Germination Characteristics of Prairie Dropseed, Blanketflower, and Hairy Goldaster in Response to Prechill and Temperature Treatments

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This thesis is approved as a creditable and independent investigation by a candidate for the Masters of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

Germination Characteristics of Prairie Dropseed, Blanketflower, and Hairy Goldaster in Response to Prechill and Temperature Treatment

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Native plant materials centers across the United States create concentrated sources of native species seeds, cuttings, and plants that are readily available for use in restoration, landscaping, and other revegetation projects. In addition, these centers provide materials from local genetic sources that are adapted to the environmental conditions of the target site which makes them less likely to aggressively compete with, or decrease the genetic fitness of, extant native vegetation. Despite the increased desire to use native plant materials for re-vegetation, a general lack of information regarding germination and propagation requirements for many native species has restricted their usage. A better understanding of dormancy and germination patterns for these native species will make them increasingly available and affordable. As part of an effort to develop a native plant materials center for the Black Hills, three native species (*Sporobolus heterolepis*, *Heterotheca villosa*, and *Gaillardia aristata*) were selected to determine optimum germination conditions. Germination trials were conducted following two pre-treatment conditions (2-week prechill at 5°C and no prechill), under six temperature treatments in 2008 and seven temperature treatments in 2009. Tetrazolium

(2, 3, 5-triphenyl-2H-tetrazolium chloride) was used to determine germination potential of ungerminated seeds. Recognizing significant treatments interaction for all three species, *Sporobolus heterolepis* germination was highest following prechill treatment and at higher alternating temperatures (i.e., 15/30°C). Percent germination of *Heterotheca villosa* seeds was greatest under cooler constant temperatures (15°C or 20°C) without prechill treatment while *Gaillardia aristata* seeds had higher germination in the mid ranges of constant and alternating temperatures (20°C, 25°C, 15/25°C and 15/30°C). For all three species, prechill treatments significantly increased germination of at least one extreme of the temperature gradient (15°C or 30°C).The temperature treatments most improved by prechill treatment would not typically occur at the time of germination under natural conditions in the field.

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INTRODUCTION

The Black Hills of South Dakota is an intensively managed area and has been so for more than a century (Ball and Schaefer 2000). Most management activities in the Black Hills National Forest are concerned with timber production (Ball and Schaefer 2000); however, management plans also include consideration for wildlife habitat, rangeland improvement, fire fuels reduction, recreation activities, and control of pests, disease, and invasive species (Shepperd and Battaglia 2002). Such management activities can have a profound impact on vegetation in the Black Hills. In a recent vegetation survey, Marriott et al. (1999) found that 28 out of 68 classified communities in the Black Hills were considered globally rare (G1-G3). The unique vegetation assemblage in conjunction with the frequent occurrence of natural and anthropogenic disturbances in the Black Hills emphasizes the need for a native plant materials center as a concentrated source of locally adapted species that can be used in a wide variety of restoration efforts. However, for most native plant species, a general lack of information regarding germination and propagation requirements has restricted their usage.

Native plant materials centers across the United States create concentrated sources of native species seeds, cuttings, and plants that are available for use in restoration, landscaping, and other revegetation projects. These centers serve to increase the availability and usability of native plants by investigating techniques to increase seed production, improve propagation protocols, and enhance seedling establishment. Furthermore, native plant materials centers provide materials from local genetic sources that are adapted to the environmental conditions of the target site making them less likely

to aggressively compete with, or decrease the genetic fitness of, extant native vegetation (Erickson, 2008). Selecting native species from local populations provides consistency in natural features and processes that ultimately result in healthier, self-sustaining ecosystems (Richards et al. 1998, Chapin et al. 2002, Rogers and Montalvo 2004, Gustafson et al. 2005, Erickson 2008).

As part of an effort to develop a native plant materials center for the Black Hills, three native species (*Sporobolus heterolepis, Heterotheca villosa,* and *Gaillardia aristata*) were selected to determine optimum germination conditions. All three of these species are common in a wide variety of Black Hills plant communities, although not overly abundant. They provide community structure, ground cover, and aesthetic value while reducing the potential of accelerated erosion and providing food and habitat for wildlife. They are also water conservative, drought tolerant, and cold hardy which makes them excellent candidates for restoration and re-vegetation efforts.

Sporobolus heterolepis

Sporobolus heterolepis (A. Gray) A. Gray, commonly called prairie dropseed, is a warm-season, tufted, native, perennial grass that may produce a single, sphere-shaped utricle in each spikelet from August through September. Although *S. heterolepis* is widely distributed throughout the United States and Canada, it is not overly abundant in areas outside of the Great Plains where it is considered a characteristic species of the tallgrass and mixed-grass prairie ecosystems. *S. heterolepis* is steadily declining throughout its range due to encroachment of woody and exotic plants, urban development, and land conversion for agriculture (Engstrom 2004). In the Black Hills, it

is an indicator species of the high elevation, endemic montane grasslands that are currently listed as globally- and state-imperiled (G1S1) by the Natural Heritage Network and The Nature Conservancy (Marriot et al. 1999). *S. heterolepis* is often difficult to establish (Nuzzo 1978, Schramm, 1978; Howell and Kline 1992), and the limited information available on germination and propagation of this species is incomplete and often contradictory.

Heterotheca villosa

Heterotheca villosa (Pursh) Shinners, commonly known as hairy goldaster, is a perennial forb of the Asteraceae that produces showy yellow heads of both disc and ray flowers from late June to September. The seed unit is an achene with a pappus of capillary bristles and short, uneven scales (Barkley 1986, Larson and Johnson 1999). *H. villosa* is distributed throughout the United States from Michigan to the west coast and from North Dakota to Texas (U.S. Department of Agriculture [USDA] 2009). Although it is predominantly found in the western United States, its native range also includes portions of Canada. There are currently no published guidelines on germination and propagation of *H. villosa.*

Gaillardia aristata

Gaillardia aristata Pursh, commonly named blanketflower, is also a member of the Asteraceae. This perennial forb produces showy heads of disc and ray flowers from late July to September. The seed units consist of hairy achenes crowned by four barbed awns (Barkley 1986, Larson and Johnson 1999). The range of this species includes much of the western and northern portions of the United States as well as most of Canada

(USDA 2009). In recent years, related species and horticultural varieties of *Gaillardia* have gained popularity as ornamental species in home and urban gardens; however, little information exists regarding germination and propagation of *G. aristata*.

Temperature requirements for dormancy-breaking and germination initiation can give a clear indication of the intrinsic environmental conditions from which a seed was derived, yet identifying and satisfying those conditions has proven challenging for many seed researchers. For most species, especially native species, prechill/cold-moist stratification and alternating temperature regimes often result in increased seed germination; however, specific germination requirements of these three species are unknown. Guidelines governing the germination/propagation of *Sporobolus heterolepis*, *Heterotheca villosa*, and *Gaillardia aristata* have yet to be established. The objectives of this study are to use hand collected seeds from diverse, wild populations to 1) determine temperature regimes that result in the greatest germination percentages, 2) determine whether temperature requirements for germination change with a prechill treatment, 3) determine whether there is year to year variation in optimal germination requirements, and finally 4) determine whether germination requirements change with age of seed. Such information is important in identifying the optimal pretreatment and environmental conditions needed to overcome dormancy and induce germination, with the ultimate goal of increasing local source seed availability, usability, and affordability for restoration and revegetation projects.

LITERATURE REVIEW

While dormancy-breaking requirements are often variable and difficult to identify, germination requirements for most seeds typically include adequate moisture levels, suitable temperatures, and proper lighting (Steinbauer and Grigsby 1957, Bewely and Black 1982). The influence of a single environmental factor on seed germination and dormancy is difficult to isolate because of interacting environmental variables. For instance, temperature may interact with light, moisture, and nitrate levels to affect seed dormancy and germination (Steinbauer and Grigsby 1957, Bewely and Black 1982). The effects of temperature interacting with other environmental variables may be further complicated by constant or alternating temperatures. For example, alternating temperatures are effective in the presence of light (Bewely and Black 1982). However, light may act as a replacement for alternating temperatures for some species while in other species, light treatment simply reduces the required amplitude of alternating temperatures (Thompson and Grimes 1983). Interactions are not well known or understood and may be the result of physiological, nutritional, metabolic, or mechanical properties (Totterdell and Roberts 1979, Bewely and Black 1982). Nonetheless, temperature is often regarded as the single most important environmental factor because of its role in regulating dormancy (Bouewmeester and Karssen 1992, Vleeshouwers et al. 1995) and determining germination success (Baskin and Baskin 1988, Bouewmeester and Karssen 1992).

Dormancy and germination response to constant and alternating temperatures varies by species. However, Steinbauer and Grigsby (1957) reported that 80% of weed

seeds (no definition of ‘weed’ was provided) included in germination tests resulted in greater germination with alternating temperature regimes. An additional 75% of cultivated species showed a similar pattern (Association of Official Seed Analysts [AOSA] 2007). Such results may be explained by the congruence of alternating temperatures with environmental conditions occurring in natural settings (Baskin and Baskin 1998, Fenner and Thompson 2005). Temperature fluctuations often signify environmental changes that are essential for seed germination, such as soil depth, seasonality, and niche availability (Grimes 1979, Baskin and Baskin 1988, Van Assche and Vanlerberghe 1989). More extreme differences (greater amplitude) in temperature fluctuations naturally signify the arrival of spring and early summer, while temperatures stabilize as summer progresses. Likewise, seeds at varying soil depths gauge burial depth by the amplitude of temperature fluctuations which is less at greater soil depths and more pronounced at the surface. The presence and extent of niche openings in vegetation are also conveyed through temperature fluctuations because vegetation acts as an insulator that stabilizes ground surface temperatures (Pearson et al. 2002). Similar patterns have been recognized among wetland species where temperature alterations designate flooding or drawdown conditions (Grime et al. 1981, Schütz and Rave 1999).

Determining the effects of alternating temperatures, however, may be complicated by at least nine related factors that include amplitude, number of cycles, warming rate, cooling rate, higher temperature value, lower temperature value, length of interval at the higher temperature, length of interval at the lower temperature, and the timing of cycle(s) after imbibition (Totterdell and Roberts 1980). Totterdell and Roberts (1980) found that

germination success for *Rumex* spp. was greatly dependent upon the amplitude of alternating temperatures as well as the minimum and maximum temperature values. In that study, they found that the most productive alternating temperature sequence included a temperature ≥15°C and ≤ 25°C. In addition, greater germination success occurred when the lower temperature value was maintained at the longest interval and, as the higher temperature value increased, the required interval duration decreased. In a similar study, Van Assche and Van Nerum (1997) found that germination can be induced for some species through a single temperature shift of only a few degrees.

Cold-moist stratification/prechill is another temperature treatment that has been proven to expedite the dormancy breaking process (Davis 1930, Toole et al. 1955, Baskin and Baskin 1998) and increase the range of temperatures at which seeds can germinate (Baskin and Baskin 1988). However, temperature alone cannot produce this effect because the seeds must be fully imbibed to attain increased germination percentages. Stratification treatment can be achieved with high temperatures (20-35°C), but Baskin and Baskin (1998) have given evidence that plant species from temperate regions often require a period of cold-moist stratification, or prechill, to break dormancy. Traditionally, optimal cold-moist stratification temperatures were thought to be in the range of 0-10°C (Nikolaeva 1969, Baskin and Baskin 1998); however, Totterdell and Roberts (1979) have demonstrated that temperatures up to 15°C may also bring about a rapid loss of dormancy. Furthermore, they found that as temperature increased through the range of 1.5-15°C, induction of secondary dormancy proceeded more quickly and the 10-15°C

temperature range only produced successful germination when subsequently transferred to higher temperatures.

MATERIALS AND METHODS

Study area

The Black Hills and Bear Lodge Mountains are an unglaciated, mountainous uplift positioned along the west-central border of South Dakota and the northeastern corner of Wyoming. They are approximately 200 km north to south and 100 km east to west, covering an area of about 15,540 km^2 (Froiland 1990). The elevation ranges from 914 m in low-lying areas to 2,208 m at the highest point (Harney Peak). The climate of the Black Hills and surrounding Northern Great Plains is representative of the temperate steppe, characterized by cold winters and hot summers (Bailey et al. 1994); however, the two regions are set apart by a number of factors. The greater elevation of the Black Hills is an obvious characteristic that distinguishes the two regions. The increase in elevation produces lower wind speeds, less variation in temperature (Figures 1 and 2), and greater amounts of precipitation (Figures 3 and 4) in the Black Hills (Froiland 1990, Sheppard and Battaglia 2002). Additionally, the Black Hills receive a larger proportion of annual precipitation during the cooler months compared to the surrounding Great Plains where most of the annual precipitation occurs during the warmer months (Hoffman and Alexander, 1987). Temperature and average annual precipitation also vary slightly between the northern and southern portions of the Black Hills. In the northern Black Hills (Lead, SD), the average annual precipitation from 1971-2000 was 76 cm with a mean temperature minimum/maximum of -10°C/0°C in January and 13°C/25°C in July.

Average annual precipitation in the southern region (Custer, SD) during the same time period was 51 cm with a mean temperature minimum/maximum of -11°C/2°C in January and 11°C/27°C in July (South Dakota Office of Climatology 2010). The length of the growing season varies with elevation and latitude but typically comprises 100-154 days between May and September (Heyward 1928, Sheppard and Battaglia 2002).

The Black Hills consist of five distinct geologic formations that include the Hogback Ridge, the Red Valley, the Minnelusa Foothills, the Limestone Plateau, and the Central Area (Froiland, 1990). These five formations are comprised of a variety of soil parent materials such as: sandstone, shale, limestone, schist, slate, quartzite, granite, siltstone, dolomite and pegmatite. Although classifying specific soil types for each of the five areas is difficult because of the diverse topography and parent materials (Froiland, 1990), the major soil orders found in the Black Hills are Entisols, Mollisols, Alfisols and Inceptisols (Shepperd and Battaglia 2002). Mollisols are the most fertile and they predominantly appear in the Hogback Ridge, Red Valley, Minnelusa Foothills and the Limestone Plateau formations (Shepperd and Battaglia 2002).

Vegetation of the Black Hills is a culmination of species overlap from Cordilleran Forest, Grassland, Eastern Deciduous Forest and Northern Coniferous Forest biomes (Marriott et al. 1999). Vegetation classifications of the Black Hills tend to center around *Pinus ponderosa* because it is a dominant species that is only absent from areas that are characteristically treeless (Hoffman and Alexander 1987). Hoffman and Alexander's (1987) habitat type classification recognized eight habitat types based on *P. ponderosa*, and they included additional forest habitat classifications for areas dominated by *Quercus*

macrocarpa, Populus tremuloides, and *Picea glauca.* Plant community classification by Marriot et al. (1999) followed the U.S. National Vegetation Classification System which resulted in 68 plant associations for the Black Hills area; 15 of the 68 were dominated by *P. ponderosa*.

