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Improving Post-Wildfire Seeding Success using Germination Modeling and Seed Enhancement Technologies

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Improving Post-Wildfire Seeding Success using Germination Modeling

and Seed Enhancement Technologies

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A thesis submitted to the faculty of
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in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

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and Seed Enhancement Technologies

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Arid and semi-arid rangelands are important ecosystems that are consistently degraded through disturbances such as wildfires. After such disturbances, the invasion and dominance of annual grasses, like cheatgrass (*Bromus tectorum* L.), can lead to an overall loss of ecosystem productivity and an increase in fire frequency. To reduce weed dominance, native and introduced perennials species are typically be seeded in the fall. High mortality is seen from these seeded plant communities due to germinated seed being exposed to freezing, drought, fungal pathogens, and other biotic and abiotic stressors during winter months. We utilized wet-thermal accumulation models to first further validate the theory that germination from seeded plant populations occurs during periods of high environmental stress, and then to establish the practicality of abscisic acid seed coatings as a technology that could circumvent winter germination and mortality. In Chapter 1, we developed an excel workbook called Auto-Germ using Visual Basic for Applications, which allows users to estimate field germination timing based on wet-thermal accumulation models and field data. We then used Auto-Germ to model seed germination timing for 10 different species, across 6 years, and 10 *Artemisia*-steppe sites in the Great Basin of North America. We estimated that for the majority of the species analyzed, a mid to late-winter planting was required on average for the majority of the population to germinate in the spring. This planting time would be logistically difficult for many land managers, due to freezing and/or saturated soil conditions. In Chapter 2, we utilized wet-thermal accumulation models to evaluate the use of abscisic acid (ABA) to delay germination of *Pseudoroegneria spicata* (Pursh) Á. Löve (perennial native bunchgrass) across 4 years and 6 *Artemisia*-steppe sites. Germination models estimated that ABA seed treatments typically would delay germination of fall sown seed to late winter or early spring when conditions may be more favorable for plant establishment. Based on these results, we recommend both the use of wet-thermal accumulation models as a tool in educating researchers and land managers in knowing when seeding practices should occur, and the further study of ABA seed coatings as a technology that may improve plant establishment of fall sown seeds.

Keywords: germination rate, restoration, seeding, thermal time, wet-thermal accumulation model, seed enhancement technology, abscisic acid

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TABLE OF CONTENTS

CHAPTER 1

Use of Auto-Germ to model germination timing in the Sagebrush-steppe

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ABSTRACT

Germination timing has a strong influence on direct seeding efforts, and therefore is a

closely tracked demographic stage in a wide variety of wildland and agricultural settings.

Predictive seed germination models, based on soil moisture and temperature data in the seed

zone are an efficient method of estimating germination timing. We utilized Visual Basic for

Applications (VBA) to create Auto-Germ, which is an Excel workbook that allows a user to

estimate field germination timing based on wet-thermal accumulation models and field

temperature and soil moisture data. To demonstrate the capabilities of Auto-Germ, we calculated

various germination indices and modeled germination timing for 11 different species, across 6

years, and 10 *Artemisia*-steppe sites in the Great Basin of North America to identify the planting

date required for 50% or more of the simulated population to germinate in spring (1 March or

later), which is when conditions are predicted to be more conducive for plant establishment. Both

between and within the species, germination models indicated that there was high temporal and

spatial variability in the planting date required for spring germination to occur. However, some

general trends were identified, with species falling roughly into three categories, where seeds

could be planted on average in either fall (*Artemisia tridentata* ssp. *wyomingensis* and *Leymus*

cinereus), early winter (*Festuca idahoensis, Poa secunda, Elymus lanceolatus, Elymus*

elymoides, and *Linum lewisii*), or mid-winter (*Achillea millefolium, Elymus wawawaiensis* , and

Pseudoroegneria spicata) and still not run the risk of germination during winter. These predictions made through Auto-Germ demonstrate that fall may not be an optimal time period for sowing seeds for most non-dormant species if the desired goal is to have seeds germinate in spring.

INTRODUCTION

Seed germination timing strongly impacts the success of direct seeding efforts in wildland systems by influencing exposure to pathogens, nutrients and soil moisture, temperature, light, herbivory, and other biotic and abiotic factors (Gornish *et al.* 2015; James & Carrick 2016). For these reasons, several studies have tracked germination timing in the field to better understand and improve seeding outcomes (Gerrit 1991; Abbott & Roundy 2003; James, Rinella & Svejcar 2012; Boyd & James 2013). However, tracking seed germination timing in the field can be challenging, resource intensive, and time-consuming. Additionally, knowledge gained from short-term field germination studies is often lacking due to high annual variability in weather conditions at the time of the experiment (Hardegree *et al.* 2016a). Subsequently, to gain general inferences from germination studies, labor intensive studies need to be repeated for multiple years.

Researchers have turned to predictive germination models for a more efficient method of estimating germination timing (Hardegree & Van Vactor 1999; Bradford 2002a; Allen *et al.* 2007; Hardegree *et al.* 2017). In recent years, models have been developed that assume there are naturally occurring processes within the seeds themselves already in place to regulate germination timing (Finch-Savage & Leubner-Metzger 2006a). It has been shown that the majority of these processes are a function of temperature and moisture (Bradford 1990; Allen, Debaene-Gill & Meyer 1992; Hardegree *et al.* 1999; Hardegree *et al.* 2008).

Progress towards germination can be predicted through a wet-thermal accumulation model where soil moisture must exceed a base water potential (Ψ_b) for germination to occur. (Finch-Savage, Steckel & Phelps 1998; Roundy *et al.* 2007; Rawlins 2009; Rawlins *et al.* 2012) The base water potential used is derived through laboratory experimentation (Roundy *et al.* 2007). Though there are many factors that influence the rate of seed germination and number of germinable seeds, adjusting Ψ_b is expected to correct for impacts from environmental conditions, after-ripening and seasonal changes in dormancy cycling (Bradford 2002b). Subsequently, once Ψ_b is determined, seed germination timing and number of germinable seeds may be accurately predicted from soil temperature. Field trials have validated wet-thermal accumulation models (Rawlins *et al.* 2012a; Rawlins *et al.* 2012b), and confirmed their utility in predicting seed germination in a number of settings, with a wide variety of species (Hardegree *et al.* 2016b; Cline, Roundy & Christensen 2017a; Cline, Roundy & Christensen 2017b). Despite the simplicity of wet-thermal accumulation models, a relatively large amount of data and processing is required to develop the models and estimate seed germination timing in the field.

