

GERMINATION RESPONSE OF TWELVE ACCESSIONS OF *BOUTELOUA
CURTIPENDULA* (MICHX.) TORR. (*POACEAE*) TO A SIMULATED WINTER
TEMPERATURE REGIME

by

Wyatt Thomas Philabaum

Copyright © Wyatt Thomas Philabaum 2022

A Thesis Submitted to the Faculty of the

SCHOOL OF NATURAL RESOURCES AND THE ENVIRONMENT

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2022

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Master's Committee, we certify that we have read the thesis prepared by: Wyatt Thomas Philabaum
titled: Germination Response of Twelve Accessions of Bouteloua curtipendula (Michx.) Torr. (Poaceae) to a Simulated Winter Temperature Regime

and recommend that it be accepted as fulfilling the thesis requirement for the Master's Degree.



Jeffrey Fehmi

Date: Jul 29, 2022



Steven E. Smith

Date: Jul 29, 2022

Elise Gornish

Elise Gornish

Date: Jul 29, 2022

Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

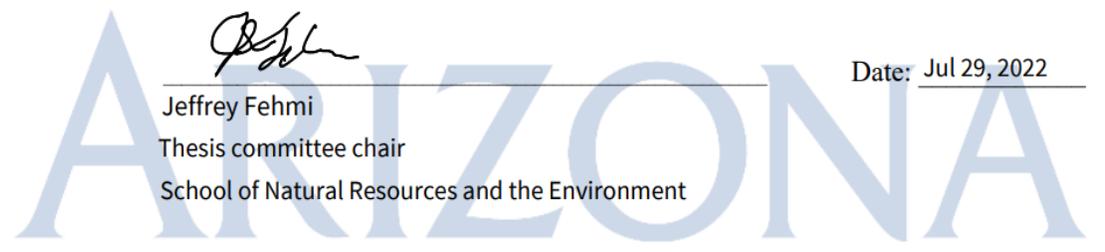
I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.



Jeffrey Fehmi

Thesis committee chair
School of Natural Resources and the Environment

Date: Jul 29, 2022



ACKNOWLEDGMENTS

Foremost I would like to thank my major advisor, and committee chair, Dr. Jeffrey S. Fehmi for his invaluable patience, guidance, and feedback. I could not have undertaken this research without him. Additionally, this research would not have been possible without the guidance and expertise of Dr. Steven E. Smith, who also supplied me with a large portion of the seed I needed for this research. I could not have undertaken this research without the support of my defense committee, who provided me with their expertise and reassurance. Finally, this endeavor would not have been possible without the support from the Harry Wayne Springfield and Tommy Hart Memorial scholarships, who financed this research.

I would be remiss in not mentioning my fiance Heather. Her belief in me kept me motivated, and allowed me to push forward. Her continued support kept me grounded, and kept my spirits high.

LAND ACKNOWLEDGEMENT

We respectfully acknowledge the University of Arizona is on the land and territories of Indigenous peoples. Today, Arizona is home to 22 federally recognized tribes, with Tucson being home to the O'odham and the Yaqui. Committed to diversity and inclusion, the University strives to build sustainable relationships with sovereign Native Nations and Indigenous communities through education offerings, partnerships, and community service.

TABLE OF CONTENTS

ABSTRACT	8
CHAPTER	
I INTRODUCTION.....	9
II METHODS.....	12
III RESULTS.....	16
IV DISCUSSION.....	17
REFERENCES.....	34

LIST OF FIGURES

FIGURE	PAGE
1. Map of Collection locations for all twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>).....	22
2. Total germination percentages of twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>) under the Winter temperature regime.....	23

LIST OF TABLES

TABLE	PAGE
1. Location data for twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>) with botanical variety and expected impact of location	24
2. All germination percentages for all treatments for each of twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>) with seed source, mean seed mass(mg) and seed age.....	27
3. Temperature Regimes for Winter and Summer regimes utilized in this experiment	29
4. ANCOVA results for total germination percentage of twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>) under the simulated summer treatment.....	30
5. Estimated Marginal Means of total germination percentage under the simulated Summer treatment.....	31
6. ANCOVA results for total germination percentage of twelve accessions of <i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Poaceae</i>) under the simulated winter treatment.....	32
7. Estimated Marginal Means of relative germination percentage under the simulated Winter treatment.....	33

Abstract

Twelve accessions of *Bouteloua curtipendula* were tested in a laboratory growth chamber to examine the effect of simulated winter temperature on the germination response of the selected accessions of *B. curtipendula* var. *curtipendula* (Michx.) Torr. and *B. curtipendula* var. *caespitosa* Gould & Kapadia, two botanical varieties of disparate origin. Accessions of the variety *curtipendula* had significantly higher total germination percentages compared to accessions whose variety was var. *caespitosa* under the winter treatment. Latitude was also significant with accessions whose origin was farther north exhibiting higher relative germination percentages under the winter treatment. Mean total germination percentage was significantly higher for accessions whose grains were from NPGS seed production facilities under the winter treatment compared to the mean of accessions from wild-collected populations. Mean seed (grain) mass was highly correlated ($r_s = 0.774$) with relative germination percentages, suggesting seed vigor may be an important explanatory factor in germination under stressful conditions.

Keywords Grass germination, total germination response, relative germination response, GRIN-Global National Plant Germplasm System, botanical variety, seed vigor

Introduction

Bouteloua curtipendula (Michx.) Torr. (sideoats grama) is one of the most phenotypically diverse and widely distributed warm-season bunchgrasses in North America (Gould & Kapadia 1964, Tomas de Pisani 2004). Within its natural range, *B. curtipendula* is utilized by wildlife and livestock, but generally comprises a minor component of naturally occurring rangeland plant communities (Ruyle and Young, 1997). The value of *B. curtipendula* in rangeland communities is surpassed by its relative importance as an agronomic pasture grass. *Bouteloua curtipendula*'s unique morphology and upright growth habit are desirable traits for typical farm cultivation, and this has resulted in *B. curtipendula* being a permanent seeded addition in many pastures in the central and southern Great Plains (Smith et al. 2004).

