

Germination of seeds of *Streptanthus bracteatus* A. Gray, Bracted Twistflower (Brassicaceae), a Rare Central Texas Endemic

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ABSTRACT

Streptanthus bracteatus A. Gray, Bracted twistflower (Brassicaceae), is a rare central Texas endemic that appears to produce dormant seed; consequentially, acquiring seedlings for field and greenhouse studies is difficult. Factors promoting germination, including light levels and time to germination, were examined in the current studies. Germination of *S. bracteatus* seeds was significantly greater in the low light treatment than in the high light treatment (non-parametric Wilcoxon rank-sum test, $P = 0.0173$). Percent germination in the low light was 45% with 30/66 germinating. Germination in high light was 20% with 13/66 germinating. Almost all germinations occurred 5-9 days after seeds were wet. Based on seed mass, all seeds could have germinated: however, 67% of the *S. bracteatus* seeds were dormant. There may be another factor or combination of factors besides light level that could promote additional seed germination for this species. Published on-line www.phytologia.org *Phytologia* 96(3): 181-188 (July 1, 2014). ISSN 030319430.

KEY WORDS: seed dormancy, rare species, endemic, central Texas, Bracted twistflower, high light, low light

Many factors can affect the germination of seeds of flowering plants (Begon et al. 2006; Van Auken 2013). Seeds that fail to germinate when placed in conditions that normally promote growth are broadly termed dormant (Bradbeer 1988). There are various factors or conditions that can lead to dormancy, including immaturity of seeds, unfavorable growth conditions, and physical, morphological, or chemical barriers (Villiers 1972; Baskin and Baskin 2001). However, seed dormancy appears to be an important adaptation to help preserve an adequate number of viable seeds in the soil seed bank (Leck et al. 1989; Harlan 1992; Evans et al. 1996; Veasey et al. 2004; Finkelstein et al. 2008). Dormancy is especially important in buffering natural populations from extreme environmental conditions which can have major negative effects on seedling survival (Harlan 1992; Veasey et al. 2004).

Streptanthus bracteatus A. Gray, Bracted twistflower (Brassicaceae), is a rare herbaceous annual endemic to the Edwards Plateau ecoregion of central Texas. The recent and historic range of *S. bracteatus* is thought to include Bandera, Bexar, Comal, Hays, Medina, Travis, Real and Uvalde counties in south central Texas (Fig. 1) (Leonard and Van Auken 2013). Plants have been found in a wide range of habitats including dense live oak (*Quercus fusiformis*)/Ashe juniper (*Juniperus ashei*) woodlands, savannas, and intercanopy grassland gaps or patches (Van Auken 2000; Van Auken and McKinley 2008; Leonard and Van Auken 2013). Seeds germinate in early - late fall and can continue to germinate through much of the winter (personal observation) when temperatures become moderate and soil is wet by fall showers and soil moisture becomes non-limiting (Baskin et al. 1993). Plants will over-winter as basal rosettes and bolt in March or April to form a flowering spike (Fig. 2). Lavender flowers appear late in spring, usually from April to May (Correll and Johnston 1979). Fruits are siliques (Fig. 3) and can measure up to 15 cm in length. Siliques mature from late May to July and then dehisce, releasing the seeds (Correll and Johnston

1979). Most reports of germination of *S. bracteatus* seed are anecdotal, but germination of seeds from greenhouse-grown plants seem to be high (Zippin 1997). Seed collected from plants in the field appear dormant, thus making seedlings for ecological field and greenhouse studies difficult to obtain.