To overcome the logistical challenges associated with predicting seed germination timing, we created a programmed workbook called "Auto-Germ" that allows users to efficiently process seed germination data and predict seed germination timing in the field. Our workbook utilizes Visual Basic for Applications (VBA) in Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) to create wet-thermal accumulation models as well as calculate various other germination indices from laboratory constant temperature trials. Auto-Germ also provides users with an interface to apply the wet-thermal accumulation models to estimate germination timing in the field from historic soil moisture and temperature data sets.

Auto-Germ's predictive germination modeling capabilities has the potential to educate practitioners in knowing how their planting dates may influence germination timing and

subsequently the growing conditions that impact seedling establishment. The *Artemisia spp.* (sagebrush)-steppe ecosystem in the Great Basin region of the western United States is an example of an imperiled ecosystem that would benefit from improved restoration practices (Suring, Rowland & Wisdom 2005; Hardegree *et al.* 2016a). In this region, seeding is used to reclaim degraded sites that have been impacted by wildlfires, invasive species, and various human disturbances (Noss 1995; Knick *et al.* 2011; Davies *et al.* 2014). In the *Artemisia*-steppe, seeding typically occurs in autumn, with the expectation that seeds will remain dormant in the soil and then germinate in the spring (Richards, Chambers & Ross 1998; Crawford *et al.* 2004; Madsen *et al.* 2016). However, planting too early in the year can result in seeds germinating prior to winter and then experiencing high mortality over the winter period (James & Svejcar 2010). Winter mortality may occur as a result of freezing conditions (James, Svejcar & Rinella 2011; Boyd & Lemos 2013). Roundy and Madsen (2016) determined that across 14 sagebrush steppe sites there was an average of 58 freeze-thaw periods for the upper 1-3 cm of soil between October and March. Seedbed freezing conditions have been shown to alter the physiological responses of big sagebrush (*Artemisia tridentata* Nutt. (Asteraceae)) in the Great Basin (Loik & Redar 2003), and has the potential to further inhibit plant survival of perennial grasses such as bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Love) (Boyd & Lemos 2013). Mortality may also occur to seedlings over the winter period as a result of drought, pathogens, and expenditure of seed carbohydrate resources (James et al., 2011; Madsen et al., 2016). Subsequently, in this region understanding the seeding date required to prevent premature germination and subsequent winter mortality is paramount to improve the effectiveness of restoration projects.

Our objectives were to provide instructions on how to use Auto-Germ and demonstrate the utility of the program through a case study that 1) calculated various germination indices

under different constant temperatures on 10 different species commonly used for restoration projects in the Great Basin, and 2) for these same species modeled seed germination timing across 6 years and 10 *Artemisia*-steppe sites to estimate the planting date required for 50% or more of the simulated population of seeds to germinate in spring (March 1st or later) when conditions are predicted to be more conducive for plant establishment.

<div align="center">METHODS AND MATERIALS</div>

Instructions for Operating Auto-Germ

There are four main steps for processing data in Auto-Germ, which include: 1) entering laboratory data, 2) wet-thermal model creation, 3) entering field data, and 4) model application. Each step is initiated by clicking a button in Auto-Germ on the Home worksheet (note macros and content must be enabled to use Auto-Germ). Auto-Germ provides instructions on the Home worksheet for each step (Fig. 3-1-APPENDIX 1).

Step 1 – Germination Count Data Input

The first step is to input germination counting data from constant-temperature laboratory trials into the Data Entry worksheet (Figure 3-2-APPENDIX 1), which is accessed by clicking the Data Entry button. To input new data, click the Start Over button on the Data Entry worksheet. In Auto-Germ, the data organization must match the sheet setup, where column A is temperature in Celsius, column B is replicate (or block), column C is plot ID, column D is treatment, column E is the number of seeds planted per sample, and everything from column F to the right is measurement dates and their respective germination counts. The planting date is entered into cell B8. The workbook processes up to 100 germination date entries and 1,000 samples. Under each measurement date, enter the number of seeds that germinated between the

<div align="center">5</div>

last counting time and the current one. Do not enter cumulative germination counting data on this sheet. Entries in the in the columns labeled as rep/block and plot ID are optional. If the user does not want to produce wet-thermal accumulation models, germination metrics will be calculated through Auto-Germ without temperature data. Auto-Germ will not operate if empty cells are included under the columns labeled as temperature, treatment, seeds planted, planting date and the germination measurement columns. The treatment column can be used to signify a number of different variables. For example, if seed treatments are being analyzed the type of seed treatment would be placed in this column. If species were being compared the treatment column would contain the name of the species.

Step 2 – Wet-thermal Model Creation

Once the data is entered, return to the Home worksheet and click the Make a Model button, and enter in the pop up-window the lower and upper germination percentage and interval size to model. The workbook can model any range of germination percentages from 1 - 99%. The four new worksheets created are called Germination Metrics, Data Averages, Standard Error, and Polynomial Equations. Once the calculations are completed, a pop up window notifies that the data is ready to be viewed. Click the View Data button under the Workbook Options heading to view the worksheets in a new workbook that can be saved, or click the worksheet tabs on the bottom of the screen. The Germination Metrics sheet displays the whole data set sorted by treatment, temperature, and calculated germination metrics. The calculated metrics for each sample include the number of seeds that germinated, final germination percentage, mean germination time, coefficient of variation of the germination time, mean germination rate, uncertainty of germination, synchrony of germination, and time to reach each percent germination (Ranal *et al.* 2009).

Mean germination time is calculated as:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where:

$\bar{t}$ = mean germination time

t_i = time from the start of the experiment to the ith observation

n_i = number of seeds germinated in the ith time

k = last time of germination

The coefficient of variation is calculated as follows:

$$CV_t = \frac{s_t}{\bar{t}} * 100$$

where:

CV_t = coefficient of variation of the germination time s_t = standard deviation of the germination time

$\bar{t}$ = mean germination time

The mean germination rate is calculated by taking the inverse of the mean germination time.