A key factor in the survival of *B. curtipendula* in both rangeland and pastureland sites is rapid germination under suitable conditions (Smith et al. 2004, Fehmi et al. 2014). Germination is often inhibited by temperature even while moisture conditions are favorable because near the minimum germination temperature, the time to achieve germination is increased (Roundy & Biedenbender, 1994). This could be detrimental in areas where dominant species' time to germination has the potential to exceed typical soil moisture availability (Jordan & Haferkamp 1989). It is possible that the local adaptation of within *B. curtipendula*, including germination speed and requirements, has contributed to the relative success and prolific distribution of the warm-season bunchgrass throughout North America (Gould & Kapadia 1964, Tomas de Pisani 2004, Smith et al. 2004, Halbrook et al. 2012).

Bouteloua curtipendula's phenotypic diversity has led taxonomists to establish three botanical varieties (Gould & Kapadia, 1964). Of these varieties, var. *caespitosa* and var. *curtipendula* have extensive distributions in the United States. The third variety, var. *tenuis* is limited to mesic areas of southeastern Texas and northeastern Mexico (Tomas de Pisani 2004, Halbrook et al. 2012). The two dominant varieties, var. *caespitosa* and var. *curtipendula*, have remarkably similar appearance and ecological function, but may differ significantly in biomass allocation, reproductive strategy, and morphology (Gould & Kapadia 1964, Tomas de Pisani 2004). Each variety is adapted to a larger plant community with var. *caespitosa* being referred to as the “phalanx” form and generally produces less aboveground biomass with a short but densely foliated aboveground morphology (more flowering culms, more tillers, and a basal foliage distribution; Tomas de Pisani, 2004). It is adapted to southwestern grasslands and desert communities and its densely foliated aboveground biomass may provide self-shading that would limit evapotranspirative demands on an individual. The other dominant variety, var. *curtipendula*, is referred to as the “guerrilla” form. It is adapted to short and tall grass prairie communities of the central and northern United States and produces more aboveground biomass than var. *caespitosa* (Tomas de Pisani, 2004) and its rhizomatous and stoloniferous growth allows var. *curtipendula* to dominate many mesic areas of the Great Plains (Gould & Kapadia 1962, Tomas de Pisani 2004).

The var. *caespitosa* may be unique in that it is facultatively apomictic (Gould, 1959). Apomixis is defined as an asexual reproductive process that paradoxically occurs within the embryo sac or in nucellar tissues (Nogler, 1984). Apomixis results in fertile seeds, with the distinct difference being that the resultant apomictic embryo is derived solely from maternal tissue (Koltunow, 1993). It is important to acknowledge that apomixis does not imply a lack of

adaptive potential. Facultative apomicts like *B. curtipendula* have the potential to produce a novel cytotype upon sexual reproduction that could allow for apomictic populations to adapt to new or changing environments (Halbrook et al. 2012) although it is unclear how common these adaptations occur. It is expected that sexual outcrossing, and possibly to a lesser extent, apomictic populations of *B. curtipendula* could have variability in their relative performance and germination response (Tomas de Pisani, 2004).

Germination response is influenced by a multitude of factors, the foremost being environmental factors like temperature and precipitation, and factors like seed vigor, age, and post-harvest dormancy (Donohue 2002, Zettlemoyer et al. 2017). Environmental factors like temperature and precipitation are geographically variable and significantly influence the timing and success of germination (Donohue 2002, Fehmi et al. 2014, Zettlemoyer et al. 2017). Geographic variation may influence local adaptations in germination, and this may contribute to population-level differences between ecotypes or different taxonomic groups (Gallagher & Wagenius, 2016). Seedlot specific factors like seed age, and vigor, post-harvest dormancy, and storage conditions may also influence germination performance (Egli & Rucker, 2012).

There is little consensus on the relative importance of seed origin and its effect on germination response, and research into these effects for *Bouteloua curtipendula* is lacking along with warm-season bunchgrasses generally (Smith et al., 2000). This study aims to determine how seed origin affects germination response of accessions of *B. curtipendula* collected along a north-south gradient in the United States. It is hypothesized that under a winter treatment characteristic of December conditions in low-elevation deserts of the Southwest, total germination response will be higher for accessions of *B. curtipendula* whose origin is further north (var. *curtipendula*) than those whose origin is further south (var. *caespitosa*).

Methods

Accessions Origin and Collection

Accessions are defined as plant material from a single species collected from a single population at a single location at a given time. The accession samples came from two sources. The first source provided a majority of the var. *caespitosa* accessions (Table 1), which came from S.E. Smith's personal collection. Seeds (grains) were collected by S. E. Smith and K. H. Halbrook from wild populations, and represented a bulk of seeds from at least 10 plants in the population.

The second set of accessions came from the GRIN-Global National Plant Germplasm System (National Plant Germplasm System, 2022) and included all of the var. *curtipendula* accessions and one var. *caespitosa* accession (Table 1). Seed from the NPGS was collected from wild populations on their original collection date but was grown by the Department of Agriculture in seed production plots in Griffin, GA in later years.

Preliminary Viability Testing

Seed quality was determined immediately prior to the start of the experiments. Standard germination was determined in rolled paper towel ragdolls inside of a plastic bag held at a constant temperature of 30°C with the final count taken after 7 days as described by AOSA

(AOSA, Rules for Testing Seeds 2019; see Table 2). A total of 40 seeds of each accession were used to test preliminary viability with no replication.