The three species selected for this study were reported by Marriot et al. (1999) to be found among several of the 68 plant associations. Five of the 28 globally rare plant associations found in the Black Hills contained at least one of the three species. *Sporobolus heterolepis* was recorded among seven total plant associations, three of which were considered rare. Additionally, *S. heterolepis* is an indicator species for the rare Black Hills Montane Grassland association (Marriot et al. 1999). *Heterotheca villosa* was found among 11 total plant associations, three of them rare, and *Gaillardia aristata* was recorded in two rare plant communities.

Seed collection, storage, and treatments

Seeds for this study were hand collected from various locations throughout the Black Hills during the summers of 2007, 2008, and 2009 from mid-August through mid-September when the natural dispersal mechanisms had developed. Inert material was separated from the seeds using a Seedburo® South Dakota seed blower, and further seed cleaning was performed manually. Clean seeds were stored dry at -12°C to maintain viability.

Filled seeds were randomly selected for germination trials that began January 2008 and 2009. Some of the seed lots in this germination study were tested in a different year than the seeds were collected. Where a pair of years is written *a/b*, *a* indicates the

year that the seeds were collected and *b* indicates the years in which germination trials were conducted. For example, seeds collected and immediately tested in 2008 will be referred to as 2008/08, while seeds collected in 2008 and tested in 2009 will be referred to as 2008/09. Similarly, the 2007 and 2009 seed collections tested in 2009 only will be referred to as 2007/09 and 2009/09, respectively. At the time of evaluation, the 2007/09 and 2008/09 seed lots had been in freezer storage for two years and one year, respectively. No *G. aristata* seeds were collected in 2007 and only a limited amount of filled seeds was available in 2008; therefore, germination trials were conducted in 2008 with the 2008 seed collection and in 2009 with only the 2009 seed collection.

Using standardized laboratory germinating techniques, four replications of 50 seeds (*S. heterolepis* and *H. villosa*) and 25 seeds (*G. aristata*) were placed in 12cm x 12cm plastic germination boxes on double layer germination blotter paper for the 2008/08 germination trials. Germination trials were conducted under two pre-treatment conditions (prechill and no prechill) and six different temperature treatments, which consisted of three alternating temperature settings (15/25°C, 15/30°C, and 20/30°C) and three constant temperatures (15°C, 20°C, and 25°C) controlled within ± 1 °C. Prechill treatment was applied for two weeks on 3-layers of moistened blotter paper at 5°C. After two weeks, the prechilled seeds were transferred to one of the six temperature treatments. During one 24-hour period, each of the temperature treatments was provided 8 hours of light and 16 hours of darkness. The alternating temperature cycles incorporated the light period during the highest temperature interval. Germination trials were carried out for a total of 28 days, with germination counts taken at 14 and 28 days.

In 2009, germination tests were repeated as previously described on the 2007, 2008, and 2009 seed collections except that the number of replications was increased from four to eight with 50 seeds planted in each germination box. Additionally, a constant temperature treatment of 30°C was incorporated into the germination trials.

For *S. heterolepis,* seed germination was recorded based on the presence of an elongated coleoptile with enclosed leaves, an attached endosperm, and an elongated primary root. Seeds of *H. villosa* and *G. aristata* were evaluated based on the presence of an elongated primary root and secondary root structures, an elongated hypocotyl, and photosynthetically active, leaf-like cotyledons. All of these seedling characteristics indicate the ability to produce and sustain a functional plant (AOSA, 2007).

Tetrazolium tests for viability and dormancy

Tetralozium (TZ) tests were conducted by soaking seeds for 4-12 hours in a 1.0% solution of 2, 3, 5-triphenyl-2H-tetrazolium chloride (TCC) solution. Seeds were allowed to imbibe distilled-water (dH_2O) prior to testing for initial viability. For viability tests at the end of germination trials imbibition had softened seed coats which facilitated dissection of the embryo and application of TZ to exposed tissues. Hydrogen ions produced by cellular respiration from living tissue, combined with TZ to form an insoluble red formazan dye (Cottrell 1947, Lambou, 1953, Baskin and Baskin 1998). The result was a red stain of living tissue that indicated viability in both dormant and germinating seeds (Baskin and Baskin 1998).

Initial viability TZ tests were conducted on the 2007/09, 2008/09, and 2009/09 seed collections prior to the start of the 2009 germination trials, but not prior to the 2008

germination trials. Subsequent TZ tests were conducted on seeds that failed to germinate for all 2008 and 2009 germination trials. Seeds that remained ungerminated following germination trials but were determined to be viable through TZ tests were considered dormant seeds.

Data analysis

Germination percentages are known to result in a binomial distribution where the deviation from normality is greater among the small and/or large percentages (0-30% and 70-100%) (Zar 2010). To achieve a more normal distribution, all germination percentages were arcsine transformed prior to statistical analysis while actual germination means are reported. Light bank failure in the 20°C and 25°C germination chambers paired with an insufficient amount of replacement seeds resulted in an unbalanced data set for the 2008/08 *Gaillardia aristata*. Because of the unbalanced nature of several of the datasets, the General Linear Model procedure (SAS Institute 2008) was used to analyze the effects of pre-chilling, temperature, and their interactions on arcsine transformed mean percent germination values. When the procedure detected a significant difference ($\alpha \leq 0.05$), mean values were compared using the LSMEANS procedure (SAS Institute 2008) with a Tukey adjustment to control the Type I experimental error rate.

Hierarchical cluster analysis was used to evaluate the relationships among the germination results of all three species tested in 2009 using Sorensen distance measurement and flexible beta linkage ($\beta = -0.25$) (McCune and Grace 2002). Data from 2008 were not included in the cluster analysis because of missing data. The resulting

cluster analysis dendrogram was subjectively pruned into what appeared to be natural groupings.

RESULTS AND DISCUSSION

Sporobolus heterolepis

Irrespective of prechill and temperature treatments, total mean germination percentages of seeds collected in 2008 and 2009 were comparable and ranged from 30-32%, with the exception of the seeds collected and immediately tested in 2009 (2009/09), which averaged 70% germination (Table 1). *S. heterolepis* seeds were soaked for 12 hours in TZ solution to attain an observable red stain that indicated seed viability; however, *S. heterolepis* seeds contain chlorophyllous embryos that occasionally failed to stain red despite obvious viability. Such seeds were considered viable and dormant when the surrounding tissues were turgid. Initial TZ tests of the 2007/09, 2008/09, and 2009/09 seed collections revealed viability levels (i.e., potential germinability) of 74%, 99%, and 93%, respectively. A comparison of the potential germination of the initial TZ tests with the actual percentages of the 2009 germination trials showed that actual percentages were 59% (2007/09), 68% (2008/09), and 25% (2009/09) less than the potential (Table 1). Disparity between potential germination and actual percentages of *S. heterolepis* was also reported by Howell and Kline (1992), and Fedewa and Stewart (2009). Howell and Kline (1992) suggested that the discrepancy between initial viability and final germination in their study may be the product of more thorough seed screening for the viability test than the germination experiment. The seeds in the present study, however, were selected using the same process for both viability and germination tests.

Fedewa and Stewart (2009) found that initial viability of the seeds used in cold-moist stratified treatment experiments (43%) was similar to the final germination results for the 0-day, 30-day and 60-day stratified seeds (35%, 50%, and 45%, respectively). However, field germination in soil treatments (1:1:1 soil, peat, and perlite mix; non-compacted field soil; and compacted field soil) was much less (3%, 11%, and 14%, respectively) than the initial viability (74%) estimates. *S. heterolepis* seeds in the soil treatments were similarly stratified so it is unlikely that the difference observed between the amount of viable seeds and actual germination percentages was due to cold-moist stratification treatment. Fedewa and Stewart (2009) suggested that an unknown property associated with the soil media, a fungal infection of the seed coat, or residual dormancy may have contributed to reduced germination percentages. While soil was not used for this experiment, the seeds in this study had fungal growth that seemed more extensive in warmer temperature treatments, and this may have contributed to reduced germination percentages.

In temperate regions of North America, spring and early summer provide environmental conditions that are most conducive to seed germination of most species; therefore, it is quite reasonable to expect that cold-moist stratification (prechill treatment) would increase seed germination because it is a simulation of winter conditions experienced by these species. Recognizing the interactions of temperature with prechill treatment, prechill treatment generally increased seed germination by 56% (2008/08), 66% (2008/09), and 31% (2009/09), while germination of seeds collected in 2007 was similar (Table 1). In similar studies, Larson (2002) reported no substantial increase (11%

non-prechilled, 12% prechill treated) in seed germination for prechilled seeds, while Fedewa and Stewart (2009) reported relative increases of 43% (35% non-prechilled, 50% prechill treated) for 30-day stratification treatment and 29% (35% non-prechilled, 45% prechill treated) for 60-day stratification treatment. These results suggest that the duration of the prechill treatment may not need to be longer than two weeks given that the range of germination percentages achieved in this study (29-80%) for prechill treated seeds were similar to results of Fedewa and Stewart (2009). Moreover, Baskin and Baskin (1998) point out that there are few studies that investigate the optimal cold stratification interval for temperate grassland species; however, of the available studies, the requisite period appears to be rather brief. Additionally, Totterdell and Roberts (1979) and Bouwmeester and Karssen (1992) found that dormancy can be re-induced through extended stratification periods.

Despite the seemingly beneficial effect that prechill treatment has on germination when taken as a whole, determining the exact conditions that result in optimal germination for *S. heterolepis* was difficult because several instances of treatment interaction were observed among the 2008 and 2009 germination trials. Germination trial results for the 2008/08 collection indicates that the effect of the prechill treatment is highly dependent upon temperature (Figure 5; prechill*temperature interaction, $F = 16.3$; $df = 5, 36$; $P < 0.01$). Mean germination percentages for the 2008/08 *S. heterolepis* seeds ranged from 3-56% for non-prechilled seeds and 17-53% for prechill treated seeds. Significant treatment interactions were observed for the 15°C, 20°C, and 20/30°C treatments where germination percentages were higher with prechill treatment compared

to non-prechill. The largest difference between prechill and non-prechill occurred at the 20/30°C treatment. The 15/30°C treatment produced the greatest germination among the non-prechilled seeds but decreased by 24% with prechill conditions. Regardless of prechill treatment, the 15°C treatment yielded the lowest germination percentages of all the treatment combinations.

Collectively, germination percentages of the 2009/09 seed lot were among the highest compared to all of the other seed collections (Figure 6). Overall mean germination values ranged from 39-80% among the non-prechilled seeds and 48-91% for the prechill treated seeds. For the most part, prechilling significantly increased germination at all temperature regimes, with the exception of the 15/30 °C, 20 °C, and 15 °C temperature regimes where germination between prechill and non-prechill treatments was similar (prechill*temperature interaction, $F = 10.14$; $df = 6, 98$; $P < 0.01$). Milberg and Andersson (1998) suggested that variation in germination within a single seed collection event can be evened out through prechill treatment. This seems to be the case for *S. heterolepis* seeds collected and analyzed in 2009. Among the prechill treated samples, the 15/25°C temperature treated seeds displayed the greatest germination (91%) and all temperature treatments, with the exception of the 15°C treatment, resulted in germination percentages of 81% or greater. Similar to the 2008/08 seed lot, the 15°C treatment produced the lowest germination percentages in both prechill and non-prechill treatments.

Unlike the 2008/08 and 2009/09 seed lots, there was no significant treatment interaction for the 2008/09 seed lot (Figure 7; prechill*temperature interaction, $F = 0.33$;

df = 6, 98; P = 0.92)). Overall germination percentages for the 2008/09 seed lot (freezer-stored for one year) ranged from 11-32% (non-prechill) and 23-48% (prechill). The germination patterns were similar in both of the pre-treated groups but overall germination was improved by 40% through prechill treatment (Figure 7). The greatest mean germination percent of prechill and non-prechilled seeds was observed at the 15/30°C treatment (38%) while the lowest was observed at 15°C (17%).

Overall germination of the 2007/09 seed lot (freezer-stored for two years) was similar to the 2008/09 seed lot, which was relatively low (Figure 8). Mean germination percentages ranged from 27-42% for non-prechill treatment and 25-33% for prechill treatment. Because the 2008/08 collection also had low germination percentages, the low germination percentages were not likely an effect of an additional year of storage. Both Larson (2002) and Springer (2001) reported *S. heterolepis* seeds as being short-lived. Springer (2001) projected that seed viability was one year or less; however, germination percentages of the present study were similar for 2008/08 and 2008/09 seed collections and viability was likely maintained by freezer storage. Treatment interaction was only observed at the 15/25°C temperature treatment where germination without prechill was significantly higher than all other treatment combinations (prechill*temperature interaction, F = 2.19; df = 6,98; P < 0.05). Collectively, prechill treatment resulted in a 9% decrease in germination compared to the non-prechilled samples. Greater germination without prechill treatment may be the result of two years of dry storage. Baskin and Baskin (1998) reported that seeds from temperate regions that often require a cold-moist stratification treatment to break dormancy can produce a similar result through

afterripening, but the requisite period of afterripening is usually much longer than cold-moist stratification.

Figure 9 illustrates the variability in treatment responses among the seed lots as well as a few general patterns. With few exceptions (15/30 NP and 20 NP), germination for the 2009/09 seed lot was 24% greater under prechill treatment regardless of temperature, alternating temperature, or constant temperature. Overall, the highest and lowest constant temperatures (15°C and 30°C) resulted in the lowest percent germination among all the seed lots, but was improved slightly by prechill treatment for the 2008/08 and 2008/09 seed lots, and substantially for the 2009/09 seed lot. Percent germination for the 2007/09 seed lot was consistent among all treatment combinations, with the exception of the 15/25°C non-prechill treatment, which responded to the treatments similar to the 2008/08 seed lot. For the most part, germination of the 2007/09 seed lot under prechill conditions was significantly less than the 2008/08 and 2008/09 seed lots, regardless of temperature. Germination values between the 2008/08 and 2008/09 seed lots were similar only under prechill conditions. The exception was for the 25°C non-prechill treatment (25 NP), which was the only treatment combination where the three seed lots (2007/09, 2008/08, and 2008/09) were similar.

Heterotheca villosa

Woosaree and James (2004) reported germination percentages of about 80% for *Heterotheca villosa* seeds included in seed mixes. For this study, total germination percentages of non-prechill and prechill treated *H. villosa* seeds for all four germination trials (2008/08, 2009/09, 2008/09, and 2007/09) ranged from 53-83% (Table 2). Initial

seed viability tests were conducted prior to 2009 germination trials where *H. villosa* seeds were soaked for 4 hours in TZ solution to produce clear viability and dormancy results. Viability levels from those tests were 79%, 91%, and 65% for the 2007/09, 2008/09, and 2009/09 seed lots, respectively. Comparison of the actual percentages obtained in the germination trials with the potential viability of the seeds showed that actual percentages were 8% (2007/09) and 24% (2008/09) less than, and 11% (2009/09) greater than the potential percentages.