The uncertainty of germination is calculated as:

$$U = -\sum_{i=1}^{k} f_i * log_2 f_i$$

where:

U = Uncertainty of the germination process

$$f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

n_i = number of seeds germinated on the ith time

k = last time of observation

The synchrony of germination was calculated as follows:

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\sum n_i,2}}$$

where:

Z = synchrony of germination

$$C_{n_i,2} = \frac{n_i(n_i - 1)}{2}$$

$C_{n_i,2}$ = combination of the seeds germinated in the ith time, two by two

n_i = number of seeds germinated on the ith time

The time to reach each percent germination was calculated as follows:

$$T_N = \left[\left(\frac{t_a - t_b}{n_a - n_b} \right) (N - n_b) \right] + t_b$$

where:

T_N = time (days) to subpopulation germination

t_a = incubation day when subpopulation germination was reached

t_b = incubation day before subpopulation germination was reached

n_a = number of germinated seeds on day that subpopulation germination was reached

n_b = number of germinated seeds on day before subpopulation germination was reached

N = number of germinated seeds equal to the percentage of the total subpopulation of interest

The Data Averages worksheet displays the same metrics for the average of each treatment and temperature combination. The Standard Error worksheet displays the standard error for each calculation on the Data Averages worksheet. The Polynomial Equations worksheet contains second-order polynomial equations with their associated coefficient values (A, B and C), the R^2 value for each germination percentage of each treatment, and the corresponding graphs depicting germination rate as a function of temperature (Figure 3-3-APPENDIX 1). To create new polynomial equations for different times to set germination percentages using the same data set, the newly created sheets either need to be exported or deleted.

Step 3 – Field Data Input

To estimate seed germination timing in the field from your polynomial equations, the user needs to create worksheets containing their field soil temperature and water potential data. Click the See Sample Data button on the Home worksheet to see how field data worksheets should be formatted. Create separate worksheets for separate sites and planting years. The format of the data must match the example data in the worksheet, where column A is the measurement date and time, column B is temperature, and column C is water potential. The user must input their own field data worksheets to apply the model. The field data worksheets must be located in-between the Home and Data Entry worksheets. If there are any other worksheets besides field data in this location, the program will not operate correctly.

Step 4 – Field Germination Predictions

At this point, two options are available for the user to choose from. The first option is to predict the time to reach the previously specified germination percentages based on a planting

date. The second option is to predict the dates a certain germination percentage is reached based on a range of planting dates. Before clicking either button, make sure that steps 1 - 3 are complete and that the Polynomial Equations worksheet is located in the workbook somewhere after the Data Entry worksheet. If Polynomial Equations are missing or has a changed name, Auto-Germ will not operate.

To predict the times to reach the previously specified germination percentages, click the Choose Planting Date button on the Home worksheet. Enter the planting date to model for in the pop-up window. The minimum water potential threshold can be changed from the default value of -1.5 MPa, based on the species being evaluated. The new worksheet created is named Planting Date (Figure 3-4-APPENDIX 1). The tables on the left of Planting Date show the predicted dates when the corresponding germination percentages will occur for each treatment according to each individual field data sheet. The graphs of the tables are located on the right.

To predict the dates a certain germination percentage is reached, click the Choose Germination Percentage button on the Home worksheet. Enter the percent germination and the range of planting dates to model in the pop up window. The minimum water potential threshold can also be changed from the default value of -1.5 MPa. The new sheet is named % Germination (Figure 3-5-APPENDIX 1). The tables on the left of % Germination show the predicted time to reach the specified percent germination, given the specified range of planting dates. Each table corresponds to a field data sheet. The graphs of the tables are located on the right.

Workbook Options

Workbook Options is the last heading on the Home sheet. The View Data button will create a new workbook that contains all of the data generated from steps 2 and 4, but will not remove any new worksheets. The new workbook containing generated data may be saved. The

Export Data button will export the data that was generated in steps 2 and 4 to another workbook that can be saved, and data will be removed from Auto-Germ. The Start Over button will completely reset Auto-Germ and delete all the data generated, but will not affect worksheets located before Data Entry.

Case Study

<u>Laboratory Methods</u>

We developed wet-thermal-time models for 10 seedlots of species commonly used in restoration projects in the Great Basin. We included eight perennial grasses; bluebunch wheatgrass*,* Great Basin wildrye (*Leymus cinereus* (Scribn. & Merr.) Á. Löve)*,* Idaho fescue (*Festuca idahoensis* Elmer ssp. *idahoensis*)*,* Sandberg bluegrass (*Poa secunda* J. Presl), Snake River wheatgrass (*Elymus wawawaiensis* J. Carlson & Barkworth), thickspike wheatgrass (*Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould), and bottlebrush squirreltail (*Elymus elymoides* (Raf.) Swezey), two forb species; Lewis flax (*Linum lewisii* Pursh) and western yarrow (*Achillea millefolium* L. var. *occidentalis* DC.)*,* and one shrub species; Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young). Seed was purchased from certified lots at Granite Seed (Lehi, UT, USA). A range of constant temperatures was used to germinate the seeds (5, 10, 15, 20, and 25 °C). The study was setup using a randomized block split-plot design, with temperature comprising the split plot. Seven repetitions were used for each species, at every temperature. In each repetition, 25 seeds were placed in a 9 cm diameter petri dish that contained a single layer of blotter paper. Five ml of water was initially added to each petri and additional water was added as petri dishes dried throughout the study. Petri-dishes were closed in plastic bags by block to prevent the loss of water. Germinated seeds were counted every 1-3 days, for 60 days. Seeds that had germinated were counted, recorded, and removed

from the petri dishes. Germination count data was then processed in Auto-Germ to develop wet-thermal accumulation models for each species.

Auto-Germ was used to calculate final germination percentage, T_{50}, synchrony, and mean germination time. A mixed model analysis was used to first determine the significance ($p<0.05$) of species, incubation temperature, and their interactions (unless determined to not be significant) for the four indices. In the model, blocks were considered random, while incubation temperature and species were both considered fixed. We tested for differences in responses to species at the incubation temperatures of 5, 10, 15, 20, and 25 °C using a Tukey pairwise comparison test ($P<0.05$). Final germination was squared and the log of T_{50}, synchrony, and mean germination time was taken to normalize the data.

Field Germination Predictions

Wet-thermal accumulation models for each species was applied to historical soil temperature and water potential data from the Sagebrush Step Treatment and Evaluation Project (SageSTEP) (Cline 2014) to determine how planting date influenced germination timing. We selected from the SageSTEP network ten different sites to model seed germination timing that were within *Artemisia*-steppe and *Pinus* spp.- *Juniperus* spp.(pinyon-juniper) woodland communities that had been treated with prescribed burns (Moses Coulee, WA, Saddle Mountain, WA, Bridge Creek, OR, Hart Mountain, OR, Marking Corral, NV, Owyhee, NV, Blue Mountain, CA, Greenville Bench, UT, Onaqui, UT, and Stansbury, UT) (McIver & Brunson 2014). At each of these sites, hourly measurements were made at approximately 1-3 cm below the soil surface to estimate soil temperature using thermocouples and soil water potential using gypsum blocks (Delmhorst Inc., Towaco, NJ, USA).