Determination of Temperature Regimes

Seeds from the various accessions were exposed to one of two simulated temperature regimes. The temperature regimes corresponded to either summer or winter for southeastern Arizona. Summer conditions are defined as conditions favorable for immediate seedling growth (Smith et al. 2000). The ideal summer regime ranged from 22.8 to 35.0 °C, reaching the minimum temperature at hour six of the daily temperature cycle, and a maximum temperature at hour 15 of the temperature cycle. The winter regime ranged from 4.0 - 14.9 °C, reaching a minimum temperature at hour four of the temperature cycle, and a maximum temperature at hour fifteen. Both temperature regimes had a gradual rate of change. The regimes were based on temperature data gathered from AZMET station Sahuarita (31.924, -110.949) for August and December of 2019 (The Arizona Meteorological Network, 2020). See Table 3 below for temperature regimes.

Plant Management and Assessment of Germination

Twenty seeds from each accession of *B. curtipendula* were placed in separate 90 mm Petri dishes, each with a sheet of Whatman Grade 2 Filter Paper (Cytiva Life Sciences, Marlborough, MA, USA) and subjected to either one of two temperature regimes within a replicate that was conducted five times (see temperature regimes below) over a period of 5 months. Seed was selected based on quality such that no broken or otherwise apparently damaged seeds were utilized. Each petri dish was watered such that they remained moist throughout the experiment.

Petri dishes were checked daily for germination and all germinated seeds were promptly recorded and removed from the petri dishes. A seed was considered germinated if it produced a visible radicle and hypocotyl. A replication was considered to be complete once all seeds had germinated or perished, or once germination activity had ceased for longer than 7 days. Total germination is defined as the total number of seeds that germinated. A single value of total germination percentage was derived from each of the twelve accessions under the summer temperature treatment, which was then used to calculate relative germination. Relative germination was defined as the germination percentage within a replicate for an accession under the winter treatment/total germination percentage for that accession.

Determination of Latitude

Latitude was determined by plotting the centroid of the accession's origin location on Google Earth. Accession origin locations were pulled from either the GRIN NPGS, NRCS collection records, or notes from the original collector of the accession. This location was then converted into latitude and longitude. An accession's latitude became the Latitude. Accessions with a latitude above 35 degrees were considered a northerly accession, and accessions with a latitude under 35 were considered southerly accessions. See Table 1 below for origin designations.

Determination of Seed Mass

Twenty individual seeds from each accession were measured, and used to produce a mean seed mass per accession. Mean seed mass was then used to calculate the coefficient of variation for seed mass, which measures the consistency of seed mass within a seedlot. Seed mass was used as a measure of seed vigor, with larger seeds generally considered more vigorous and of higher quality.

Statistical Methods

Temperature treatments were analyzed separately. A randomized complete block design (RCBD) with five replications was implemented for each temperature treatment. Replications were used as the blocking factor and accessions and botanical varieties as independent variables.

Replications were all performed in the same growth chamber. An ANCOVA was used to determine the effect of latitude (covariate), and botanical variety on germination response. An ANCOVA was developed for both the relative germination and summer germination percentages. The interaction between relative germination percentages, Latitude, and botanical variety was determined with Tukey HSD pairwise comparisons. Post-hoc pairwise comparisons of means separations were produced with Tukey's HSD using the multcomp package (Hothorn, Bretz and Westfall 2008). R procedures were used for ANOVA and TukeyHSD calculations (R Core Team 2022). Post-hoc seed mass measurements were conducted to assess the effect of seed vigor on germination response.

Results

Preliminary viability testing revealed initial viabilities to be over 90% for all accessions (Table 2). Total germination of seeds under summer treatment was similar between both botanical varieties (90.4 ± 3.82 vs. 97.5 ± 5.04 , mean \pm SE, $p = 0.387$, $F_{1,53} = 0.760$, Table 4 & 5). Latitude was found to be significant under the summer ANCOVA ($p < 0.001$, $F_{1,53} = 4.57$). Germination of seeds under the summer treatment was defined as 100% to calculate relative germination for winter (Fig. 1). The effect of an accession's botanical variety on relative germination percentage under the winter was significant ($p < 0.001$, $F_{1,53} = 14.84$). Accessions of the variety *curtipendula* had significantly higher mean total germination percentages compared to var. *caespitosa* under the winter treatment (98.6 ± 9.55 vs 38.6 ± 7.24 , mean \pm SE, $p < 0.001$, $F_{1,53} = 14.84$, Table 6 & 7). Latitude was also significant under the winter ANCOVA, with accessions whose origin was farther north exhibiting higher relative germination percentages (98.6 ± 9.55 vs 38.6 ± 7.24 , mean \pm SE, $p < 0.001$, $F_{1,53} = 13.59$). Replication (the blocking factor) was not significant under either ANCOVA.

Seed mass measurements revealed distinct differences between mean seed mass between accessions. Accessions whose source was a "farm plot" had higher mean seed weights than accessions whose source was "wild collected" ($0.873\text{mg} \pm 0.155$ vs. $0.602\text{mg} \pm 0.208$, mean \pm s.d, Table 2). Coefficients of variation for seed mass were also far lower in "farm plot" when compared to "wild collected" seed as well ($cv = 18.3\%$ vs. $cv = 34.6\%$). Rank correlation between seed mass and relative germination was high and statistically significant ($r_s = 0.774$).

Discussion

Variability in germination response of 12 accessions of *B. curtipendula* from across its distribution range showed seed sourced from NPGS farm plots produced significantly higher relative germination percentages than was observed from wild collected accessions under the simulated winter treatment. Four of the six northerly accessions (Killdeer, Pierre, El Reno, Trailway) produced the highest total germination percentage of all accessions under the winter treatment (Fig. 2). This significant decrease in total germination percentage could be explained by the effect of the local adaptations sustained by the accession population due to the accession's origin location, but as discussed below, this was difficult to disentangle from issues such as seed age and vigor.

