

## Research Article

# Changes in *Senna obtusifolia* Germination Requirements over 12 Months under Field Conditions

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*Senna obtusifolia* seeds were collected in Fall 2003 and immediately field sown to assess dormancy alleviation and effect of after-ripening over a 12-month period on light and temperature requirements for germination. Seeds did not exhibit physical dormancy at maturation and readily germinated over a broad range of light and thermal conditions. Dormancy gradually increased during Winter months, resulting in only a small fraction of the population capable of germination by early Spring. Dormancy break did not occur at a specific time of the year nor did dormancy alleviation increase over the 12-month period following maturation. Conditions during Spring and Summer coincided with thermal requirements for germination of the nondormant fraction of the population. *Senna obtusifolia* seeds were nonresponsive to red and far-red lights, and seeds did not acquire a light requirement following burial for 6 months.

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## 1. Introduction

Environmental conditions during seed maturation and following dispersal interact to influence the germination phenology of many species [1]. Many Summer annual species follow a seasonal germination pattern in which the conditions that promote germination are most prevalent during Spring and early Summer than other times of the year [2]. Furthermore, at least a fraction of the population of some species, including *S. obtusifolia*, requires daily temperature fluctuations of 10 to 15°C for germination to proceed [3].

Physical dormancy in legumes is most likely caused by a combination of radially elongated palisade cells that are tightly packed and are chemically impregnated with waxes to prevent water uptake to the embryo [4–6]. Legume seeds become permeable when the “water gap” is dislodged or the macrosclereid, which comprises the lens (strophiole), pulled apart [5, 7]. Because the lens is the location of imbibition in

legumes, it is thought to function as a “signal detector” for seasonal changes as well as recognition of burial depth due to thermal fluctuations [5]. This break in dormancy in water-impermeable legumes can be caused by highly fluctuating temperatures when preceded by a chilling period [8] or high temperatures [9]. Hence, seed can germinate over a wide range of conditions, but only when physical dormancy has been alleviated.

Light transmitted through and reflected by vegetation has a lower red : far-red (R : FR) ratio than full sunlight because of the selective absorption and reflection of light by chlorophyll [10]. Seeds respond differently to R and FR lights. For example, germination of some species is inhibited by prolonged exposure to direct sunlight (R : FR  $\cong$  1.2) [11, 12], while others are inhibited by exposure to a lower R : FR ratio [13, 14].

Seed germination and emergence depend on endogenous and exogenous factors. Viable seeds are dormant when all environmental conditions are appropriate for germination

but seeds fail to germinate. Thus, dormancy plays an important ecological role in preventing seed germination, being a major contributor to seed persistence of some species in soil [15].

This research was conducted to determine whether break in dormancy of field-sown *S. obtusifolia* seeds occurs at a certain time of the year. Secondly, we wanted to determine the germination response of field-sown *S. obtusifolia* seeds to light and temperature over a 12-month period.

## 2. Materials and Methods

Seeds of *S. obtusifolia* were harvested in late October of 2003. Plants that visually appeared mature were shaken to dislodge seeds from pods into a paper bag. Seeds were airdried for approximately 1 week and placed in fine mesh nylon bags (20 by 20 cm). In November 2003, polyvinyl chloride pipes 50 cm in diameter were cut to a 30-cm length and buried 15 cm in a field at the Clemson University Simpson Research Center in Pendleton, SC, USA. Nylon bags containing *S. obtusifolia* seeds were placed inside the pipes on the soil surface.

To simulate the incorporation of seeds during soil disturbance in Spring, one half of the bags was buried at 10-cm depth in each pipe in May 2004. The remaining seeds were kept in nylon mesh bags in each pipe on the soil surface in Spring and beyond to simulate conditions in the absence of soil disturbance.

Seeds were retrieved from the pipes at approximately 0, 3, 6, 9, and 12 months after maturation. Seeds on the soil surface were evaluated five times over a 12-month period, whereas buried seeds were evaluated only twice (9 and 12 months after maturation). Buried seeds were exhumed at night with aid of a green light, constructed by covering a flash light with one blue filter and one yellow filter. Once recovered, seeds were wrapped in aluminum foil and placed inside a box to avoid light exposure during transport from the field to the laboratory. Exhumed seeds were cleaned under a green light by rinsing soil from the seeds, and only nongerminated, intact seeds were used for the experiments. Fifty seeds were placed between sheets of filter paper in a 9-cm-diameter Petri dish for the light and temperature experiments, with three replicate samples within each experimental treatment.