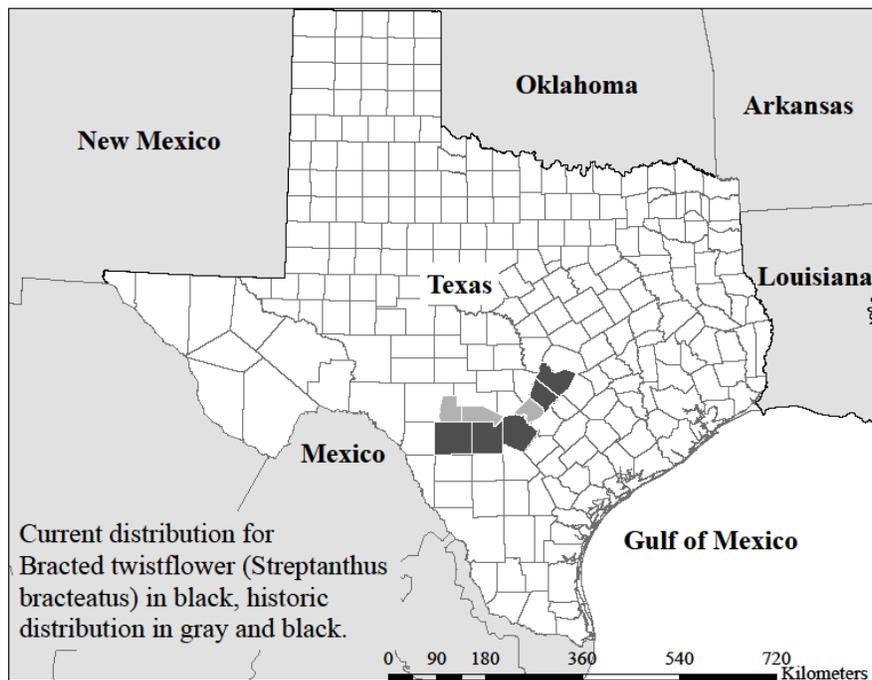


Figure 1. Distribution map for the Bracted twistflower (*Streptanthus bracteatus*). Current distribution in black and previous distribution in gray and black.

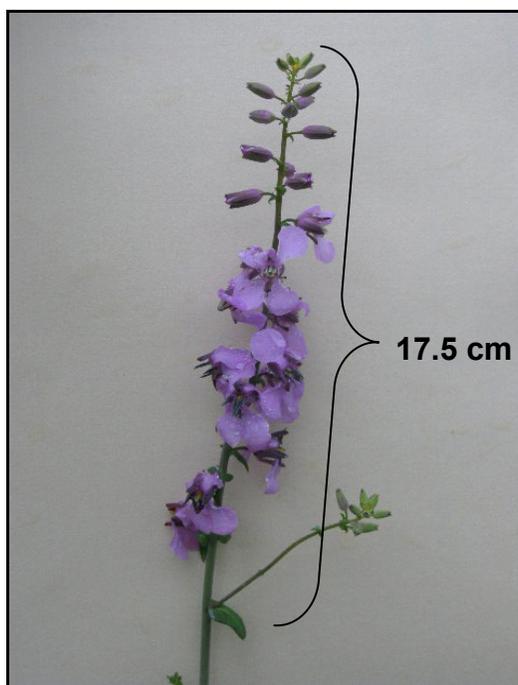


Figure 2. Picture of *Streptanthus bracteatus* inflorescence spike. Plants bloom in April and May in south Texas (Picture taken by senior author, April 2010).



Figure 3. Silique of *S. bracteatus* (9.4 cm long) containing up to 62 flattened seeds (Picture by senior author June 2010).

Simple germination tests can be conducted on seeds of any species and should be started soon after they are collected and then placed in a light-temperature regime that mimics or brackets natural habitat conditions for that species (Baskin and Baskin 2001). However, sometimes this is difficult to do, especially with rare species where plants, fruits and seeds may be uncommon and frequently hard to find. Various factors can be used to break dormancy and promote germination, including modified light levels, chilling, warming, or chemical exposure (Bradbeer 1988; Baskin and Baskin 2001).

Light level is very important in inducing germination in many species (McMillan 1986; McMillan 1987; Orth et al. 2000), as is temperature (Baskin et al. 1993). Photoperiod and specific wavelengths of light can also initiate germination of certain species (Orth et al. 2000). In non-dormant seeds, neither light nor darkness increases germination (Baskin and Baskin 1988; Baskin and Baskin 2001). In dormant seeds, germination is usually higher in high light, rather than in low light or dark treatments (Grime et al. 1981; Baskin and Baskin 1988; Baskin and Baskin 2001).