The relative germination response results for the winter treatment found here are similar to those of Roundy & Biedenbender (1994) who used a growth chamber study to test total germination percentage and minimum germination temperature of a single accession of *B. curtipendula*, Vaughn (a var. *caespitosa* cultivar that originated in New Mexico), under a simulated winter treatment with a lower minimum temperature (1 °C) than here (4 °C). Total germination percentage for Vaughn in Roundy & Biedenbender's (1984) study was higher than the mean relative germination percentage in our study (80% vs. 64%), but the variation in germination percentages between accessions in the study here was significant. Roundy & Biedenbender's unexpected cold temperature germination of the presumed var. *caespitosa* Vaughn is quite similar to the unexpected cold temperature germination of the presumed var. *caespitosa* Uvalde found here in this study.

One var. *caespitosa* accession, Uvalde, also produced some of the highest relative germination percentages in the experiment that was unforeseen considering the accession is geographically the furthest south of all the accessions tested from an area with significantly warmer winters than the simulated winter in this study. Tomas de Pisani (2004) reported the origin location of the Uvalde accession may exist in a zone of sympatry between two varieties of *B. curtipendula* but Uvalde is very likely a var. *caespitosa*. Seed age may have had an effect on the germination response of Uvalde. In a combined laboratory and field study with *B. curtipendula*, Shaidhae et al. (1969) reported that seed viability, total germination, and emergence of this species declines by more than 20% after just two years of storage. The seeds of Uvalde utilized in this experiment were grown out and collected in 2011, making the Uvalde seeds the most recently produced seeds utilized in the experiment, which could explain its comparatively high performance under the decreased temperatures of the winter treatment. This is still unclear though as other seeds utilized in this experiment, like the seeds of Pierre, are presumed to have been produced prior to 1989 and still exhibited similar relative germination percentages (Uvalde, 100% vs Pierre, 98%). Egli and Rucker (2012) reported that high-vigor seed lots of exhibited total germination percentages 43.5% higher than low-vigor seed lots under cold-test stress conditions, so high seed vigor within the seed lot could explain the increased performance of Uvalde under winter conditions (i.e., stress conditions for a var. *caespitosa*) as well.

Jordan & Haferkamp (1989) reported that the minimum temperature for germination for *B. curtipendula* was 8.9 °C based on a constant-temperature growth chamber study but they used a single accession of *B. curtipendula*, NM-28, a var. *caespitosa* sourced from the USDA-NRCS Plant Materials Center in Tucson, AZ. It is important to note that Jordan & Haferkamp utilized a

constant temperature regime, where the study here used a gradual temperature regime.

Bartholomew (2014) similarly reported the minimum temperature for germination to be 9.1 °C in a similar growth chamber study but used only a single accession, Butte, a var. *curtipendula* from Holt County, Nebraska. Bartholomew utilized a constant temperature regime as well. Five of the 12 accessions tested in our study produced significantly higher total germination rates than other accessions despite being under the reported minimum germination temperature(s) for the species for more than 15 hours of the daily temperature cycle (Fig. 2, Table 3). The remaining seven accessions produced total germination percentages far lower while under the minimum germination temperature for the species. This decreased germination response could possibly be explained by the effect of accession origin on total germination percentage, but is more likely explained by decreased seed vigor. The remaining seven accessions originated from southern populations whose local adaptations may not confer success under lower temperatures.

While the accession origin seems a plausible explanatory factor, the effects of seed age, and seed vigor must also be considered. The correlation between seed mass (a measure of seed vigor) and relative germination under the winter treatment was quite high ($r_s = 0.774$), which suggests that seed vigor is an important explanatory factor.

There was a lack of significant differences between the mean values of relative germination percentage between Yellowstone (a northerly var. *curtipendula* accession) and Niner and Sheriff (two southerly var. *caespitosa* accessions). When compared to other northerly var. *curtipendula* accessions, Yellowstone produced relative germination percentages 53-59% lower. This outcome was generally unanticipated. Low total germination of Yellowstone could again be attributed to compromised seed quality, extended seed age, or low seed vigor. Smith et al. (1987) similarly reported in a growth chamber experiment utilizing *Medicago sativa* L. that mean

germination percentages of 15-year-old seed were 33% lower than five-year-old seed. The seed of the Yellowstone accessions utilized in this experiment are presumed to have been produced in 1998, about 24 years ago, which suggests the increased age of the seed may have had an effect on the measured germination response. Egli and Rucker (2012) reported in a growth chamber study with corn that low-vigor seedlots held under low seedbed temperatures corresponded to significantly slower germination rates and total germination percentages.

The atypical performances of both Uvalde and Yellowstone are difficult to explain. The differences observed may be due to a combination of seed origin effects and seed vigor effects in combination with the artifacts of extended storage and variable storage conditions and varying maternal environments between accessions. It is interesting that the seed age effects on germination may be expressed in some temperature regimes and not others, but this is well documented. Grabe (1976) states cold-tests are often used to identify low-vigor seedlots, with poor performance under the cold-test suggesting low vigor. Variations in seed quality and age are likely affecting germination of accessions independent of any inherent tendencies for cold germination that might exist.

Future research is needed to further disentangle the effect of seed origin, botanical variety, and seed vigor, and quality on germination response. The objective of this study was to determine how the simulated temperatures in a controlled environment affect the germination of seeds of *B. curtipendula* with various origins. It is interesting that the effect of seed vigor only manifested under cold temperatures (i.e, stress conditions). The performance of accessions with high germination percentages under stressful conditions is most likely explained by seed vigor effects, but could be driven by local adaptations. A study incorporating seed produced from a common garden containing all accessions could remove the confounding effect of variability in

maternal environments, seed vigor, seed age, and storage conditions between accessions. This may be difficult considering the outcrossing accessions would need some sort of isolation to prevent interbreeding. A field study echoing the treatments utilized in this controlled environment study could provide implications more readily applicable to actual restoration or reseeded activities. Additionally, the twelve accessions included in this study represent a small portion of the accessions recorded in the GRIN NPGS. Inclusion of an increased number of accessions from other regions across the distribution range may reveal findings regarding seed origin and its effect on germination response and may reveal specific accessions that could benefit restoration activities in other regions within the distribution range of *B. curtipendula*.