Mean percent germination for the 2008 germination trials ranged from 16-66 percent (data not shown). While prechill treatment resulted in a 14% decrease ($P = 0.055$) in mean germination (Table 2), only the main effect of temperature treatments significantly affected germination outcome (Figure 10). The highest mean germination percent (63%) was observed at a constant temperature of 15°C while the lowest (28%) and only significantly different result ($P < 0.01$) occurred at a constant 25°C.

Analysis of the 2009 germination trials indicated that the prechill and temperature treatments significantly interacted to influence germination for all three seed lots tested (2007/09, 2008/09, and 2009/09) (Figure 11; prechill*temperature interaction, $F = 11.2$; $df = 6, 98$; $P < 0.01$). Germination percentages of the 2009/09 seed collection were generally higher than all of the other trials where mean percent germination ranged from 71- 91% for the prechill treatment and 49-95% among the non-prechilled seeds. Treatment interaction was observed for the 20/30°C and 30°C treatments (Figure 11), where germination was lower for the prechill treatment under the 20/30°C temperature treatment, but significantly increased for the 30°C treatment. Similar to the 2008/08 seed

lot, the only significantly different result ($P < 0.01$) corresponded to the lowest germination percentage, which occurred at the non-prechill, 30°C treatment.

Germination percentages for the 2008/09 seed collection ranged from 22-87% (Figure 12). Treatment interaction was observed at the 30°C and 15/30°C temperature treatments, which showed a significant increase when subjected to prechill treatment (prechill*temperature interaction, $F = 9.99$; $df = 6, 98$; $P < 0.01$). All other temperature treatments only slightly increased or remained the same with prechill treatment. Like the 2008/08 and 2009/09 seed lot, the highest constant temperature (non-prechill, 30°C treatment) resulted in the lowest germination percentage.

With a few exceptions, overall germination of the 2007/09 seed lot was relatively high. Significant treatment interactions were observed for this seed collection (Figure 13; prechill*temperature interaction, $F = 20.6$; $df = 6, 98$; $P<0.01$) where prechill treatment resulted in a 17% overall decrease in germination and also had the effect of increasing variation in the range of germination percentages (42-89%). The non-prechill treatment group had a greater overall range in germination means (20-89%), which amounted to a 78% difference in germination between the pre-treatment groups; however, only the 30°C treatment produced low germination (20%) while all other temperature treatments resulted in germination of 71% or greater. Under prechill conditions, germination of the 15/30°C, 25°C, and 20°C temperature treatments significantly decreased while the 30°C temperature treatment significantly increased by about 60 percent.

Although *Heterotheca villosa* germination trials revealed variability in treatment responses among the seed lots, a few general patterns were observed (Figure 14). Seed

germination was relatively high (≥ 60%) for most of the treatments and seed lots with the exception of the highest constant temperature treatment (25°C for 2008/08 and 30°C for all others) that produced consistently low germination percentages. Prechill treatment was effective at increasing germination of the high temperature treatments but seemed to have a variable effect on other temperature treatments among and between seed collection years. This was especially apparent with the 2007/09 seed lot where prechill treatment reduced germination. The reduction of germination for 2007/09 prechill treated seeds are somewhat inexplicable because pre-treatments were the same for the 2008/09 and 2009/09 seed lots. The germination reduction could be an effect of two years of cold, dry storage though it is unclear why prechilling would have reduced germination for some temperature treatments and not others. Regardless, the 15°C treatment (15P and 15NP) consistently produced higher germination for all *H. villosa* seed lots (2008/08, 2007/09, 2008/09, and 2009/09) suggesting normal seed germination in early spring or late fall when temperatures are cooler.

Gaillardia aristata

Overall germination of *Gaillardia aristata* was 6% for the 2008 germination trials and 61% for the 2009 germination trials (Table 3). Complications with the 20°C and 25°C germination chambers resulted in missing data for those temperature treatments in the 2008 trial and insufficient seed stock prohibited re-trial. A potential effect of the missing data is that pre-treatment is the only factor that had a highly significant effect on 2008 *G. aristata* seed germination, where prechill treatment increased overall germination by 63% (Figure 15).

After 4 hours of exposure to TZ solution, *G. aristata* seeds produced clear viability and dormancy results. Initial tetrazolium (TZ) tests of the 2009/09 seed lot confirmed a 79% level of viability which was 25% greater than the observed germination percentage (61%). Prechill had the effect of increasing overall germination by about 14% (from 57% to 65%); however, interaction between prechill and temperature treatments was observed for the 20/30°C, 15/30°C, 30°C, and 15°C temperature treatments where germination percentages of the 20/30°C significantly decrease while the other three treatments (15/30°C, 30°C, and 15°C) increased with prechill treatment (Figure 16; prechill*temperature interaction, $F = 16.93$; $df = 6, 98$; $P < 0.01$).

For all treatments, the 2008 and 2009 seed lots displayed marked variation in germination responses between seed collection years (Figure 17). Germination for the 2008 seed lot was consistently lower than the 2009 for all treatment combinations. Establishing a germination pattern for *G. aristata* was complicated by missing data; however, for both seed lots the non-prechill, 15°C treatment produced similarly low germination percentages. Based on the 2009 germination trial data, the 30°C treatment appeared to have a comparable affect. Prechill treatment enhanced germination of both the 15°C and 30°C treatments, although germination at least for the 30°C treatment was still lower than for other treatments. Germination in the 15/30°C treatment also significantly increased with prechill treatment but was in the mid range of germination percentages. Furthermore, germination of the non-prechill, 15/30°C treatment (15/30NP) was only slightly greater than both the 30°C treatments (30NP and 30P) and the non-prechill, 15°C treatment (15NP). Germination results from this study indicate that the

temperatures of 15°C and 30°C, singly or in combination, lie on the periphery of the range of suitable germination temperatures for *G. aristata*. Results of the 2009 germination trials also showed that both the 20°C and 25°C produced high percentages of seed germination; however, there is no available data from 2008 to support these conclusions.

SUMMARY AND ECOLOGICAL IMPLICATIONS

Results from this study have established baseline germination requirements for *S. heterolepis, H. villosa* and *G. aristata* in a laboratory setting where environmental factors were strictly controlled. Although all three species are commonly found in the same habitat, they exhibited different germination requirements with varying dormancy levels. While determining optimal germination requirements was complicated by treatment interactions, cluster analysis of the treatments provided a mechanism of evaluating the collective responses of the species to the prechill and temperature treatments (Figure 18A). For example, the non-prechill, 30°C treatment consistently resulted in reduced germination for all three species relative to other temperature treatments but pre-chilling (Figure 18A) proved to enhance germination at 30°C. With only one exception (15°C temperature treatment) the three species appeared to respond similarly to the prechill treatment with no consistent response to alternating or constant temperature. While germination of the subject species was not studied in the field, it is likely that the most productive temperature treatments for *S. heterolepis, H. villosa* and *G. aristata* would correspond to soil temperatures at the time of seedling emergence or, at the very least, to the appropriate season (Washitani and Masuda 1990). Often, lower temperature limitations are a reflection of ecological adaption (ecotype) while upper temperature limits may result from physiological constraints determined by the environment experienced by the parent during seed maturation (Brändel 2004).

Significant variation in germination has been shown to exist between individual plants within a population and between seed collection years that cannot be fully

explained by genetic adaptation to environmental conditions (Andersson and Milberg 1998). This variation may be the result of environmental conditions experienced by the parent plant during seed development. Temperature, nutrient availability, moisture availability, light availability/quality, and even the position held by the seed within the inflorescence or the age of the maternal plant at the time of embryogenesis can impact seed dormancy and germination (Gutterman 1992). Extreme environmental conditions experienced at the time of seed development, such as high temperatures and water-stress (i.e., drought) often result in low seed dormancy and, subsequently, high germination (Allen and Meyer 2002, Figueroa et al. 2010); conversely, exposure to low temperatures typically increases seed dormancy (Allen and Meyer 2002, Lacey 1996). Allen and Meyer (2002) concluded that these extreme environmental conditions result in shorter developmental periods where mechanisms that would normally enhance seed dormancy fail to develop completely. For example, in *Plantago lanceolata* low temperatures during seed development resulted in greater seed coat mass (Lacey et al. 1997) and increased seed dormancy (Lacey 1996). Similar to Allen and Meyer (2002), Lacey et al. (1997) concluded that cooler temperatures result in a prolonged developmental period that allows the seed coat to further develop, which leads to greater seed dormancy. Additional evidence suggests that with earlier exposure of the developing seed to cooler temperatures comes a lower final germination percentage (Figueroa et al. 2010).

As previously mentioned, germination requirements are a reflection of ecological adaption. Cluster analysis of germination trials using germination data recorded from the 2009 germination trials for all three species indicated that patterns of germination across

seed lots were generally most similar within a species (Figure 18B). *S. heterolepis* seeds collected in 2009 and analyzed in 2009 (SPOHET09) appeared to be the exception where patterns of germination were more similar to patterns of germination for the *G. aristata* seed lot tested in 2009 (GAIARI09) and all of the *H. villosa* (HETVIL07, HETVIL08, and HETVIL09) seed lots. A possible explanation is that germinability for all three species collected in 2009 was exceptionally high, while *H. villosa* seeds had high germinability in general.

According to Larson (2002), *S. heterolepis* seems to have more than one optimal germination temperature. Such variation in seed dormancy and germination requirements allows for survival in a variable environment (Andersson and Milberg 1998). As one might expect, fewer instances of germination occurred at the temperature gradient extremes because a constant temperature of 15°C or 30°C is least likely to occur in late June to early July when *S. heterolepis* seed would probably germinate under field conditions. Rather, those temperatures are more likely to occur in the early spring (15°C) or late summer (30°C) when chances of seedling survival would be greatly reduced. Overall, the germination trials varied in response to treatment between collection years, and can probably be explained as a result of environmental conditions experienced by the parent plant during seed development (Fenner 1991, Fenner 1992, Andersson and Milberg 1998).

In a natural setting, Engstrom (2004) claims *S. heterolepis* is easily propagated by seed; however, Nuzzo (1978) found that *S. heterolepis* established poorly when directly seeded but survival improved when seedlings were started in a greenhouse and then

transplanted. Another study found that competition within the first year of planting and competition from introduced species significantly increased germination success of *S. heterolepis*, while competition with native forbs and grasses significantly reduced germination success and seedling survival (Howell and Kline 1992). Similar to Nuzzo (1978), results from this study may indicate difficulty in establishing *S. heterolepis* from seed. A better approach may be to transplant mature individuals into established vegetation (Fedewa and Stewart 2009); once established *S. heterolepis* plants can persist for 15 to 20 years (Springer 2001).

Germination behavior of *H. villosa* in this study is analogous to the disc achenes of two dimorphic (having both ray and disc achenes), annual *Heterotheca* species (*H. subaxillaris*, Baskin and Baskin 1976; and *H. latifolia*, Venable and Levin 1985). Both species germinated early, fast, and with a high overall germination percentage. Germination of the disc flower achenes was shown to decrease slightly yet remained high following dry storage (*H. latifolia* disc flowers decreased from 78% to 70%; Venable and Levin 1985), similar to *H. villosa*. However, unlike *H. villosa*, *H. subaxillaris* and *H. latifolia* act as winter annuals (Baskin and Baskin 1976, Venable and Levin 1985), which tend to germinate best in higher temperatures and are adversely affected by prechill conditions. Conversely, *H. villosa* consistently germinated poorly at the highest constant temperature treatments (25°C or 30°C) and germinated best in cooler constant temperatures (15°C or 20°C). Prechill treatment increased germination under high temperature treatments but had a variable effect on other temperature treatments among and between seed collection years. Venable and Levin (1985) also found that achene

resource allocation of the dimorphic *H. latifolia* varied between the ray and disc achenes even though achene weights were similar. Disc flower achenes allocated the most resources to the embryo, secondly to the pericarp and lastly to the pappus. Ray flower achenes, however, had the greatest allocation to the pericarp and secondly to the embryo, and contributed nothing to the formation of a pappus. These structural differences greatly affect the dormancy and germination strategies for each of the seed types in that the ray achene pericarp acts as somewhat like a barrier to germination yet allows the seed to persist in the seed bank for long periods of time while disc achenes are shorter-lived but more adept at quickly colonizing niche openings.

Like most early-seral or ruderal species (Grime, 1979), *H. villosa* produces large crops of highly viable seeds that germinate rapidly in a wide range of temperatures, even at 5°C without light (i.e., prechill conditions, personal observation). Such species are often found in areas with unpredictable, large-scale disturbance events (e.g., fire and tree felling) and have short-lived seeds that are dispersed by wind in early spring (Grime, 1979). However, *H. villosa* is a perennial species that also possesses short rhizomes which enable lateral vegetative growth (Barkley 1986). All of these characteristics combined are consistent with competitive-ruderal/ruderal-perennial species that often inhabit productive grasslands or meadows (e.g., Black Hills Montane Grasslands) that are subjected to moderate seasonal disturbances (e.g., drought and grazing) (Grime, 1979). Results from this study indicate that *H. villosa* seeds can readily germinate and the most suitable propagation method may be to cast seed in early spring.

Determining the optimal germination conditions for *G. aristata* was complicated by an insufficient seed source and missing data. Germination responses from the only complete *G. aristata* dataset (2009/09 seed lot) showed that the lowest and highest temperatures (15 and 30°C, respectively) and the alternation between those two temperatures were the least suitable for germination. Similar to the findings of Baskin and Baskin (1988), prechill treatment increased germination by expanding the range of temperatures at which the seeds germinated, even though germination percentages remained low. The 25°C temperature treatment gave the best germination results. These results may not be reliable, however, because fluctuating climatic variables associated with the parental environment at the time of seed development have been shown to contribute to interannual variation in levels of seed dormancy and germination (Fenner 1991, Fenner 1992, Gutterman 1992, Andersson and Milberg 1998). An example of prechill influence is seen for the 20/30°C temperature treatment where in 2008 the non-prechilled, 20/30°C treatment (20/30NP) produced the second lowest germination percentage and the prechilled, 20/30°C treatment (20/30P) produced the second highest germination percentage. However, in 2009 the 20/30P treatment was much lower than the 20/30NP treatment. Additional germination tests of *G. aristata* are needed to determine factors affecting the variable results obtained by this study.

Further germination testing of all three species selected for this study (*S. heterolepis*, *H. villosa*, and *G. aristata*) should include a field study to compare the potential germination outcomes (i.e., as revealed by viability and germination tests in the laboratory) with the practical germination outcome (i.e., derived from germination tests

conducted in the field). Subsequent field germination tests using seed mixes and monocultures, as well as, interseeding into extant vegetation would give a more complete understanding of the performance of these three species in a restoration setting.