Germination is promoted for many species with a cold treatment or stratification by placing seeds on a moist substrate for approximately 12 weeks at 5°C (Baskin and Baskin 2001). It is important to note the time of year when seeds normally germinate in the wild. If plants flower and produce seeds in the spring and germinate in the fall, they would probably have higher germination after being exposed to high temperature (Baskin and Baskin 2001). Temperature regimes associated with native populations may be difficult to simulate, thus making dormancy difficult to break (Graae et al. 2008).

Our hypothesis was that *S. bracteatus* would have greater germination if seeds were grown under light levels typical of the environment where they are found. The goal of this study was to determine if germination of *S. bracteatus* seeds was improved by exposing them to increased light treatments.

MATERIALS AND METHODS

Two germination studies were conducted in 2013; results were similar and combined. Seeds were obtained from the Lady Bird Johnson Wildflower Center and were collected in July 2004 from wild populations in Medina County. The seeds were placed in airtight packaging and frozen at -18°C in

September of 2006, acting as a possible cold stratification. In October of 2008, seeds were removed from the freezer and thawed. Seeds remained in the airtight packaging at 4°C. On 24 January and 11 February 2013, six *S. bracteatus* seeds were evenly spaced in twenty-two, 100 mm diameter, polystyrene Petri dishes for a total of 132 seeds. Each Petri dish was filled with Scott's® Miracle-Gro Seed Starting Potting Mix® (approximately 8 g) that was moistened with deionized water at the start of the experiment. Subsequently, the soil and seeds were misted with deionized water as needed to maintain field capacity. Half of the Petri dishes were wrapped in aluminum foil to serve as a low light treatment and half of the dishes were left unwrapped to serve as the high light treatment (66 seeds in low light, 66 seeds in high light). Seeds in 16 Petri plates were started on 24 January and six on 11 February. Petri dishes were placed in 3.79 liter plastic bags to help retain moisture. All Petri dishes were placed 18 cm below a fluorescent grow-light on a metal rack. A timer was used to keep the grow-light on for 13 hours (from 6 am to 7 pm, CST). Temperature was measured using a stainless steel thermometer with a Vernier LabQuest® data logger. Light levels were measured with a FieldScout Quantum Light Meter® (Spectrum Technologies, Inc.). The average temperature was 22.0°C. The average light level was 46 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for the high light treatment and close to zero for the low light treatment.

Seeds in the Petri dishes were checked for germination for 15 days after wetting. Examination of the Petri plates for germination in the high light treatment was completed under the grow-light. Examination in the low light treatment was completed in a dark room with a dim, red head lamp. Any seeds that had cotyledons fully extended beyond the seed coat were removed and placed in 10 cm pots containing the same potting soil as above and transferred to a greenhouse located at Phil Hardberger Park, San Antonio, Texas for other experiments.

A non-parametric Wilcoxon rank-sum test was used to test the differences in the central tendencies of germination (%) for the light and dark treatments. Total number germinated for each survey date was also recorded. Total number of germinations per two days and the sum of the total number of germinations per two days were calculated and plotted.

RESULTS

Germination of the pooled results was significantly greater in the low light treatment than the high light treatment (Wilcoxon ranked sum test, $P = 0.0173$; Fig. 4). Number of germinations in the low light was 30/66 or 45%. For the high light treatment the number of seeds germinating was 13/66 or 20%