At each of the field sites we evaluated seed germination timing for each of the 10 seedlots using the second option in Step 4 on the Home worksheet, which predicts the dates a certain germination percentage is reached based on a range of planting dates. Simulations were ran on 6 different years with daily planting dates between September 1st and March 1st. For each simulated planting date, we analyzed for the date a simulated population of seed would reach 50% germination. A base water potential threshold of -1.5 MPa was used in the simulations based off of research by (Rawlins *et al.* 2012; Rawlins *et al.* 2012b).

We used the planting date required for 50% or more of the simulated population of seeds to germination in spring (i.e. 1 March or later) as the metric to compare between species. This metric was chosen because it is estimated to be the planting date required for land managers to circumvent the limiting biotic and abiotic factors causing mortality to seedlings during the winter. We used mixed model analysis to first determine the significance ($\alpha = 0.05$) of species, site, and year for germination date (all fixed variables). We then tested for differences in responses to species, site, and year using a Tukey pairwise comparison test ($P = 0.05$).

RESULTS

<u>Germination Indices</u>

Mixed model analysis showed that incubation temperature, species, and the interaction between these two factors affected final germination percentage (F = 10.5, $P < 0.001$; F = 23.6, $P < 0.001$; F = 2.9, $P < 0.001$), synchrony (F = 49.0, $P < 0.001$; F = 52.6, $P < 0.001$; F = 5.9, $P < 0.001$), T$_{50}$ (F = 1240.9, $P < 0.001$; F = 143.4, $P < 0.001$; F = 25.6, $P < 0.001$), and mean germination time (F = 726.8, $P < 0.001$; F = 116.1, $P < 0.001$; F = 18.8, $P < 0.001$), respectively. As would be expected for cool season species in the Great Basin, germination was highest in

general around 15 °C and typically declined under the lowest (5°C) and highest (25°C) temperatures. The degree that germination percentage changed by temperature was variable for each species, with some species showing a limited change in germination with temperature (thickspike wheatgrass, bluebunch wheatgrass, Idaho fescue, and Sandberg bluegrass), while other species were more variable (western yarrow, Snakeriver wheatgrass, Lewis flax, bottlebrush squirreltail, Great Basin wildrye, and Wyoming big sagebrush; Figure 1-1). Subsequently, it was at the highest and lowest temperatures tested were there was the greatest range in germination between species. For example, at 25 °C, thickspike wheatgrass had the highest final germination percentage (96%) and Lewis flax had the lowest (34%). At 5 °C, Idaho fescue had the highest final germination percentage (90%) while bottlebrush squirreltail had the lowest (57% ; Figure 1-1).

Synchrony values fluctuated greatly between temperatures for all species (Figure 1-1). There were five species that had synchrony values above 0.40 (thickspike wheatgrass, bluebunch wheatgrass, western yarrow, bottlebrush squirreltail, and Sandberg bluegrass). Both Great Basin wildrye and Wyoming big sagebrush had consistently the lowest values of synchrony (0.08 – 0.18; Figure 1-1).

Both T_{50} and mean germination time followed similar patterns, where all species had the highest values at 5 °C, and then decreased until 20 and 25 °C when many species had slight increases in the two variables (Figure 1-2). The greatest difference between consecutive temperatures for both T_{50} and mean germination time occurred with Wyoming big sagebrush between 5 and 10 °C (32 and 31 days). Out of all the species, Wyoming big sagebrush had the highest T_{50} and mean germination time at 5 °C (41 and 48 d, respectively), but then these values quickly decreased as temperature increased; by 25 °C, this species produced one of the fastest germinating times (2 and 4 d, respectively). Great Basin wildrye had the second highest T_{50} and

mean germination times at 5 $^\circ$C (22 and 25 days), but relative to the other species it maintained high values as temperature increased. Western yarrow was typically the fastest germinating species as shown by T_{50} and mean germination time values. However, at 10 $^\circ$C western yarrow mean germination time was lower for bluebunch wheatgrass by 7 days and at 25 $^\circ$C, T_{50} was lower for Wyoming big sagebrush by 2 days (Figure 1-2).

Field Predictions

Wet thermal accumulation models appeared to have sufficient accuracy to predict germination time (adjusted R^2 = 0.71– 0.98). Species (F = 23.2, $P < 0.001$), site (F = 146.4, $P < 0.001$), and year (F = 79.3, $P < 0.001$) affected the planting date required to have 50% or more of the simulated population of the seeds germinate after 1 March. The site that produced the earliest average planting date across all species was Marking Corral (28 October), while the site that produced the latest average planting date across all species was Bridge Creek (7 February; Figure 1-3). Seven of the sites had average planting dates in mid-fall to early-winter (September to November), while the other three sites had average planting dates much later in the season (January to February; Figure 1-3). All years had similar ranges, with 2011-2012 having the earliest average planting date (27 October), and 2014-2015 having the latest (6 January; Figure 1-4).

Analysis by individual species showed each species had average planting dates as early as September, and as late as February to have 50% or more of the simulated population of the seeds germinate after 1 March (Figure 1-5). While there was extreme variability across all species in the date required for the majority of seed to germinate by spring or later, certain species consistently required later planting dates than others. Western yarrow had the latest

average planting date (24 December), with the interquartile range of the data falling between 15 November and 16 February. The only other two species that had average planting dates in December were Snakeriver wheatgrass (5 December) and bluebunch wheatgrass (4 December). These species, while having later average planting dates than all other species besides western yarrow, had some of the largest interquartile ranges (19 October-9 February and 20 October-7 February respectively). Thickspike wheatgrass (28 November), Idaho fescue (21 November), Lewis flax (19 November), Sandberg bluegrass (18 November), and bottlebrush squirreltail (14 November) all had average planting dates in November. Great Basin wildrye (29 October) and Wyoming big sagebrush (25 October) had the earliest average planting dates, with interquartile ranges that began in mid-September (14 September, 15 September), and ended as early as late November – early December (23 November, 6 December; Figure 1-5).

DISCUSSION

Our programmed Excel workbook enables researchers to quickly process large data sets to estimate field germination timing using a wet-thermal accumulation model in combination with field data. Auto-Germ is applicable to most germination data sets from non-dormant seed. Our program may provide both land managers and researchers with a better understanding of how seeds interact with unique soil temperature and moisture regimes. The germination indices calculated in the case study further demonstrated the importance of understanding this interaction. High variability was seen across all indices and temperatures, showing that individual species react uniquely to differences in soil temperature and moisture. Both with final germination percentage and synchrony, temperature greatly affected the values, demonstrating how different species may be better acclimated to particular environments. Similarly with T_{50} and mean germination time, while a distinct relationship could be seen between the indices and

temperature across all species, certain species consistently maintained faster germination times than others.