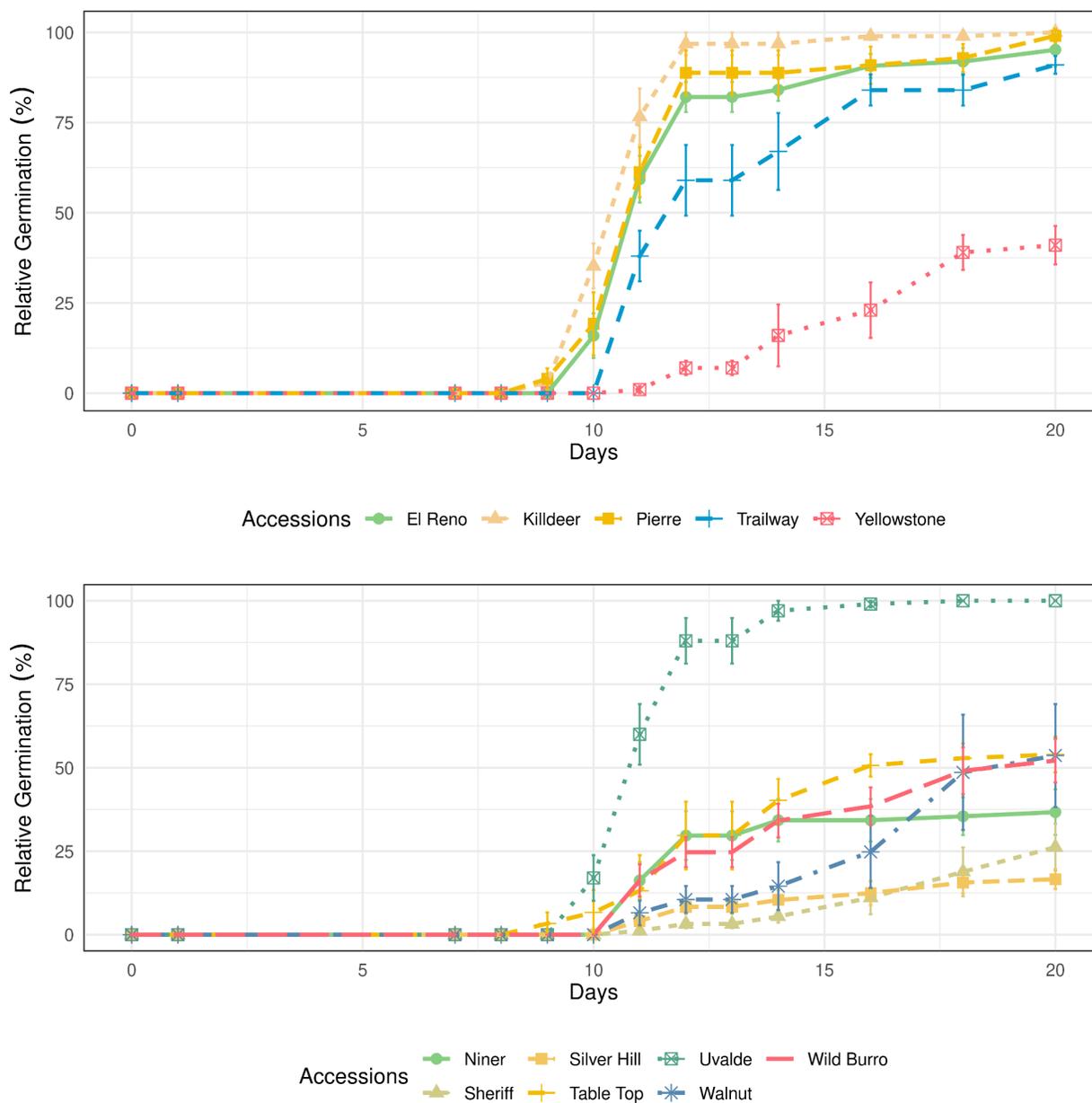


Figure 1: Total relative germination percentages of twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (Poaceae) from two botanical varieties (var. *curtipendula*, above and var. *caespitosa*, below). Error bars represent standard errors.