For the light quality experiment, seeds were moistened for at least 1 hour prior to light treatment. Light treatments evaluated included (1) R light for 15 minutes, (2) FR light for 15 minutes, (3) R light for 15 minutes immediately followed by FR light for 15 minutes, and (4) FR light for 15 minutes immediately followed by R light for 15 minutes. The intensity of the light exposed to the seeds during treatment was 2.1 and 12 Watts/m<sup>2</sup> for the R and FR light sources, respectively. Light treatments occurred inside chambers illuminated with filtered R or FR lights. Petri dishes containing seeds that received R or FR lights were wrapped in aluminum foil immediately after artificial light exposure. Additionally, a dark control was wrapped in foil and was never exposed to R or FR light. All Petri dishes

were placed in a greenhouse maintained at 24 to 30°C for 12 days after which germination was evaluated. Viability of all nongerminated seeds was evaluated using a 1% (wt/v) 2,3,5-triphenyltetrazolium chloride solution. Viability data were used to express germination as a percentage relative to the number of viable seeds.

For the temperature experiment, germination was evaluated at constant and fluctuating temperatures. Seeds in Petri dishes were hydrated with distilled water and placed in incubators in the absence of light at constant temperatures of 10, 15, 20, 25, 30, 35, and 40°C, or at alternating temperatures of 2.5/17.5, 7.5/22.5, 12.5/27.5, 17.5/32.5, 22.5/37.5, and 27.5/42.5°C (12/12 h minimum/maximum temperature in the dark). Germination and viability were assessed after 12 days. Additionally, the percentage of hard seeds was determined from those that did not germinate in the 27.5/42.5°C temperature regime based on the absence of swelling and resistance of seeds to crushing with forceps.

The arrangement of the plots in the field for the light and temperature experiments was a randomized complete block with split-split plot design with four replications of burial depth and retrieval date. For the light experiment, burial was the main plot, retrieval date was the subplot factor, and light treatment was the sub-subplot factor. For the temperature experiment, burial was the split-split plot, retrieval date was the subplot factor, and mean temperature regimes and temperature amplitude were the sub-subplot factors. Germination data were arcsine square root transformed and subjected to ANOVA in SAS. Since buried samples were evaluated only twice, separate analyses were conducted for these samples by excluding the first three retrieval dates. A one-way ANOVA was used to determine whether germination differed across light treatments within each retrieval date for seeds on the soil surface or buried. When ANOVA indicated a significant treatment effect ( $P < .05$ ), transformed means were separated using Fisher's protected Least Significant Difference test at the 5% level of significance.

## 3. Results

**3.1. Germination Response to Light Quality.** The main effect of retrieval date (month) was significant ( $P < .0001$ ) because germination was high (48% to 85%) immediately following maturation and later decreased in January and April (Figure 1). Germination was also significantly influenced by the main effects of light and burial and the month by light interaction. The effect of light quality on germination within each retrieval date and burial depth was nonsignificant for all evaluation dates except April, but even then germination was 10% or less for each light treatment.

Averaged over light treatments, 63% of the *S. obtusifolia* seeds were capable of germination at maturation in November. Germination averaged 8% for seeds from the soil surface compared to 3% for buried seeds (July and October), averaged over light treatments and retrieval dates. Moreover, there was no difference in germination among light treatments of seeds exhumed from the soil surface or from buried ones, indicating that burial does not result

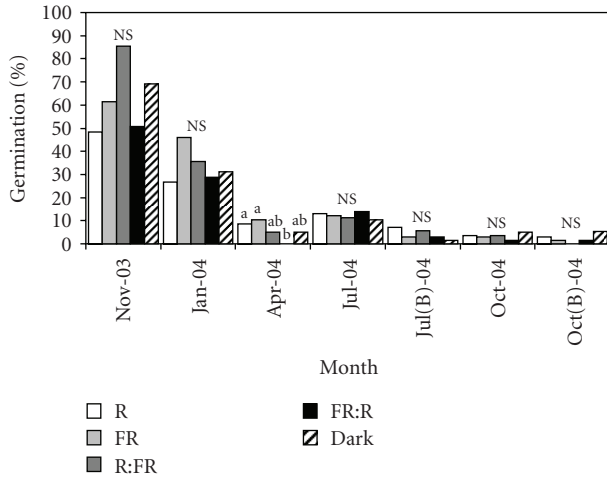


FIGURE 1: *Senna obtusifolia* percentage germination in response to light environment with after-ripening following maturation in late October 2003. One half of the *S. obtusifolia* seeds were buried in soil 10 cm deep in Spring 2004; therefore, Summer and Fall evaluations included seeds from the soil surface and buried ones. Abbreviations: B: buried; FR: far-red light; NS: nonsignificant; R: red light. Means with different lowercase letters within a month and burial depth are significantly different at  $\alpha = 0.05$ .

in a light requirement different from that of seeds lying on the soil surface. These results led us to conclude that R and FR lights neither stimulate nor inhibit *S. obtusifolia* germination. Furthermore, *S. obtusifolia* does not acquire a light requirement following a 6-month burial.

