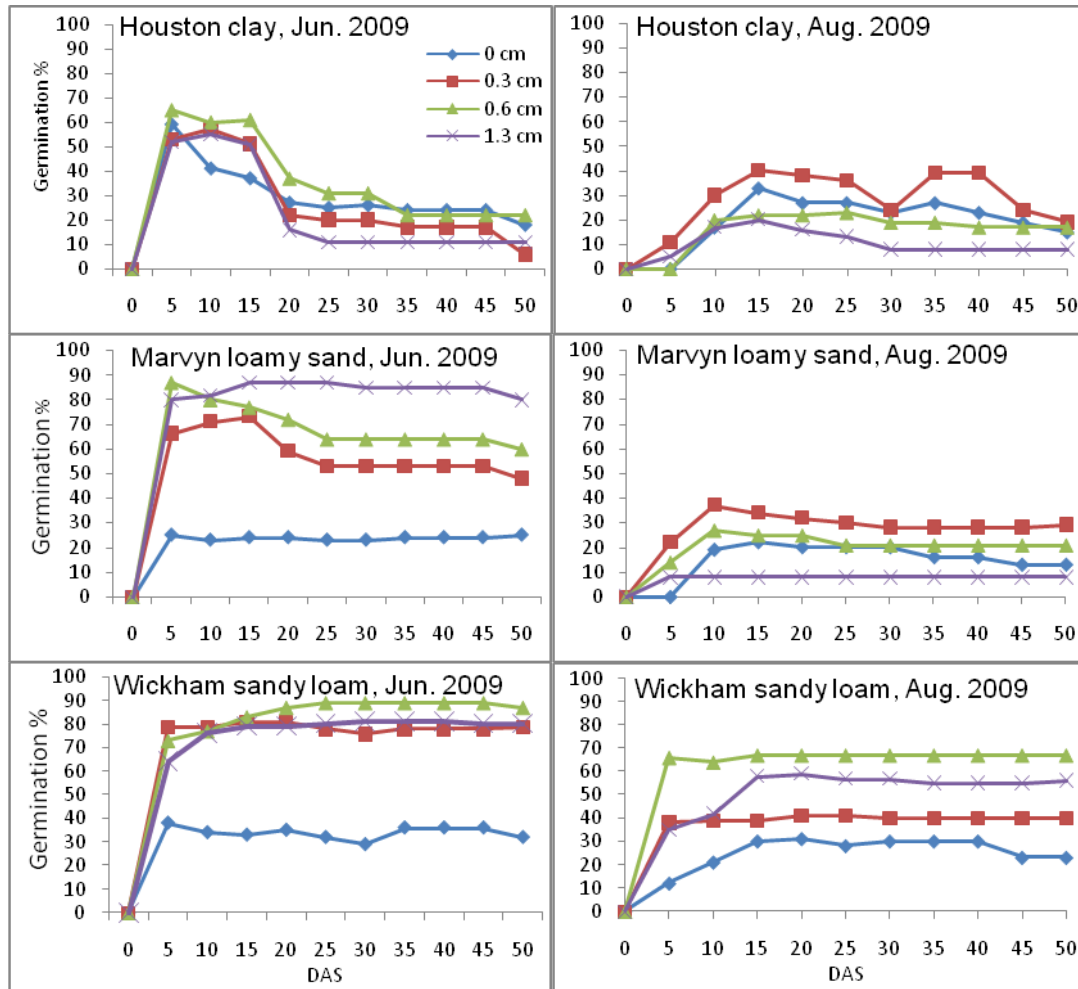


Figure 4. The effect of sowing depth (0 cm, 0.3 cm, 0.6 cm, or 1.3 cm) and soil type (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on cumulative germination percentage within days after sowing (DAS) of *Gaillardia pulcella* in three runs: 5 Aug.- 4 Oct. 2008, 1 Jun.-10 Aug. 2009, and 20 Aug.-10 Oct. 2009.