In the case study, the use of Auto-Germ allowed for germination predictions to be made for 11 species across 10 sites and 6 years. This breadth of study, allows for researchers to understand the germination patterns of species across large temporal and spatial spectrums without the investment of man power and resources required for multi-year field studies. Being able to quickly understand how species react to field temperature regimes can be extremely useful for areas such as the Great Basin, where there are high volumes of disturbed sites that require restoration.

Wet-thermal accumulation models, while more simplistic than other hydrothermal models, have been shown to be useful tools in predicting seed germination. Hardegree et al. (2017) demonstrated that even though wet-thermal models can overestimate germination rates (more so than other hydrothermal models) when soil water potential is between 0 Mpa and the water potential threshold (for this study, -1.5 Mpa), these errors are minimized by the majority of field germination occurring between 0 and -0.2 Mpa. This smaller range diminishes the magnitude of overestimation, since a relatively small portion of time would be spent at water potentials where the models would have the highest degree of potential error (Hardegree *et al.* [IN REVIEW]). Rawlins et al. (2012) further validated wet-thermal models by accurately predicting whether or not germination of six seeded species would occur by mid-spring. Both authors noted that while wet-thermal models have the potential to be extremely useful in estimating germination timing, more research needs to be conducted to understand their limitations. For this reason, it should be noted that predictions developed from Auto-Germ should be used as rough assessments to help guide further research and management.

In this study, the predictions made demonstrated the necessity of tailoring restoration practices for individual species commonly used throughout the Great Basin. To have the majority of germination occur after 1 March, all species involved in the study required planting dates that ranged from September to February. This variability was due largely to the number of locations used in the study, many of which had different soil temperature and moisture regimes. This similarity in range throughout the entire area of study is not representative of what occurred at individual sites. Each species reacted differently to the soil microclimatic data that was used for modeling at each site. For example, at the Hart Mountain, OR, site, species that exhibited lower T_{50} and mean germination time values, such as western yarrow, Snakeriver wheatgrass, and bluebunch wheatgrass, on average all required planting dates by mid-November – early December for the majority of the simulated population to germinate after 1 March. Conversely, species with higher T_{50} and mean germination time values, such as Great Basin wildrye and Wyoming big sagebrush, could be planted much earlier in the season (late September), and typically not have the majority of the seeds germinate over the winter. This demonstrates two key points, firstly that restoration plans developed for a species at one site or year do not translate to sites and years with different soil temperature and moisture regimes. The optimal planting date (the date required for the majority of germination to occur after 1 March) for a species such as bluebunch wheatgrass varies greatly between sites like Bridge Creek, OR and Marking Corral, NV where the climates are different. The same principle can be applied to variability seen on a year to year basis. The annual environmental changes at individual sites create vastly different results for planting dates. The second key point is that at any given site, understanding the germination characteristics of individual species can greatly increase the success rates of restoration projects. Planting Wyoming big sagebrush in mid-October may be late enough in the season to circumvent winter germination at multiple sites in the area of study,

18

however; for a species such as bluebunch wheatgrass, which germinates more quickly, a planting date in mid-December might be more suitable.

Our findings validates winter mortality as a major contributor to the lack of spring emergence seen in restoration efforts. Not including Wyoming big sagebrush and Great Basin wildrye, 50% or more of the required planting dates for spring germination occurred by November or later. This means that land managers who seed areas in mid to late fall would run the risk of having germination occur outside of more favorable spring conditions. Premature germination could potentially be mitigated by planting later in the season, however this study shows that seeding would need to take place in early to late winter. Winter seeding can be logistically challenging due to freezing and/or saturated soil conditions impacting the delivery of seed from mechanical equipment. Alternatively, seed dormancy may prevent seed germination until conditions are favorable for establishment and growth (Baskin & Baskin 2001; Finch-Savage & Leubner-Metzger 2006b; Allen *et al.* 2007). Seed dormancy can be induced through the addition of the plant hormone abscisic acid (ABA), which could be used in the coating of rangeland seeds to delay germination of fall- sown seeds until spring (see chapter 2 of thesis).

Additionally, our study demonstrates the benefits that seed mixes can have in restoration efforts. Rinella and James (2017) demonstrated that seed mixes of both bluebunch wheatgrass and Sandberg bluegrass led to better establishment than individually seeded species. This study shows that at any given site, the annual differences in temperature and moisture can lead to vastly different outcomes in germination timing for individual species. As shown from the germination indices calculated in this study, the species used reacted in unique ways to different temperatures, both in the timing and spread of germination. This demonstrates how individual species may be better suited for different temperature regimes and environments. Using multiple

species could increase the probability of having seeds germinate and establish during periods of more favorable conditions.

CONCLUSION

Our study is one example of how predictive modeling has the potential to help researchers and land managers better understand when seeding practices should occur to optimize planting dates so seeds are more likely to germinate during conditions favorable for plant establishment. Germination timing between species is extremely variable, and so having a tool that can quickly and effectively predict when seeds would germinate in the field would be beneficial. Auto-Germ addressed this need and helped us gain a better understanding of the individual planting requirements of several species common to the Great Basin.