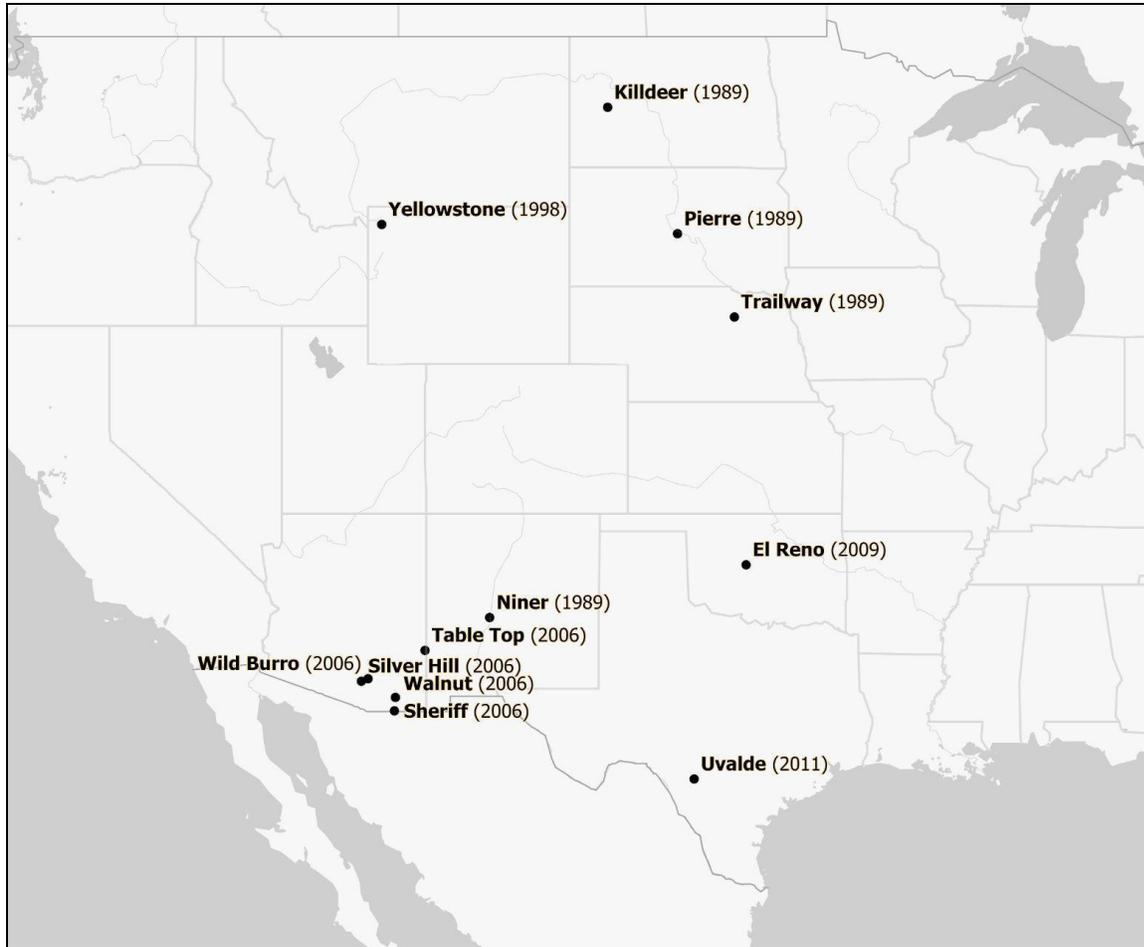


Figure 2: Map of *Bouteloua curtipendula* (Michx.) Torr. accession collection locations.

Year of collection is in parentheses.

Table 1. Location data for twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (Poaceae) with botanical variety and expected impact of location

GRIN NPGS PI #	Name	Collection origin (Lat/Long)	Collection Source	Seed Age	Botanical variety	Elevation	Expected impact of location
PI 476981	Killdeer	Dunn County, North Dakota (47.402, -102.622)	NPGS-USDA Farm plot	13 years	<i>curtipendula</i>	653 m	Presumed to be sexually Reproducing (Smith et al. 2004, Halbrook 2012), Reported by NRCS to have notable persistence in a cold, semi-arid region (Smith et al., 2004)
PI 241045	Yellowstone	Yellowstone National Park, Wyoming (44.916 -110.416)	NPGS-USDA Farm plot	24 years	<i>curtipendula</i>	1,912 m	Highest elevation collection location, Presumed to be sexually reproducing
PI 476980	Pierre	Pierre, South Dakota (44.338, -100.799)	NPGS-USDA Farm plot	33 years	<i>curtipendula</i>	587 m	Presumed to be sexually Reproducing, Reported by NRCS to have "Outstanding vigor and leafiness in South Dakota" (Smith et al., 2004)
PI 477001	Trailway	Holt County, Nebraska (42.492, -98.778)	NPGS-USDA Farm plot	33 years	<i>curtipendula</i>	647 m	Presumed to be sexually Reproducing, Reported by NRCS to have winter hardiness and persistence, and increased rust resistance, was selected for late maturity (Smith et al., 2004).
PI 421281	El Reno	Canadian County, Oklahoma (35.524, -97.992)	NPGS-USDA Farm plot	33 years	<i>curtipendula</i>	417 m	Presumed to be sexually Reproducing, Reported by NRCS to have "Outstanding vigor and leafiness in Kansas" (Smith et al., 2004)

Table 1. Location data for twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (Poaceae) with botanical variety and expected impact of location

GRIN NPGS PI #	Name	Collection origin (Lat/Long)	Collection Source	Seed Age	Botanical variety	Elevation	Expected impact of location
PI 478839	Niner	Socorro, New Mexico (34.060, -106.902)	NPGS-USDA Farm plot	33 years	<i>caespitosa</i>	1,403 m	Likely facultatively apomictic
N/A	Table Top	Table Top, Arizona (33.181, -109.113)	Native stand (wild collected)	16 years	<i>caespitosa</i>	1,664 m	Likely facultatively apomictic
N/A	Wild Burro	Wild Burro Canyon, Arizona (32.475, -111.090)	Native stand (wild collected)	16 years	<i>caespitosa</i>	854 m	Likely facultatively apomictic
N/A	Silver Hill	Silver Hill Mine, Arizona (31.688, -111.338)	Native stand (wild collected)	16 years	<i>caespitosa</i>	1,104 m	Likely facultatively apomictic
N/A	Walnut	Walnut Gulch Experimental Range, Tombstone, Arizona (31.7212, -110.184)	Native stand (wild collected)	16 years	<i>caespitosa</i>	1,180 m	Likely facultatively apomictic
N/A	Sheriff	Cochise County, Arizona (31.432, -109.871)	Native stand (wild collected)	16 years	<i>caespitosa</i>	1,523 m	Likely facultatively apomictic

Table 1. Location data for twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (Poaceae) with botanical variety and expected impact of location

GRIN NPGS PI #	Name	Collection origin (Lat/Long)	Collection Source	Seed Age	Botanical variety	Elevation	Expected impact of location
PI 469219	Uvalde	Knippa, Texas (29.294, -99.657)	NPGS-USDA Farm plot	11 years	<i>caespitosa</i>	303 m	Lowest Elevation collection location, Presumed to be sexually reproducing OR Apomictic, Reported by NRCS to have limited resistance to winters at high elevations (Smith et al., 2004)

Table 2. All germination percentages for all treatments for each of twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. with seed source, mean seed mass (mg) and seed age.