**3.2. Germination Response to Temperature.** The main effects of retrieval date (month), temperature, amplitude, and burial were significant. Furthermore, month by temperature and month by amplitude interacted to affect germination. The main effect of month was significant because germination declined from maturation through April and then remained somewhat constant (Figure 2). Germination across temperatures, amplitudes, and burial depths averaged 37%, 21%, 4%, 7%, and 2% in November 2003 and January, April, July, and October 2004, respectively. The month by amplitude interaction indicates the importance of thermal amplitude in regulating *S. obtusifolia* germination changes over time.

Averaged over temperatures, amplitudes, and retrieval dates (July and October), only 2% of the seeds that were buried germinated compared to 7% from the soil surface. Ninety one percent of the buried seeds retrieved in October were hard, which was 8 percentage points higher than for seeds from the soil surface. This may partially explain the higher germination of seeds from the soil surface. Similarly, seeds of subterranean clover (*Trifolium subterraneum* L.) were found to be softened more readily on the soil surface than when buried [16]. Taylor [16] attributed the greater softening of seeds on the soil surface to insulation of buried seeds from high temperatures and increased microbial decomposition of softened buried seeds.

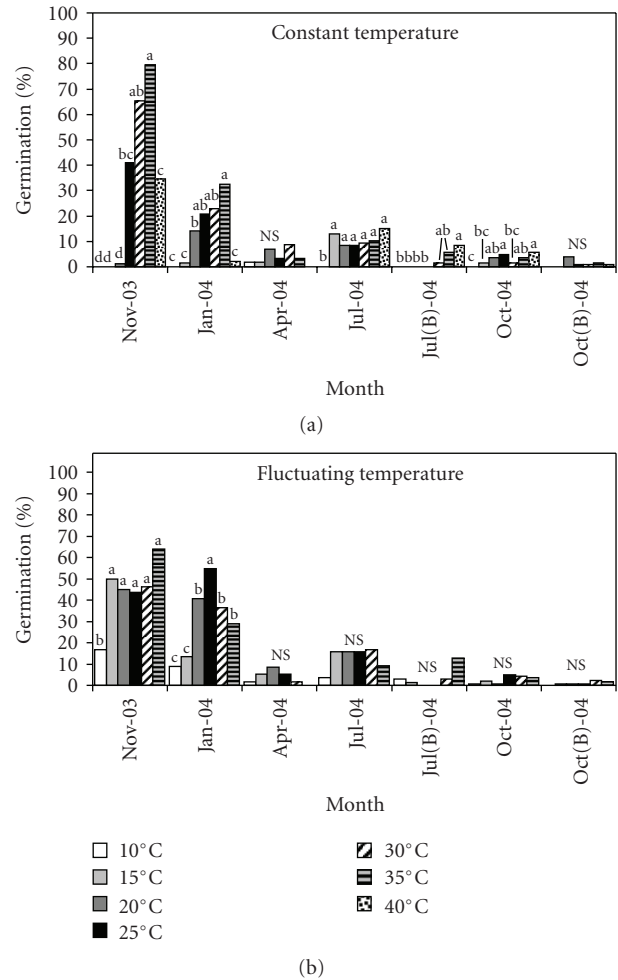


FIGURE 2: *Senna obtusifolia* percentage germination in response to constant and fluctuating ( $\pm 7.5^\circ\text{C}$ ) temperatures with after-ripening following maturation in late October 2003. One half of the *S. obtusifolia* seeds was buried in soil 10 cm deep in Spring 2004; therefore, Summer and Fall evaluations included seeds from the soil surface and buried (B) ones. The fluctuating temperature experiment did not include a mean temperature of  $40^\circ\text{C}$ . Means with different lowercase letters within a month, thermal amplitude (constant or fluctuating), and burial depth are significantly different at  $\alpha = 0.05$ .

*S. obtusifolia* germination immediately following maturation occurred at constant temperatures of 20 to  $40^\circ\text{C}$ . The optimum constant temperature for germination soon after maturation was 30 to  $35^\circ\text{C}$ , which led to 65% to 79% germination. Some *S. obtusifolia* seeds germinated at all fluctuating temperatures (10 to  $35^\circ\text{C}$ ) immediately following maturation. Germination over the range of optimum fluctuating temperatures was 44% to 64% in November 2003.