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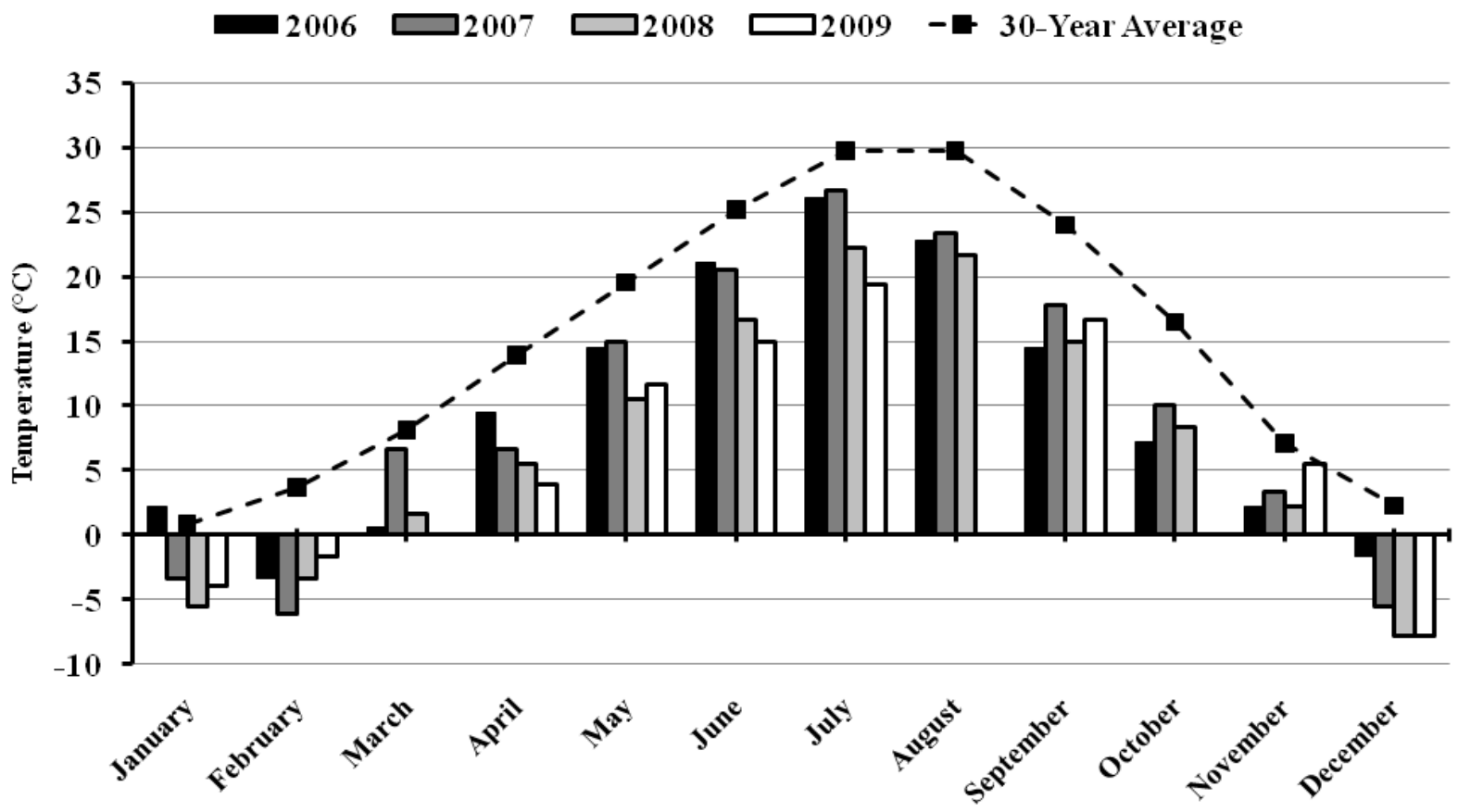


Figure 1. Monthly temperature averages in Rapid City, SD for 2006-2009 and the 30-year temperature average. Data are missing for the August and October 2009. Data were compiled through Western Regional Climate Center (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?sd6947) and the South Dakota Office of Climatology (http://climate.sdstate.edu/climate_site/climate.htm).

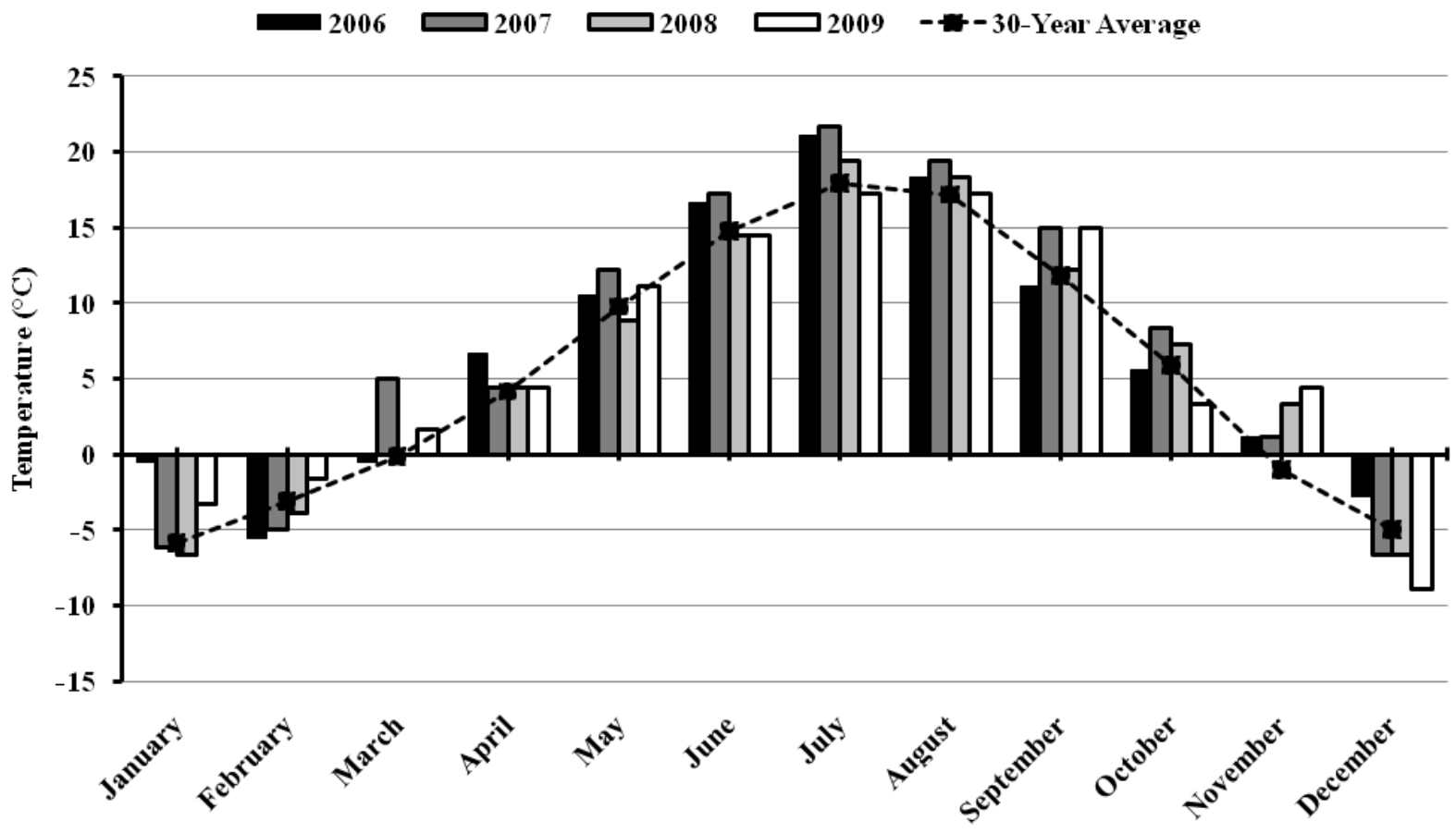


Figure 2. Monthly temperature averages in Hill City, SD for 2006-2009 and the 30-year temperature average. Data were compiled through Western Regional Climate Center (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?sd6947) and the South Dakota Office of Climatology (http://climate.sdstate.edu/climate_site/climate.htm).

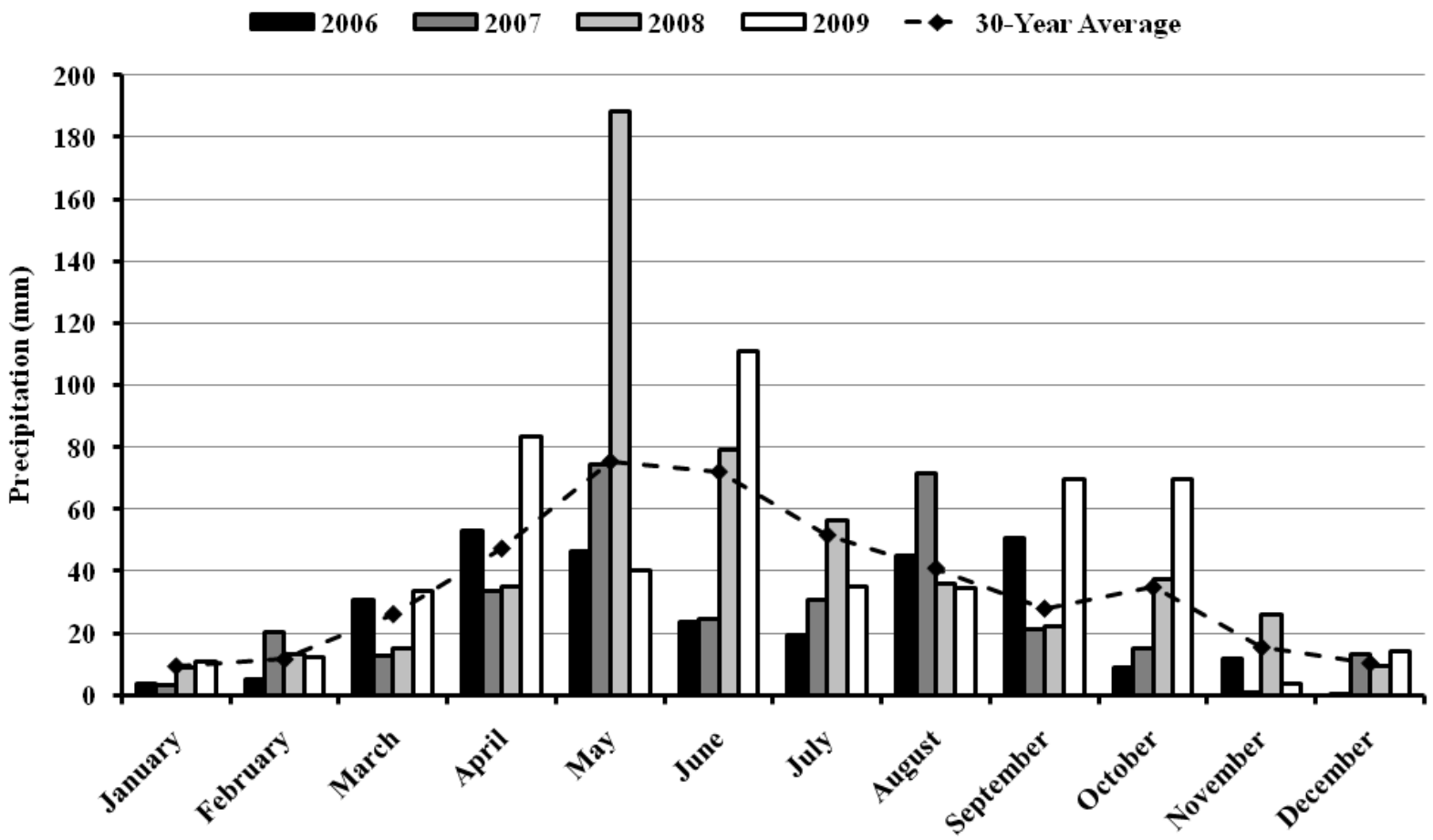


Figure 3. Monthly precipitation averages in Rapid City, SD for 2006-2009 and the 30-year temperature average. Data were compiled through Western Regional Climate Center (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?sd6947) and the South Dakota Office of Climatology (http://climate.sdstate.edu/climate_site/climate.htm).

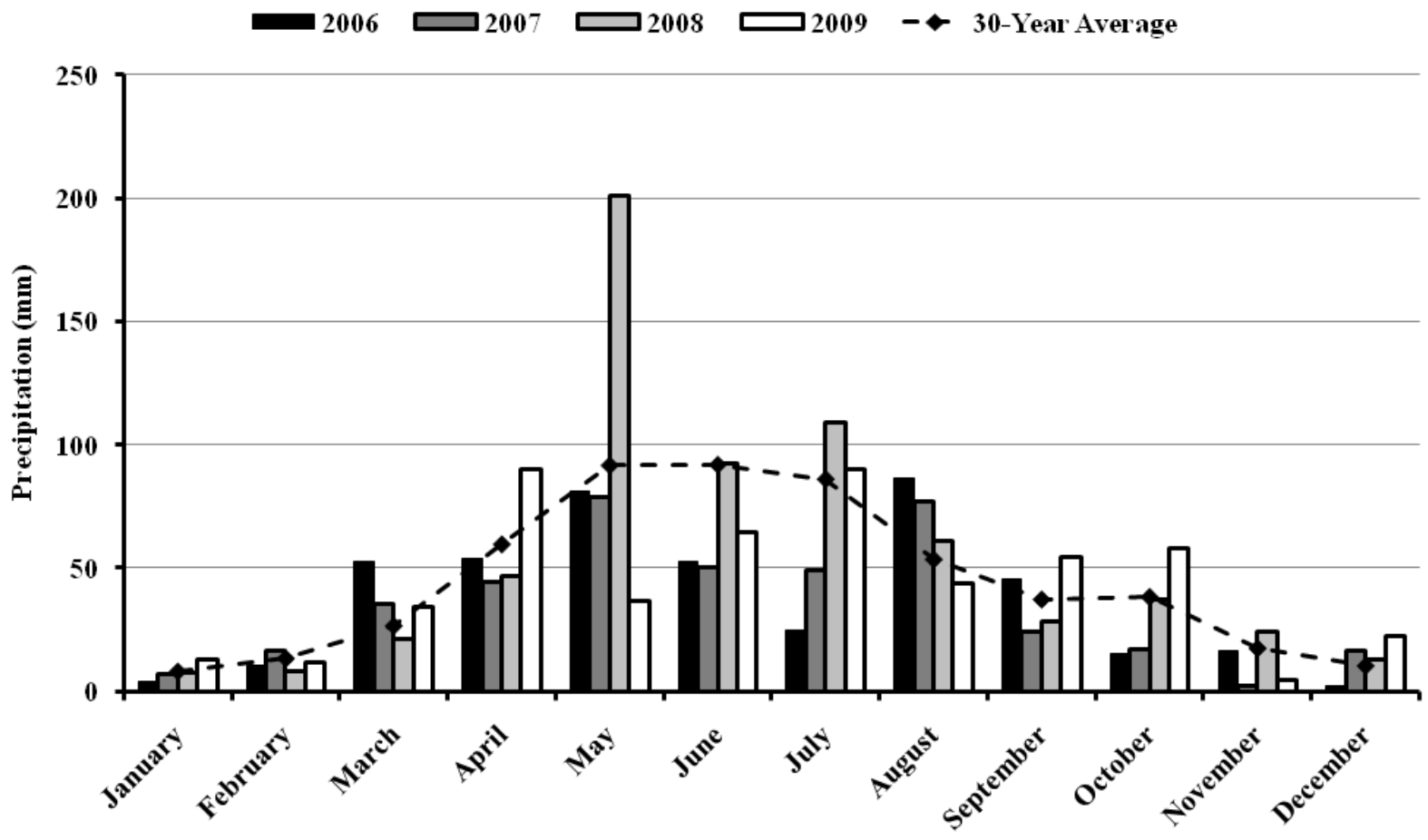


Figure 4. Monthly precipitation averages in Hill City, SD for 2006-2009 and the 30-year temperature average. Data were compiled through Western Regional Climate Center (http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?sd6947) and the South Dakota Office of Climatology (http://climate.sdstate.edu/climate_site/climate.htm).