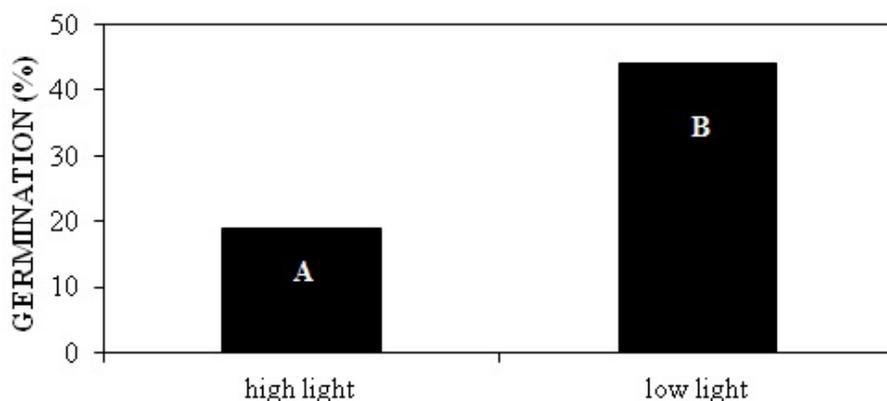


Figure 4. Percent germination for seeds of *Streptanthus bracteatus* in a germination study from January to February 2013 in high light and low light treatments. Different letters (A, B) within each bar indicate significant differences between the two light treatments (Wilcoxon ranked sum test, $P = 0.0173$).

(Fig. 4). Germinations occurred between the fourth and ninth day of treatment, peaking on day 7 (Fig. 5 and 6). After the ninth day, germination ceased for the low light treatment and was very low for the high light treatment. On the seventh day, both the low light and the high light treatment had the largest number of seeds germinating than any other day.

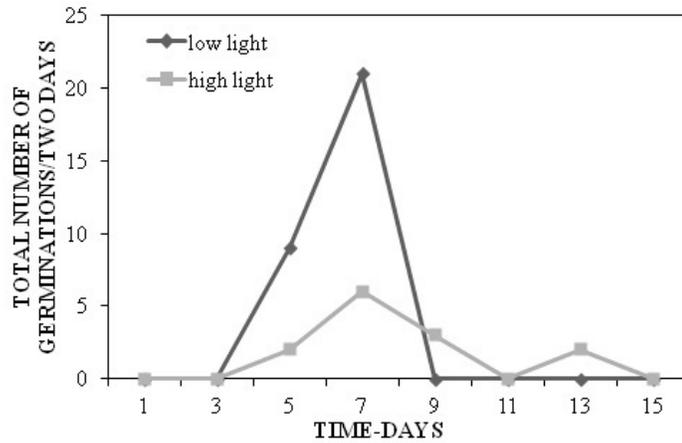


Figure 5. Number of germinations per two days for seeds of *Streptanthus bracteatus* in a germination study from January to February 2013 in low light and high light treatments.

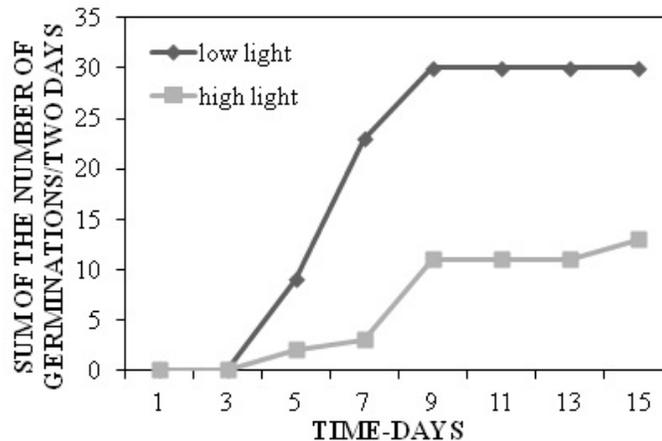


Figure 6. Sum of the total number of germinations per two day for seeds of *Streptanthus bracteatus* in a germination study at the University of San Antonio, San Antonio, Texas from January to February 2013 in low light versus high light treatments.

DISCUSSION

Most flowering plants exhibit some degree of seed dormancy (Begon et al. 2006). Mature seeds that fail to germinate even under favorable conditions require a dormancy-breaking agent to initiate germination (Villiers 1972). If dormancy occurs at the onset of seed release, then an environmental factor such as temperature or oxygen may be required for initiation of germination (Orth et al. 2000). Other dormancy-breaking factors may be necessary including hormones, photoperiod, water, nutrients, or mechanical scarification (Hilhorst and Karssen 1992; Gutterman 1994; Orth et al. 2000).