Name	Botanical variety	Collection Source	Seed Age (years)	Seed Mass (mg)	Preliminary Viability (%)	Summer Total Germination (%)	Winter Relative Germination (%)
Killdeer	<i>curtipendula</i>	NPGS-USDA Farm plot	13	1.095	98	99	100.0
Yellowstone	<i>curtipendula</i>	NPGS-USDA Farm plot	24	1.020	96	100	41.0
Pierre	<i>curtipendula</i>	NPGS-USDA Farm plot	33	0.980	100	98	98.0
Trailway	<i>curtipendula</i>	NPGS-USDA Farm plot	33	0.890	94	100	91.0
El Reno	<i>curtipendula</i>	NPGS-USDA Farm plot	33	0.800	96	95	94.0
Niner	<i>caespitosa</i>	NPGS-USDA Farm plot	33	0.735	90	89	37.0
Table Top	<i>caespitosa</i>	Native stand (wild collected)	16	0.705	92	95	54.0
Wild Burro	<i>caespitosa</i>	Native stand (wild collected)	16	0.680	94	95	52.0

Table 2. All germination percentages for all treatments for each of twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. with seed source, mean seed mass (mg) and seed age.

Name	Botanical variety	Collection Source	Seed Age (years)	Seed Mass (mg)	Preliminary Viability (%)	Summer Total Germination (%)	Winter Relative Germination (%)
Silver Hill	<i>caespitosa</i>	Native stand (wild collected)	16	0.645	96	96	16.6
Walnut	<i>caespitosa</i>	Native stand (wild collected)	16	0.584	96	59	53.8
Sheriff	<i>caespitosa</i>	Native stand (wild collected)	16	0.545	94	94	26.2
Uvalde	<i>caespitosa</i>	NPGS-USDA Farm plot	11	0.489	96	100	100

Table 3. Temperature Regimes for Winter and Summer regimes utilized in this experiment

Winter		Summer	
Average Hourly Air Temp		Average Hourly Air Temp	
HR	Air Temp (C)	HR	Air Temp (C)
1	4.8	1	24.1
2	4.4	2	23.6
3	4.2	3	23.2
4	3.9	4	23.0
5	3.8	5	23.1
6	3.9	6	22.8
7	3.7	7	23.2
8	3.8	8	25.5
9	5.9	9	27.8
10	8.6	10	30.6
11	10.5	11	32.6
12	11.9	12	34.0
13	13.2	13	35.0
14	14.2	14	34.8
15	14.9	15	34.7
16	14.9	16	34.8
17	14.1	17	33.9
18	11.6	18	31.8
19	9.4	19	29.1
20	7.8	20	26.5
21	6.4	21	25.2
22	5.1	22	24.7
23	4.6	23	24.6
24	4.3	24	24.2

Table 4. ANCOVA results for total germination percentage of twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (*Poaceae*) under the simulated summer treatment

Variable	Degrees of Freedom	Sum of Squares	Mean square	<i>F</i>	<i>p</i>
Latitude (covariate)	1	952	951.7	4.577	0.037
Botanical variety	1	158	158.0	0.760	0.387
Replication	4	454	113.5	0.546	0.703
Residuals	53	11019	207.9		

Table 5. Estimated Marginal Means of total germination percentage under the simulated Summer treatment

Botanical variety	Est. Marginal Means (%)	Group
<i>curtipendula</i>	97.5	a
<i>caespitosa</i>	90.4	a

Table 6. ANCOVA results for relative germination percentage of twelve accessions of *Bouteloua curtipendula* (Michx.) Torr. (*Poaceae*) under the simulated winter treatment

Variable	Degrees of Freedom	Sum of Squares	Mean square	<i>F</i>	<i>p</i>
Latitude (covariate)	1	10242	10242	13.590	< 0.001
Botanical variety	1	11180	11180	14.835	< 0.001
Replication	4	1179	295	0.391	0.814
Residuals	53	39943	754		

Table 7. Estimated Marginal Means of relative germination percentage under the simulated Winter treatment

Botanical variety	Est. Marginal Means (%)	Group
<i>curtipendula</i>	98.6	a
<i>caespitosa</i>	38.6	b

References

Bartholomew, P. W. (2014). Effect of varying temperature regime on phyllochron in four warm-season pasture grasses. *Agricultural Sciences*, 5:1000–1006.

<https://doi.org/10.4236/as.2014.511108>

Biligetü, B., Schellenberg, M. P., & McLeod, J. G. (2011). The effect of temperature and water potential on seed germination of poly-cross side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.) population of Canadian prairie. *Seed Science and Technology*, 39(1), 74–81. <https://doi.org/10.15258/sst.2011.39.1.07>

Cole, D. F., Major, R. L., & Wright, L. N. (1974). Effects of light and temperature on germination of Sideoats grama. *Journal of Range Management*, 27(1), 41.

<https://doi.org/10.2307/3896437>

College of Agriculture and Life Sciences. The Arizona Meteorological Network. Retrieved January 12, 2021. <https://cals.arizona.edu/AZMET/az-data.htm>

de Pisani, M. A. L. T. (2004). *Ecological implications of genetic variation in Bouteloua curtipendula* (Michx.) Torr (Doctoral dissertation, Texas A&M University).

Donohue, K. (2002). Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. 83(4), 11.

Egli, D. B., & Rucker, M. (2012). Seed Vigor and the Uniformity of Emergence of Corn Seedlings. *Crop Science*, 52(6), 2774–2782.