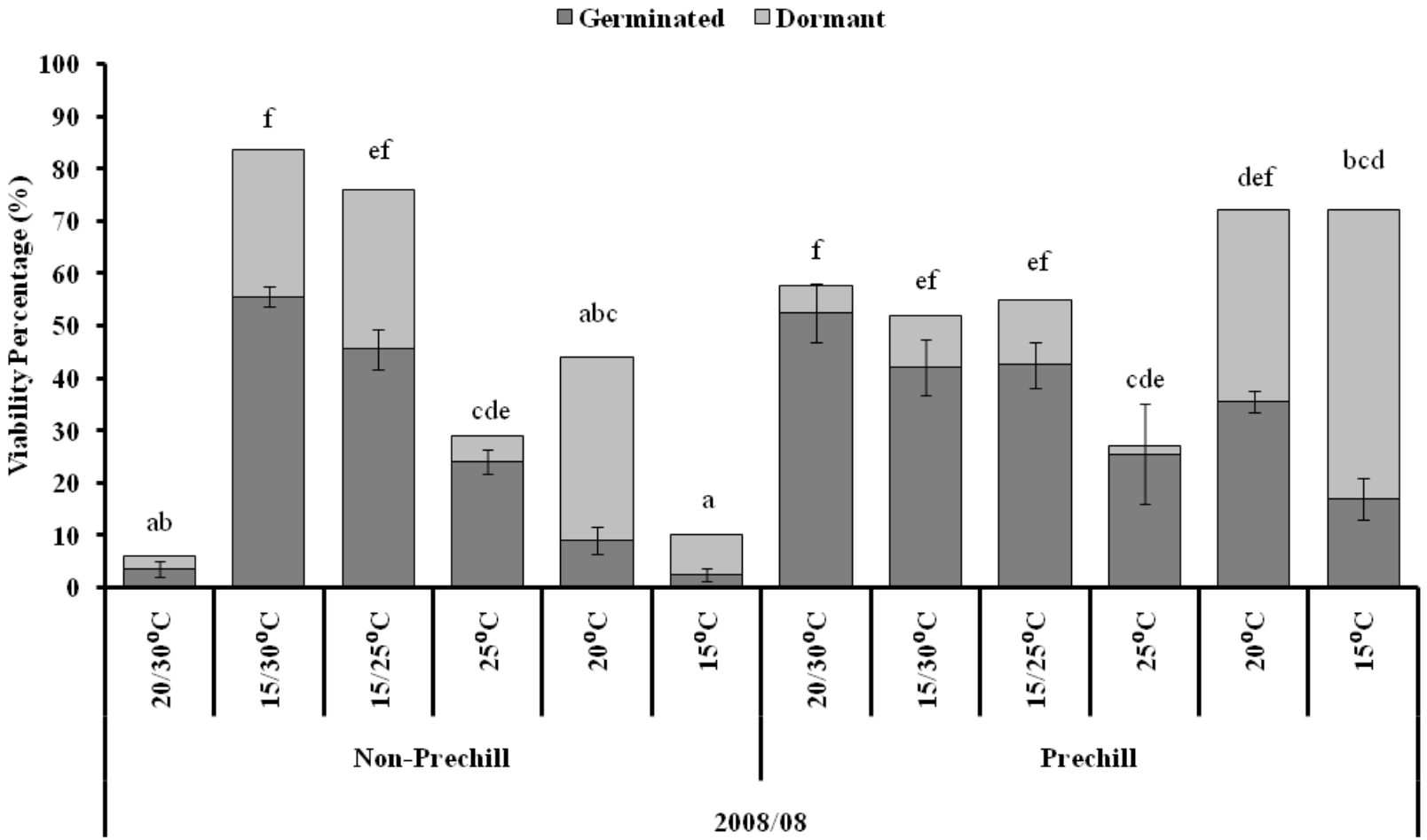


Figure 5. Total seed viability percentages (mean ± SE, n = 4), including mean germination and mean dormancy, for *Sporobolus heterolepis* seeds collected and immediately tested in 2008 (2008/08). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

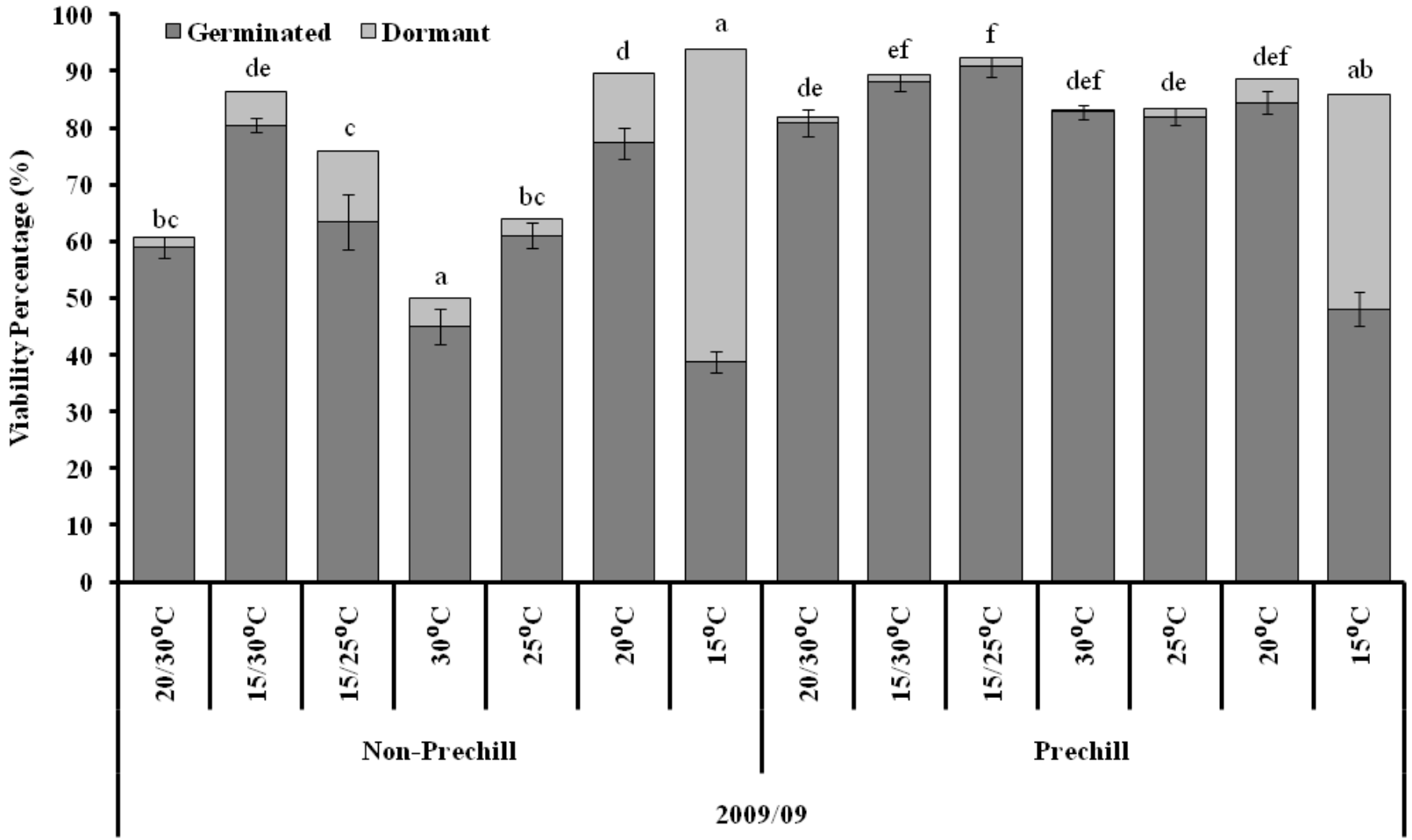


Figure 6. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Sporobolus heterolepis* seeds collected and immediately tested in 2009 (2009/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

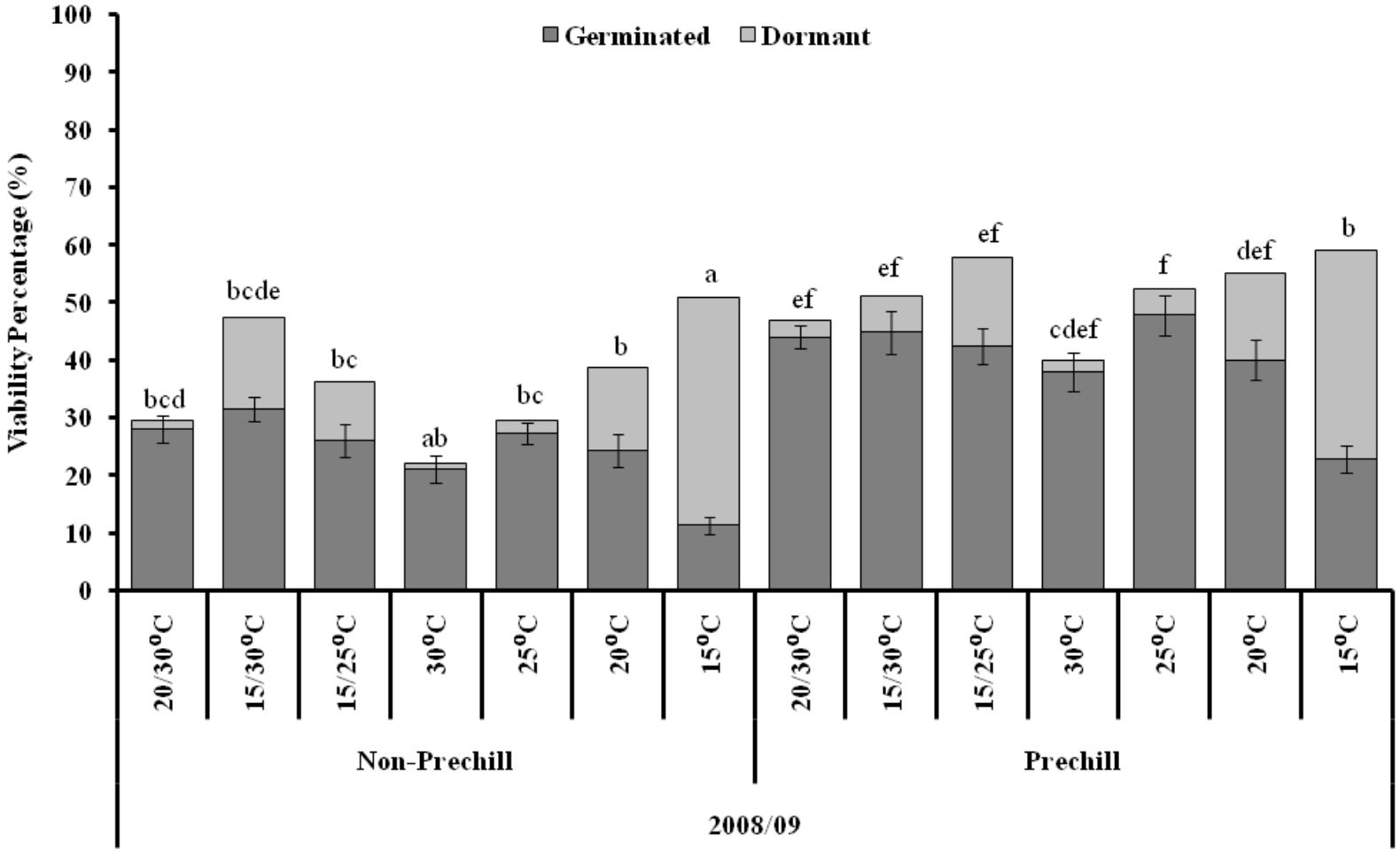


Figure 7. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Sporobolus heterolepis* seeds collected in 2008 and tested in 2009 (2008/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

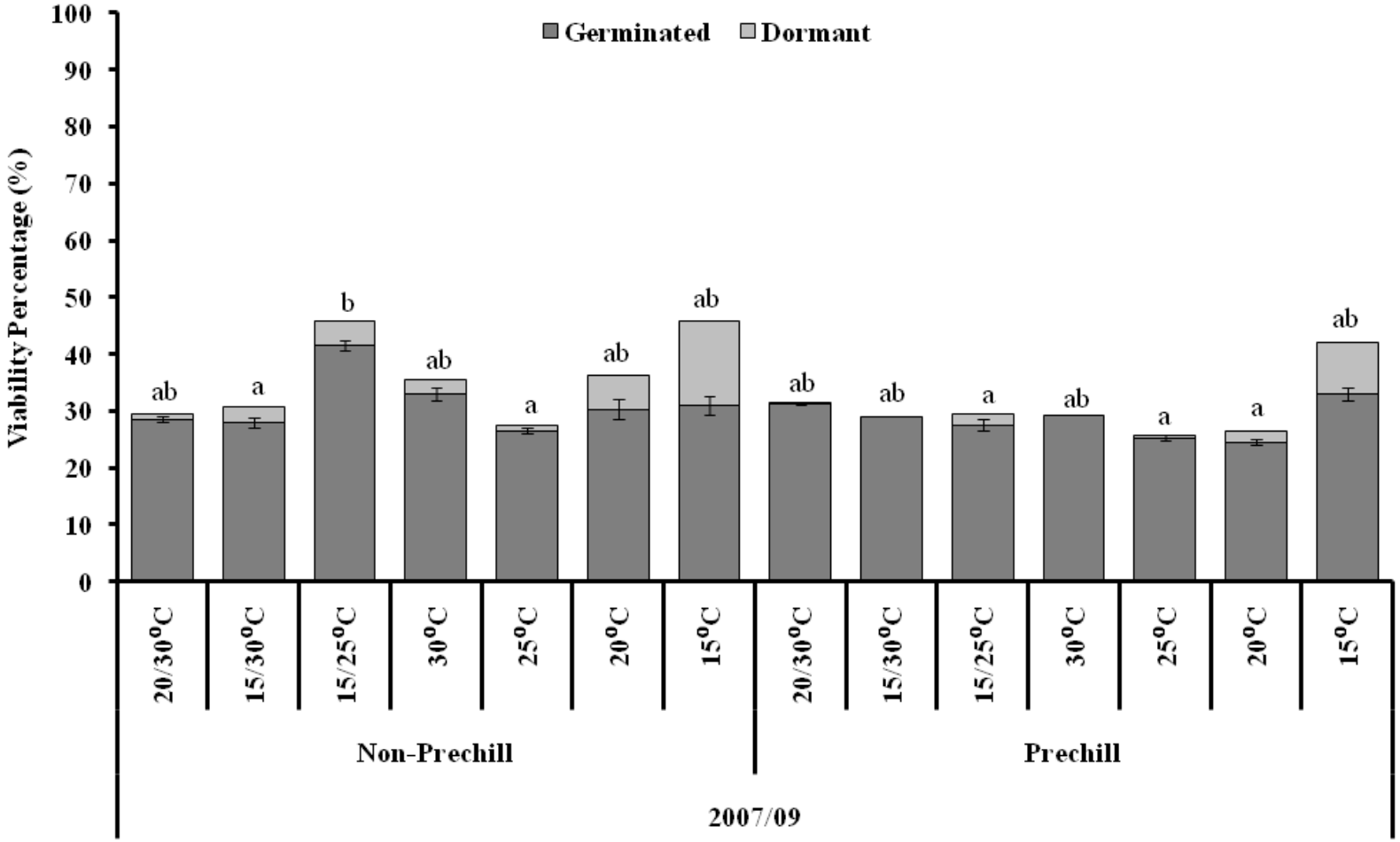


Figure 8. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Sporobolus heterolepis* seeds collected in 2007 and tested in 2009 (2007/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