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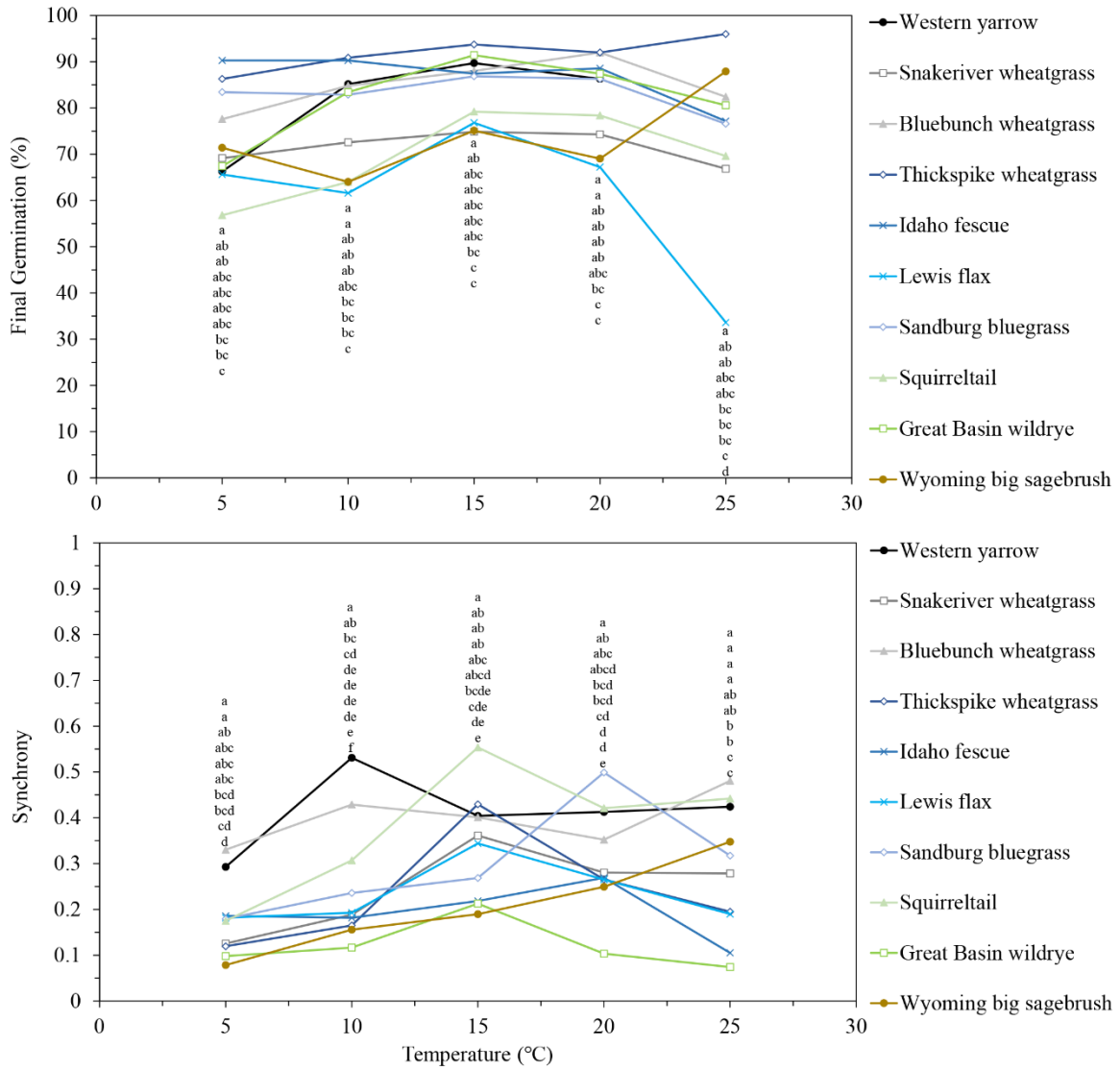*Concern*. Alliance Communications Group, Lawrence, Kansas.

Figure 1-1. Final germination percentage and synchrony at temperatures ranging from 5-25 °C for 10 different species commonly seeded in the Great Basin, USA. Values with the same incubation temperature with different letters are significantly different (*P* < 0.05) at that temperature. Letters correspond with the order of the data points in the figure.
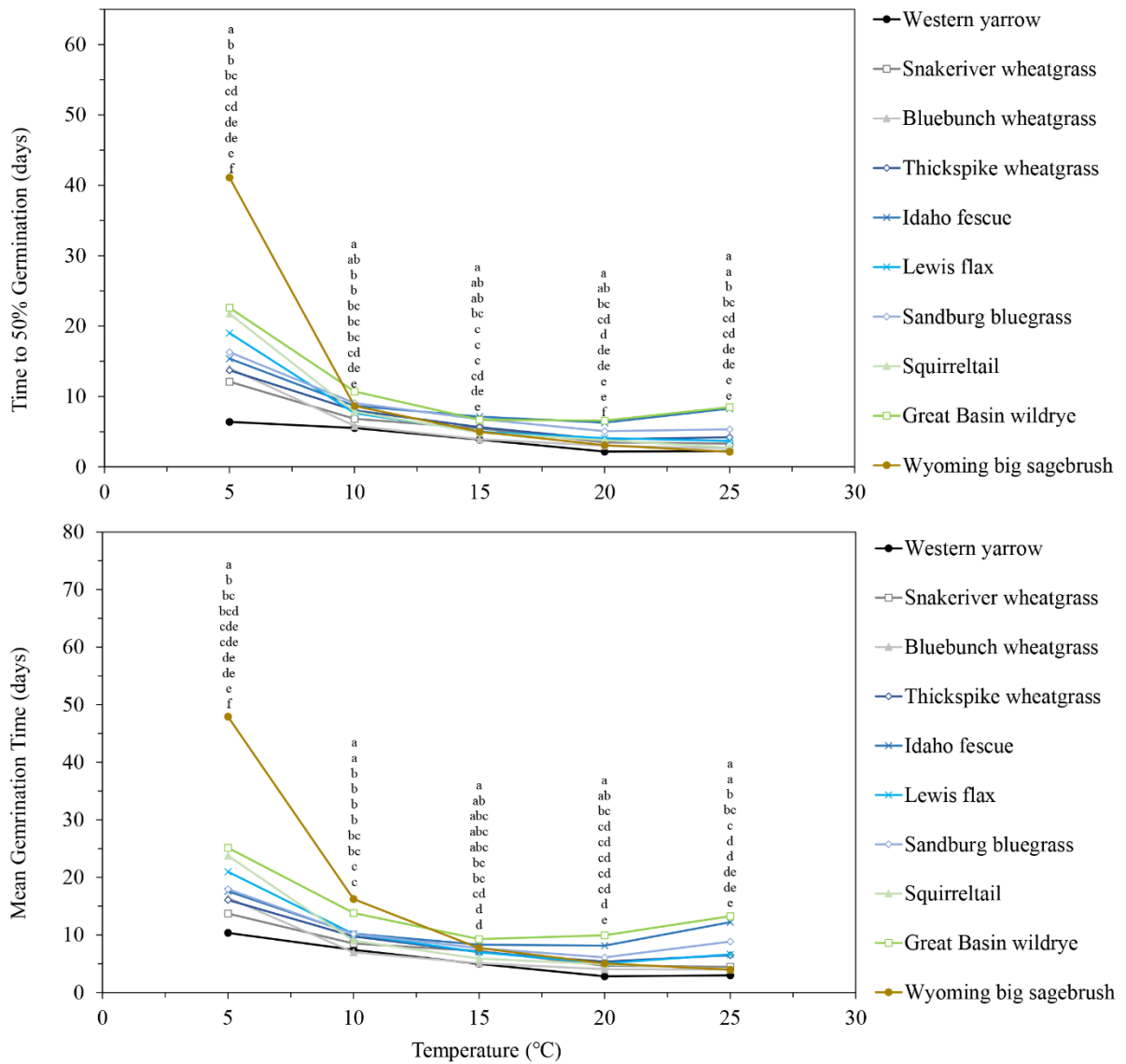
Figure 1-2. Time to 50% germination and mean germination time at temperatures ranging from 5-25 °C. Values with the same incubation temperature with different letters are significantly different ($P < 0.05$) at that temperature. The letters correspond with the data points from top to bottom. Letters correspond with the order of the data points in the figure.