Germination of seeds from the soil surface differed among constant temperatures for four of five retrieval dates, indicating that constant temperatures are more restrictive to *S. obtusifolia* germination than to fluctuating temperatures (Figure 2). Although changes in the percentage of

*S. obtusifolia* germination were observed over time, germination at constant and fluctuating temperatures occurred over a wide range of conditions, which supports the extended period of emergence under field conditions [17, 18].

#### 4. Discussion

Almost all (98%) *S. obtusifolia* seeds either germinated or imbibed water following the initial incubation period, evidence that physical dormancy was not present in November. The percentage of hard seeds increased over the 12 months from low of 2% in November to high of 91% the following October for buried seeds. These findings are comparable to the 95% hard seeds reported by others [6]. It is apparent that *S. obtusifolia* seeds become progressively dormant during Winter months following maturation, similar to pitted morning glory (*Ipomoea lacunosa* L.), another species that exhibits physical dormancy [19].

Physical dormancy as a result of a water-impermeable seed coat occurs during the dehydration phase of seed maturation [20]. Insufficient drying on the mother plant can lead to failure of some seeds to exhibit physical dormancy [21]. During the final stage of seed formation, water is lost, resulting in physical dormancy [22]. Harvested seeds in our study appeared mature at harvest, and all seeds were resistant to crushing with forceps. However, a portion of *S. obtusifolia* seeds at the time of collection had not dehydrated to the point of physical dormancy based on the ability of the seeds to imbibe water and readily germinate over a wide range of thermal conditions.

Dispersing seeds onto the soil surface in November placed them in contact with a moist substrate (the soil surface) due to the frequent rainFall and minimal evaporative losses during Winter months, hence the reason for the slow but gradual increase in the fraction of hard seeds over time. During Spring and Summer, some of the seeds lying on the soil surface imbibed water and germinated following rainfall events (personal observation). Germination of these seeds contributes to exhaustion of nonhard seeds and increases the percentage of hard seeds remaining. Once the lens of Fabaceae seeds has ruptured, these seeds can no longer exhibit physical dormancy and must soon germinate since imbibition can readily occur [7]. Pathogens would have ready entry into the seeds through the ruptured lens; therefore, those seeds that do not germinate would be susceptible to microbial infection and decay.

We suggest that *S. obtusifolia* germinate directly after dispersal, assuming environmental conditions are similar to those in this experiment and that seeds become progressively dormant during Winter months (Figures 1 and 2). If *S. obtusifolia* seeds were nondormant during Spring, this species would not persist in the soil seed bank as observed in another research [18, 23]. Rather, germination rarely exceeded 15% during Spring and Summer months, even at the most suitable temperatures for germination. These findings explain why *S. obtusifolia* forms a persistent seed bank and substantiates research showing that only a small percentage (2% to 13%) of seeds germinates

and emerges during the first year following maturation [17, 18].

In summary, break in dormancy of field-sown *S. obtusifolia* does not occur at a specific time of the year but rather conditions during Spring and Summer coincide with thermal requirements for germination; hence, germination occurs during this period. *Senna obtusifolia* is dispersed in Fall with a low dormancy level that gradually increases during Winter months as a larger fraction of the population begins to exhibit physical dormancy. Consequently, only a small fraction of seeds is capable of germinating during Spring and Summer when suitable conditions occur, which agrees well with emergence and seed persistence data [6, 17, 18].

#### Acknowledgment

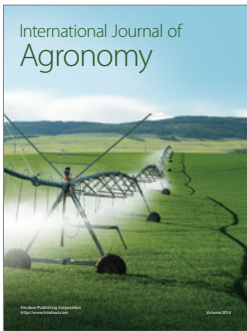
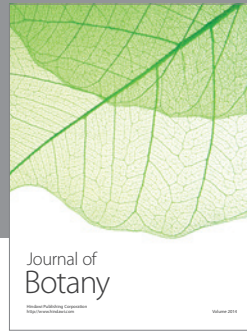
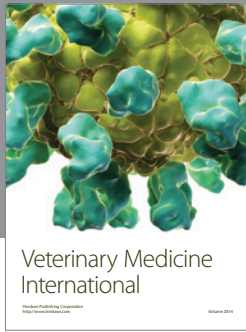
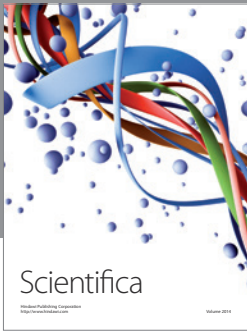
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