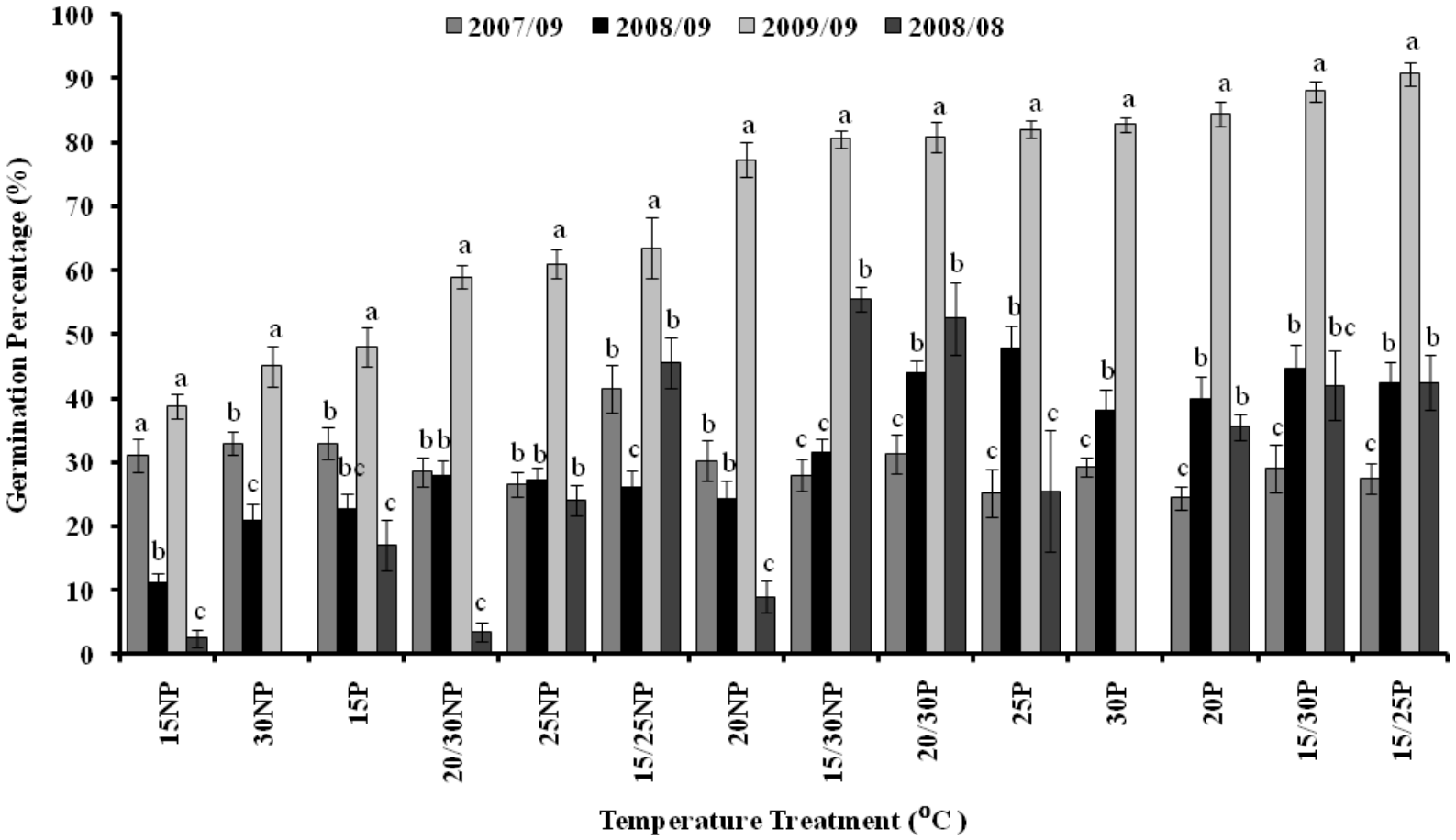


Figure 9. Mean percent germination (± SE) for 2007/09 (n = 8), 2008/09 (n = 8), 2009/09 (n = 8), and 2008/08 (n = 4) *Sporobolus heterolepis* seed collections. For ease of presentation, values are sorted by mean germination percentage for 2009/09. Means among years within temperature and prechill treatments labeled with the same letter are not different (P > 0.05). Data were not collected for 30°C prechill and non-prechill treatments in 2008.

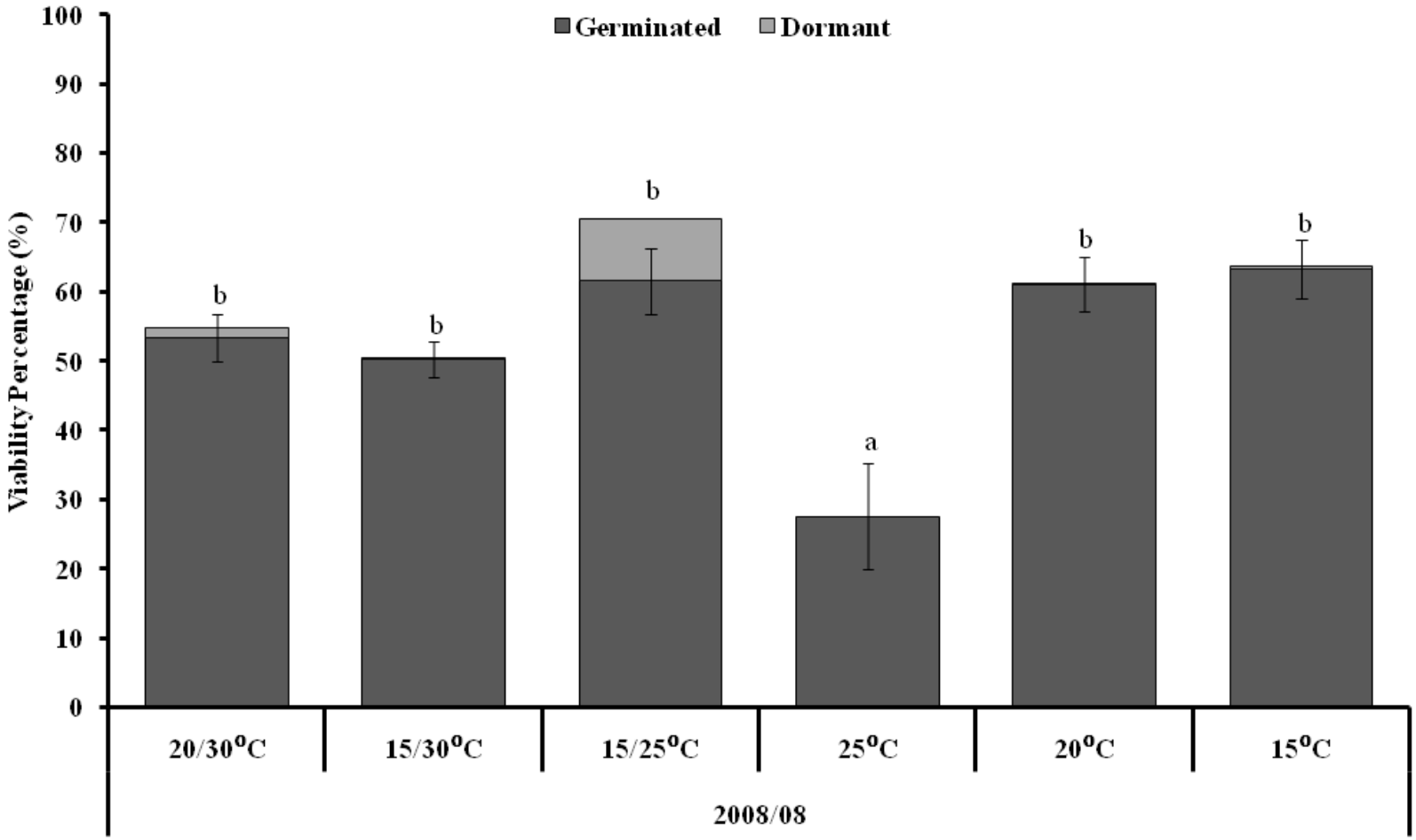


Figure 10. Total seed viability percentages (mean ± SE, n = 4), including mean germination and mean dormancy, for *Heterotheca villosa* seeds collected and immediately tested in 2008 (2008/08). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

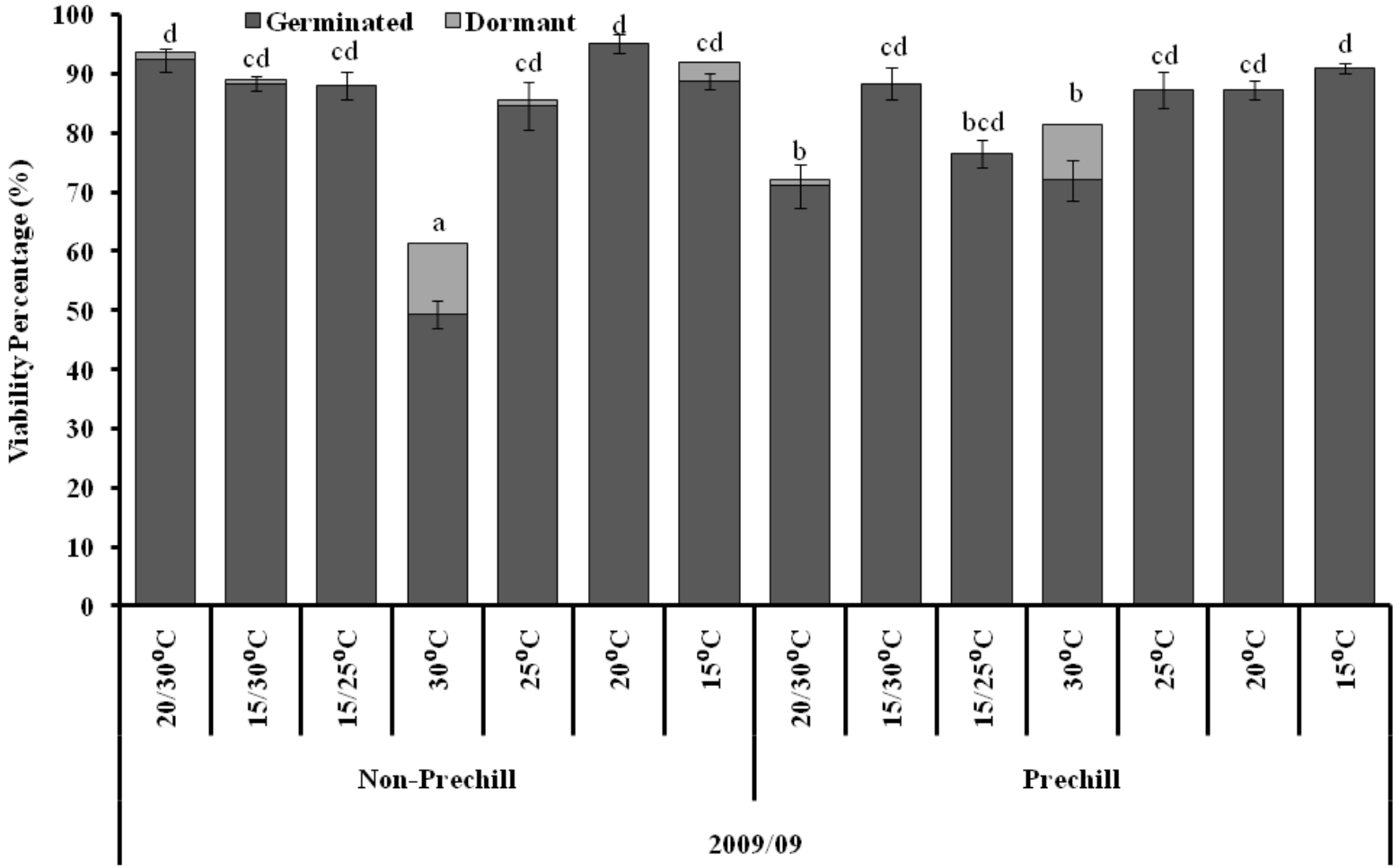


Figure 11. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Heterotheca villosa* seeds collected and immediately tested in 2009 (2009/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