Figure 1-3. Planting date by site required for 50% or more of the simulated population to germinate in March or later. Box limits represent the first and third quartiles, the black line within the box indicates the median, the blue line indicates the mean, the whiskers limits represent the 10th and 90th percentiles, and the individual dots represent outliers. Plots with different corresponding letters are statistically different ($p < 0.05$).
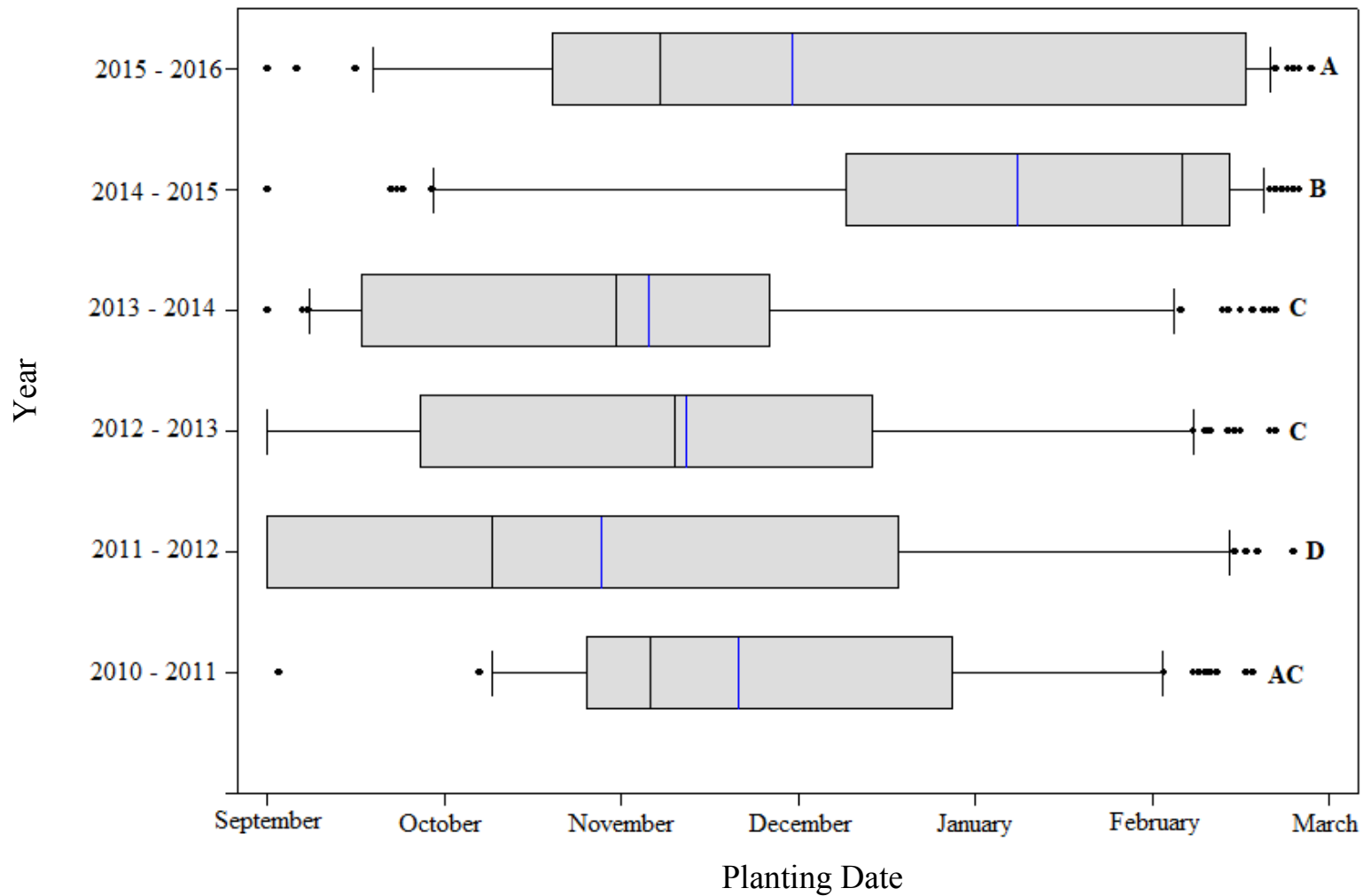
Figure 1-4. Planting date by year required for 50% or more of the simulated population to germinate in March or later. Box limits represent the first and third quartiles, the black line within the box indicates the median, the blue line indicates the mean, the whiskers limits represent