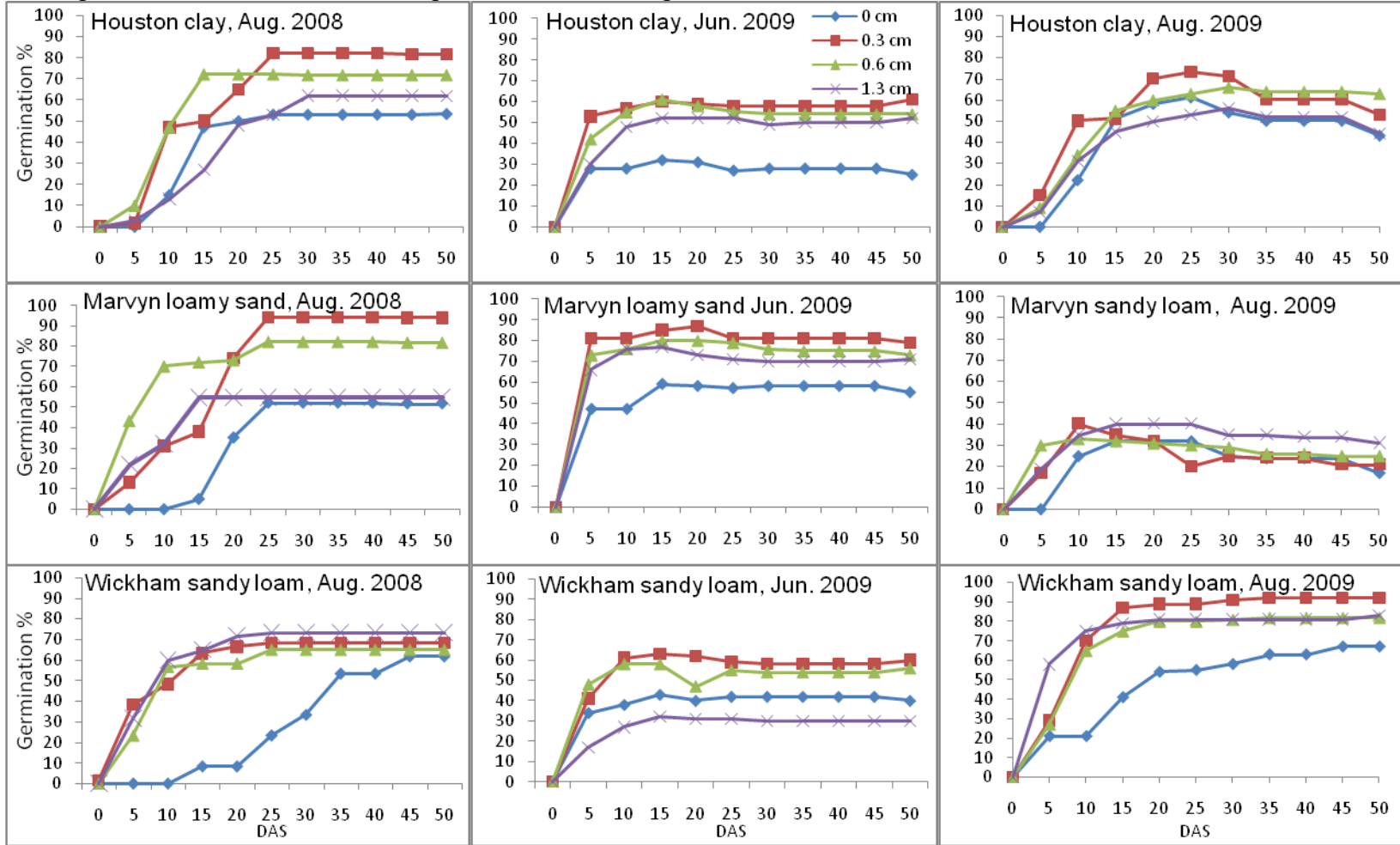


Figure 5. The effect of sowing depth (0 cm, 0.3 cm, 0.6 cm, or 1.3 cm) and soil type, (Houston clay, Marvyn loamy sand, or Wickham sandy loam) on cumulative germination percentage within days after sowing (DAS) of *Echinacea purpurea* (North Texas) in 2 runs:1 Jun.-10 Aug. 2009, and 20 Aug.-10 Oct. 2009.