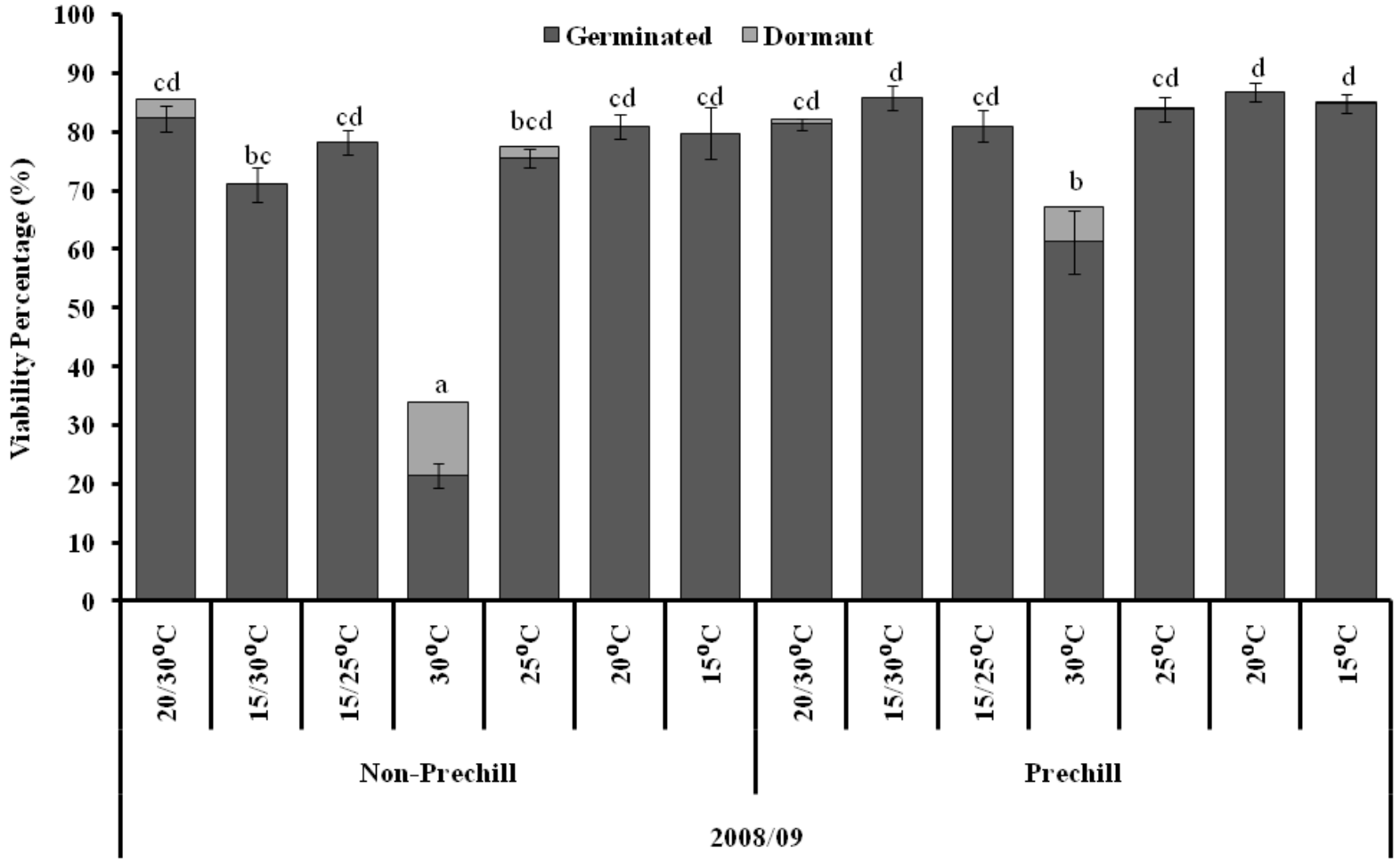


Figure 12. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Heterotheca villosa* seeds collected in 2008 and tested in 2009 (2008/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

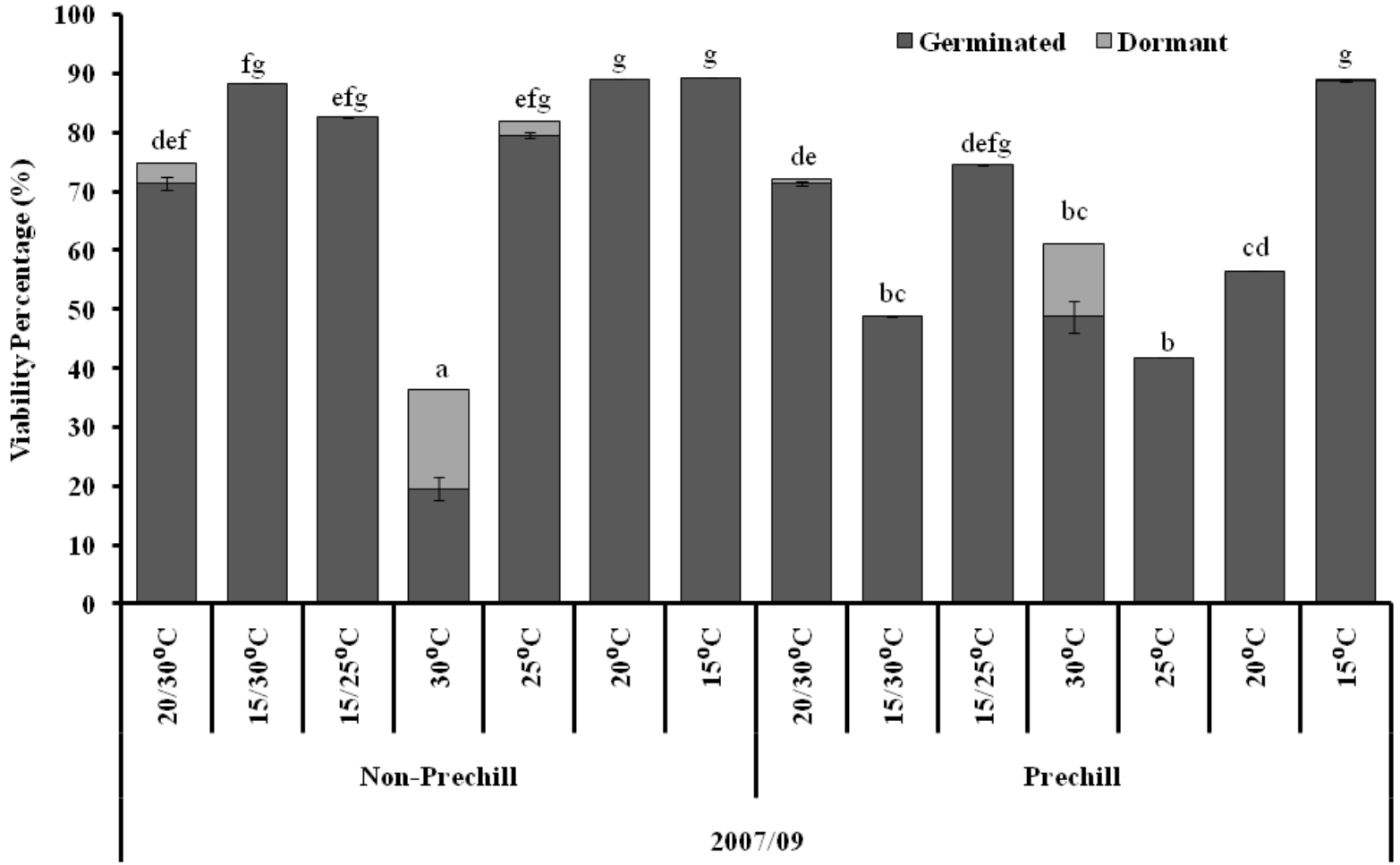


Figure 13. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Heterotheca villosa* seeds collected in 2007 and tested in 2009 (2007/09). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

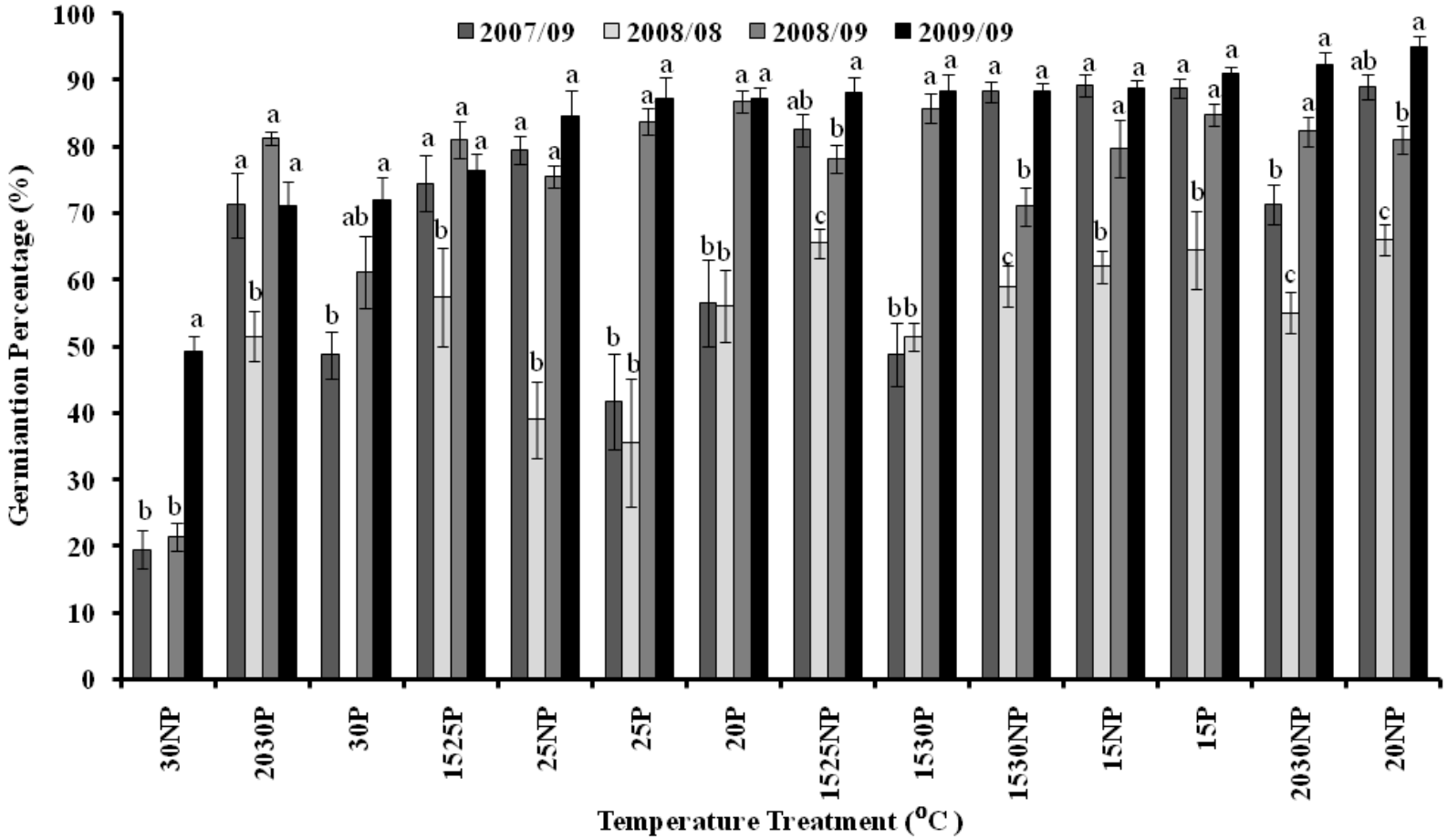


Figure 14. Mean percent germination (± SE) for 2007/09 (n = 8), 2008/08 (n = 4), 2008/09 (n = 8), and 2009/09 (n = 8) *Heterotheca villosa* seed collections. For ease of presentation, values are sorted by mean germination percentage for 2009/09. Means among years within temperature and prechill treatments labeled with the same letter are not different (P > 0.05). Data were not collected for 30°C prechill and non-prechill treatments in 2008.

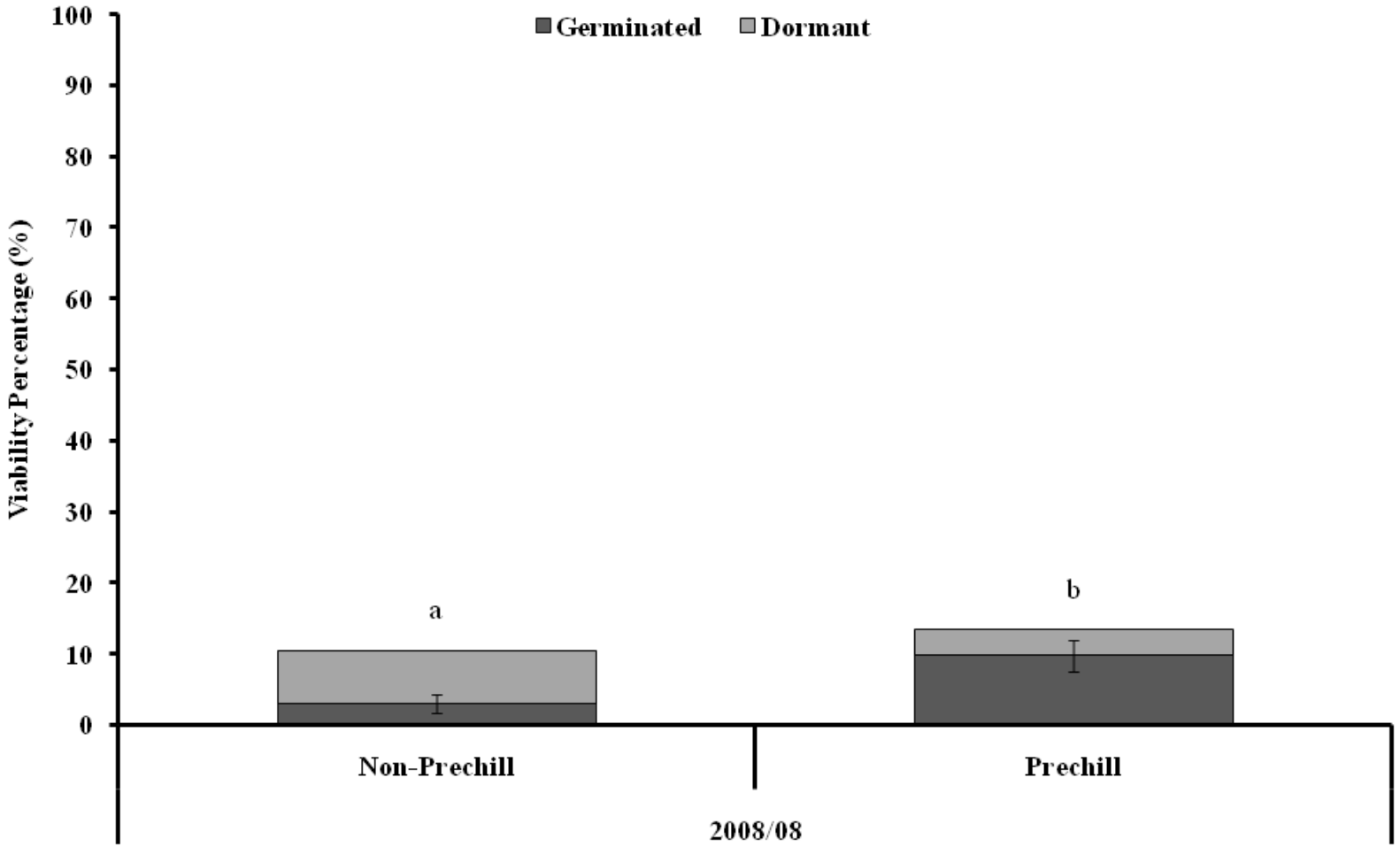


Figure 15. Total seed viability percentages (mean ± SE, n = 4), including mean germination and mean dormancy, for *Gaillardia aristata* seeds collected and immediately tested in 2008 (2008/08). Germination means labeled with the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