<https://doi.org/10.2135/cropsci2012.01.0064>

- Evans, R. A., Holbo, H. R., Eckert, R. E., & Young, J. A. (1970). Functional environment of downy brome communities in relation to weed control and revegetation. *Weed Science*, 18(1), 154–162. <https://doi.org/10.1017/S0043174500077572>
- Fehmi, J. S., Niu, G.-Y., Scott, R. L., & Mathias, A. (2014). Evaluating the effect of rainfall variability on vegetation establishment in a semidesert grassland. *Environmental Monitoring and Assessment*, 186(1), 395–406. <https://doi.org/10.1007/s10661-013-3384-z>
- Gallagher, M. K., & Wagenius, S. (2016). Seed source impacts germination and early establishment of dominant grasses in prairie restorations. *Journal of Applied Ecology*, 53(1), 251–263. <https://doi.org/10.1111/1365-2664.12564>
- Gould, F. W. (1959). Notes on Apomixis in Side Oats Grama. *Rangeland Ecology & Management/Journal of Range Management Archives*, 12(1), 25-28.
- Gould, F. W., & Kapadia, Z. J. (1964). Biosystematic studies in the *Bouteloua curtipendula* complex II. Taxonomy. *Brittonia*, 16(2), 182. <https://doi.org/10.2307/2805095>
- Grabe, D. F. (1976). Measurement of seed vigor. *Journal of seed Technology*, 1(2), 18-32.
- Halbrook, A. K. (2012). *Bouteloua curtipendula* (Poaceae): reproductive biology, phenotypic plasticity, and the origins of an apomictic species complex. [Doctoral dissertation, University of Arizona]. UA Campus Repository. <https://repository.arizona.edu/handle/10150/243112>
- Hardegee, S. P., Jones, T. A., Pierson, F. B., Clark, P. E., & Flerchinger, G. N. (2008). Dynamic variability in thermal-germination response of squirreltail (*Elymus elymoides* and *Elymus multisetus*). *Environmental and Experimental Botany*, 62(2), 120–128. <https://doi.org/10.1016/j.envexpbot.2007.07.010>

- Hardegree, S. P., Moffet, C. A., Roundy, B. A., Jones, T. A., Novak, S. J., Clark, P. E., Pierson, F. B., & Flerchinger, G. N. (2010). A comparison of cumulative-germination response of cheatgrass (*Bromus tectorum* L.) and five perennial bunchgrass species to simulated field-temperature regimes. *Environmental and Experimental Botany*, 69(3), 320–327. <https://doi.org/10.1016/j.envexpbot.2010.04.012>
- Jordan, G. L., & Haferkamp, M. R. (1989). Temperature responses and calculated heat Units for germination of several range grasses and shrubs. *Journal of Range Management*, 42(1), 41. <https://doi.org/10.2307/3899656>
- Kneebone, W. R., & Cremer, C. L. (1955). The Relationship of Seed Size to Seedling Vigor in Some Native Grass Species 1. *Agronomy Journal*, 47(10), 472-477.
- Koltunow, A. M. (1993). Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. *The plant cell*, 5(10), 1425.
- Nogler, G. A. (1984). Gametophytic apomixis. *Embryology of angiosperms*, 475-518.
- R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved August 20, 2020. <https://www.R-project.org/>
- Roundy, B. A., & Biedenbender, S. H. (1996). Germination of warm-season grasses under constant and dynamic temperatures. 49. <https://doi.org/10.2307/4002924>
- Schmuths, H., Bachmann, K., Weber, W. E., Horres, R., & Hoffmann, M. H. (2006). Effects of preconditioning and temperature during germination of 73 natural accessions of *Arabidopsis thaliana*. *Annals of Botany*, 97(4), 623–634. <https://doi.org/10.1093/aob/mcl012>

Shaidae, G., Dahl, B. E., & Hansen, R. M. (1969). Germination and emergence of different age seeds of six grasses. *Journal of Range Management*, 22(4), 240.

<https://doi.org/10.2307/3895925>

Simanton, J. R., & Jordan, G. L. (1986). Early root and shoot elongation of selected warm-season Perennial grasses. *Journal of Range Management*, 39(1), 63.

<https://doi.org/10.2307/3899689>

Smith, S. E., & Dobrenz, A. K. (1987). Seed Age and Salt Tolerance at Germination in Alfalfa ¹. *Crop Science*, 27(5), 1053–1056.

<https://doi.org/10.2135/cropsci1987.0011183X002700050046x>

Smith, S. E., Riley, E., Tiss, J. L., & Fendenheim, D. M. (2000). Geographical variation in predictive seedling emergence in a perennial desert grass. *Journal of Ecology*, 88(1), 139-149.

Smith, S. E., Haferkamp, M. R., & Voigt, P. W. (2004). Gramas. In *warm-season (C4) grasses* (pp. 975–1002). John Wiley & Sons, Ltd.

<https://doi.org/10.2134/agronmonogr45.c30>

United States Department of Agriculture - Agricultural Research Service. U.S. National Plant Germplasm System. Retrieved January 12, 2021

<https://www.ars-grin.gov/Pages/Collections>

Young, J. A., Eckert, R. E., & Evans, R. A. (1981). Temperature profiles for germination of bluebunch and beardless wheatgrasses. *Journal of Range Management*, 34(2), 84.

<https://doi.org/10.2307/3898117>

Young, J. A., Clements, C. D., & Jones, T. (2003). Germination of seeds of big and bottlebrush squirreltail. *Rangeland Ecology & Management/Journal of Range Management Archives*, 56(3), 277-281. <https://doi.org/10.2307/3897331>

Zettlemoyer, M. A., Prendeville, H. R., & Galloway, L. F. (2017). The Effect of a Latitudinal Temperature Gradient on Germination Patterns. *International Journal of Plant Sciences*, 178(9), 673–679. <https://doi.org/10.1086/694185>