Streptanthus bracteatus seeds in the current study were frozen for over eight years before germination was attempted, which is not a standard cold treatment. Storage of seeds or achenes frozen in

a gene bank is a common preservation practice (Walters et al. 2005). Yet, the effect of long-term seed storage on most species, particularly rare native species, is generally unknown including effects on seed longevity and seed reserves. There seems to be considerable enthusiasm about collecting and preserving seeds of rare plants, but not for measuring initial viability or ability to germinate. Breaking dormancy or changing environmental controls preventing germination are mostly unknown effects of long-term, low temperature storage (Walters et al. 2005). If storage of the seeds at -18°C did cause dormancy-breaking, storage frozen for eight years probably allowed *S. bracteatus* seed after-ripening to occur. The seeds were probably physiological dormant and that dormancy was broken during low temperature dry storage.

Many species of the mustard family (Brassicaceae) have non-deep physiological dormancy and require after-ripening before germination will occur (Baskin and Baskin 2001). After-ripening can occur in stored seeds with non-deep physiological dormancy, regardless of how dormancy is broken in nature. In the soil or on the soil surface, mature seeds can after-ripen and have their dormancy broken by high summer temperatures or by low winter temperatures. Specifically, after-ripening will proceed faster at room temperatures compared to low temperatures, but low temperatures do not stop after-ripening entirely. If *S. bracteatus* seeds are not allowed to after-ripen (Baskin and Baskin 2001), essentially no germination will take place within 14 days of observation (personal observation).

A considerable amount of anecdotal information regarding high germination of *S. bracteatus* seeds is reported for greenhouse-grown plants (Zippin 1997). In the field, seeds of this species disperse in summer and start germinating in late September and early October, after they have been exposed to high summer temperatures (personal observation). We report 45% germination (Fig. 4) for our pooled experiments; seeds germinated better in low light than in high light. However, caution should be used when interpreting these results as the duration and intensity of light exposure could be important. *Streptanthus bracteatus* seeds were examined with a dim red lamp multiple times throughout the current germination experiment. Seeds germinated in darkness should not be exposed to any light until the end of the germination experiment (Baskin and Baskin 2001). The dim red light could have been enough to promote germination. *Streptanthus bracteatus* may require short durations of light to promote germination, but this is uncertain at this time. The germination for this species in this study was highest at 45% in low light.

Photoperiod and specific wavelengths of light can be important in initiating germination of certain species (Orth et al. 2000). However, in non-dormant seeds, neither light nor darkness increased germination (Baskin and Baskin 1988; Baskin and Baskin 2001). In dormant seeds, germination is usually higher in high light, rather than in low light or dark treatments (Grime et al. 1981; Baskin and Baskin 1988; Baskin and Baskin 2001). Only a few species germinate at higher levels in low light or dark rather than high light treatments (Baskin and Baskin 2001). Also, germination may be dependent upon the duration of light received; for example, long exposure rather than short exposures (Pons 2000). Long high light exposure inhibits mustard seed germination in some species (Pons 2000). Lettuce (Brassicaceae) seeds are negatively affected by long exposure to high light levels but requirements vary with temperature (Baskin and Baskin 2001; Gorski and Gorska 1979). More than 80% of *Lactuca sativa* seeds germinated in light when exposed to temperatures of $10\text{-}30^{\circ}\text{C}$; yet, when placed in darkness, germination was 0% at 30°C and $>45\%$ at temperatures of $10\text{-}22^{\circ}\text{C}$ (Evenari 1952; Baskin and Baskin 2001).

Continuous exposure to high irradiance may be inhibitory during the germination process (Baskin and Baskin 2001). Germination in three species of *Brassica* (mustard) was inhibited at the light levels of $0.5\text{ mol m}^{-2}\text{ day}^{-1}$ or higher (Baskin and Baskin 2001). Short-day seeds like annuals that germinate in the fall or winter can be inhibited by continuous light, whereas long-day seeds are not inhibited by continuous light (Evenari 1965; Baskin and Baskin 2001). *Streptanthus bracteatus* is a winter annual that typically germinates in the fall when day length begins to shorten.

The percent of *S. bracteatus* seeds not germinating in the present study may be ecologically important for the long-term survival of this species in a habitat that fluctuates dramatically in both temperature and rainfall (Van Auken and McKinley 2008).

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