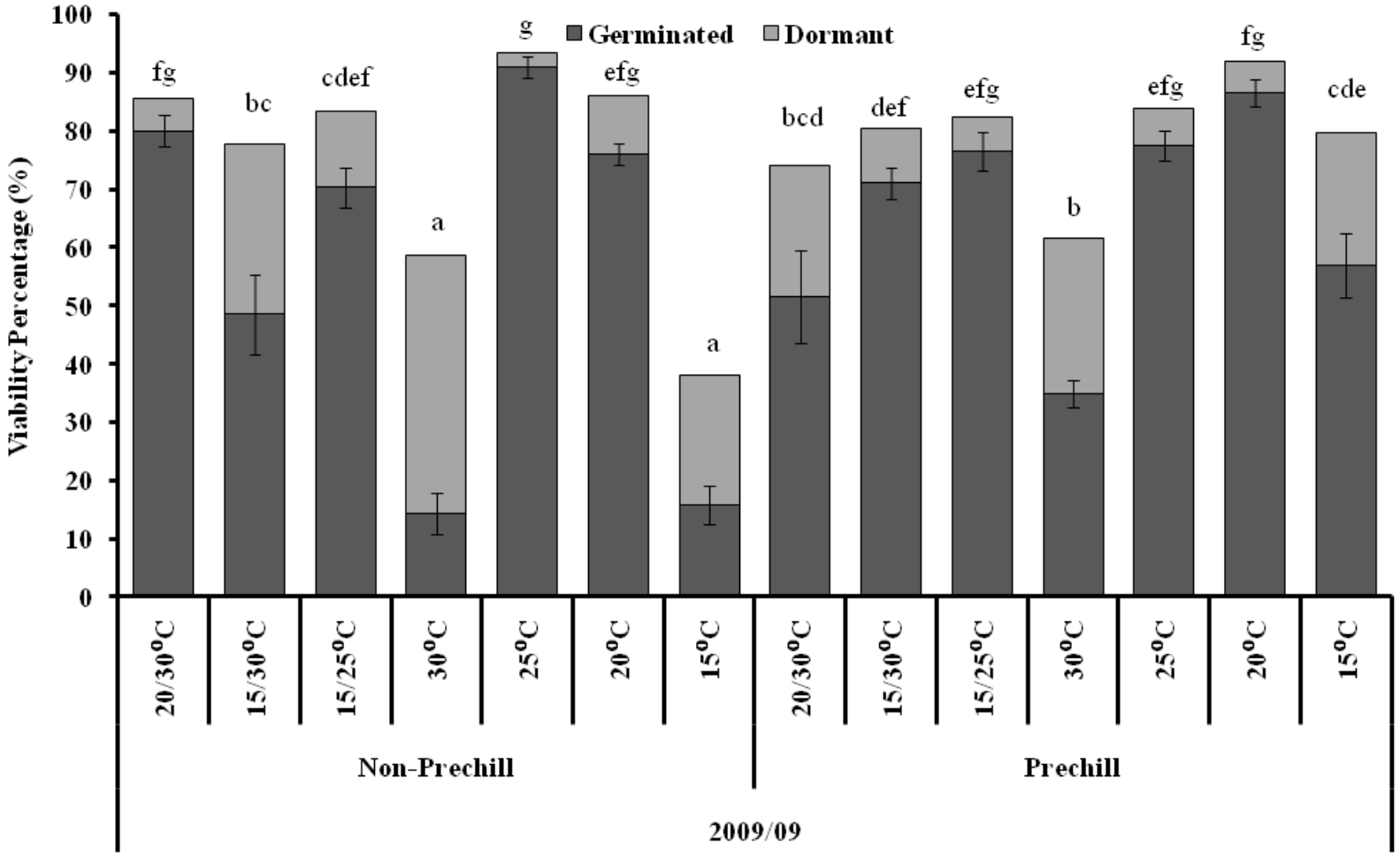


Figure 16. Total seed viability percentages (mean ± SE, n = 8), including mean germination and mean dormancy, for *Gaillardia aristata* seeds collected and immediately tested in 2009 (2009/09). Germination means followed by the same letter are similar (P > 0.05). Dormancy means are provided for descriptive purposes.

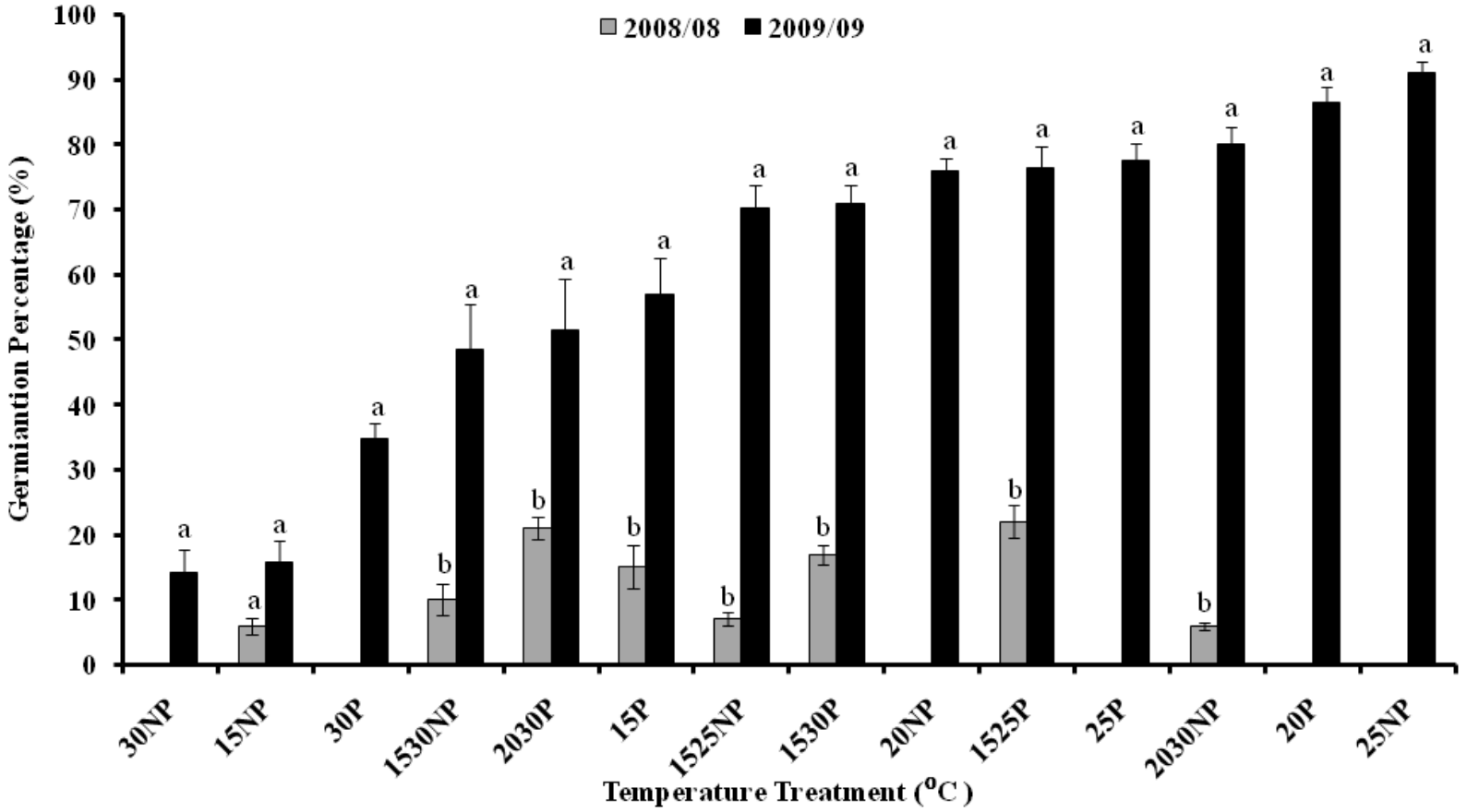
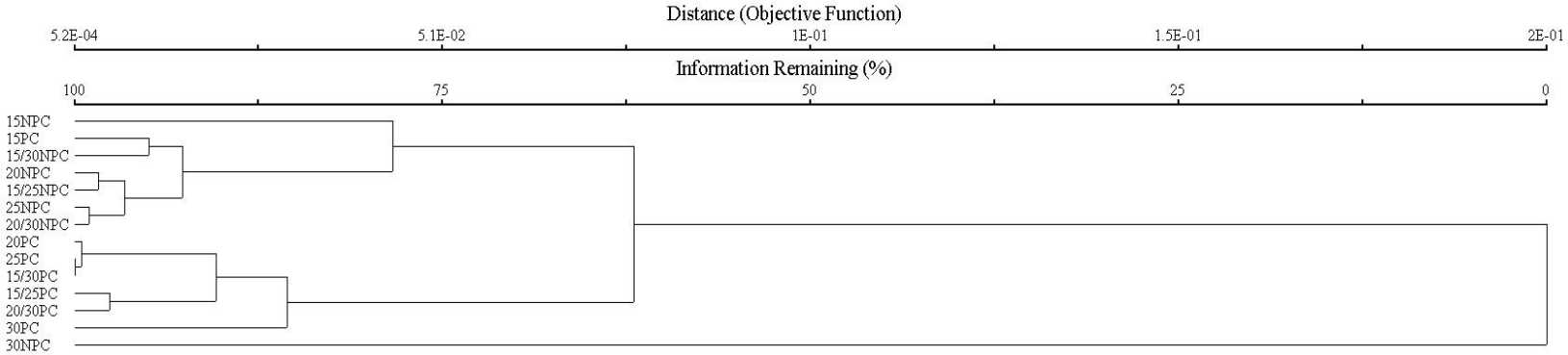


Figure 17. Mean percent germination (± SE) for 2008/08 (n = 4), and 2009/09 (n = 8) *Gaillardia aristata* seed collections. For ease of presentation, values are sorted by mean germination percentage for 2009/09. Means among years within temperature and prechill treatments labeled with the same letter are not different (P > 0.05). Light bank failure in the 20°C and 25°C growth chambers resulted in missing data and data were not collected for 30°C prechill and non-prechill treatments in 2008.

A.



B.

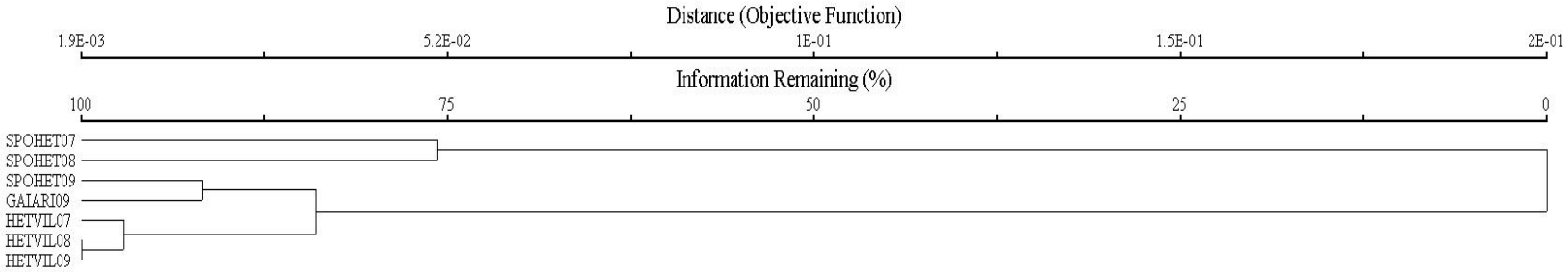


Figure 18. Cluster analysis dendrograms of treatments applied to *Sporobolus heterolepis, Heterotheca villosa, and Gaillardia aristata* seeds germinated in 2009. Dendrogram **A** displays data in relation to treatment and dendrogram **B** displays dendrogram data arranged by species and collection year.

Table 1. Initial viability, total mean germination, and germination in response to prechill and temperature treatments for *Sporobolus heterolepis* by seed lot (2009, 2008, and 2007) for each year of germination trials (2009 and 2008). Initial viability and germination results are expressed as a percent. Standard deviation (± SD) is included for all germination percentages. (n = number of germination boxes, NT = Not Tested)

	2009 Germination Trials				2008 Germination Trials	
	n	2009	2008	2007	n	2008
Initial Viability		93%	99%	74%		NT
Total Germination	112	70 (± 18)	32 (± 13)	30 (± 8)	48	30 (± 20)
Non-prechill	56	61 (± 16)	24 (± 9)	31 (± 9)	24	23 (± 20)
Prechill	56	80 (± 14)	40 (± 11)	29 (± 8)	24	36 (± 16)
Constant Temperature	64	65 (± 19)	29 (± 13)	29 (± 7)	24	19 (± 14)
Fluctuating Temperature	48	77 (± 14)	36 (± 11)	31 (± 9)	24	40 (± 19)

Table 2. Initial viability, total mean germination, and germination in response to prechill and temperature treatments for *Heterotheca villosa* by seed lot (2009, 2008, and 2007) for each year of germination trials (2009 and 2008). Initial viability and germination results are expressed as a percent. Standard deviation (± SD) is included for all germination percentages. (n = number of germination boxes, NT = Not Tested)

	2009 Germination Trials				2008 Germination Trials	
	n	2009	2008	2007	n	2008
Initial Viability		93%	99%	74%		NT
Total Germination	112	83 (± 13)	75 (± 18)	68 (± 23)	48	54 (± 16)
Non-prechill	56	84 (± 16)	70 (± 21)	74 (± 24)	24	58 (± 26)
Prechill	56	82 (± 11)	81 (± 11)	62 (± 21)	24	50 (± 19)
Constant Temperature	64	82 (± 16)	72 (± 22)	64 (± 27)	24	51 (± 21)
Fluctuating Temperature	48	84 (± 10)	80 (± 8)	73 (± 16)	24	57 (± 9)

Table 3. Initial viability, total mean germination, and germination in response to prechill and temperature treatments for *Gaillardia aristata* by seed lot (2009 and 2008) for each year of germination trials (2009 and 2008). Initial viability and germination results are expressed as a percent. Standard deviation (± SD) is included for all germination percentages. (n = number of germination boxes, NT = Not Tested)

	2009 Germination Trials		2008 Germination Trials	
	n	2009	n	2008
Initial Viability		93%		NT
Total Germination	112	70 (± 18)	32	6 (± 5)
Non-prechill	56	61 (± 16)	16	3 (± 2)
Prechill	56	80 (± 14)	16	9 (± 4)
Constant Temperature	64	65 (± 19)	8	5 (± 5)
Fluctuating Temperature	48	77 (± 14)	24	5 (± 7)