the 10th and 90th percentiles, and the individual dots represent outliers. Plots with different corresponding letters are statistically different ($p < 0.05$).
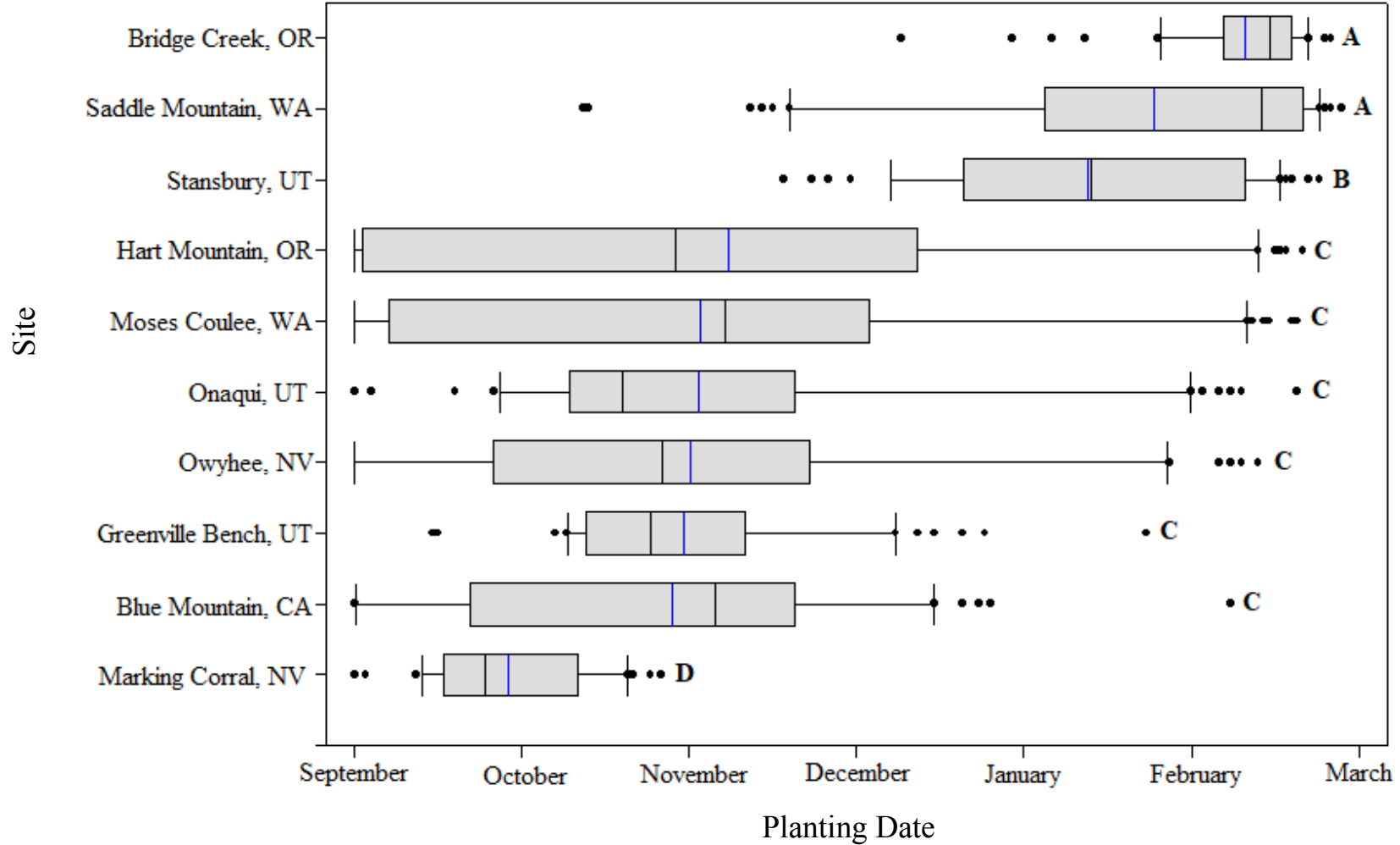
Figure 1-5 . Planting date by species required for 50% or more of the simulated population to germinate in March or later. Box limits represent the first and third quartiles, the black line within the box indicates the median, the blue line indicates the mean, the whiskers limits represent the 10th and 90th percentiles, and the individual dots represent outliers. Plots with different corresponding letters are statistically different ($p < 0.05$).
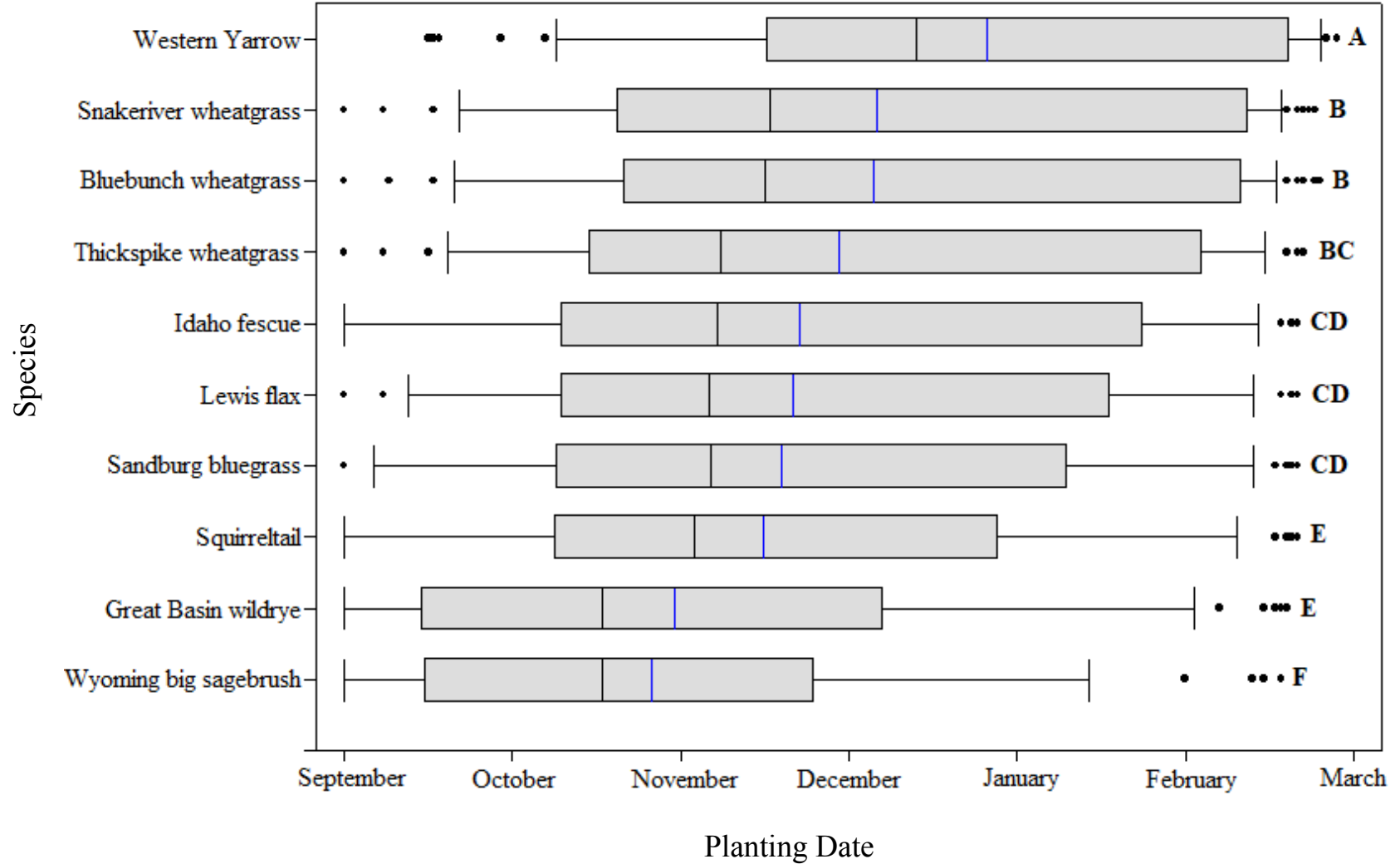
CHAPTER 2

Influence of Abscisic Acid (ABA) Seed Coating on Seed Germination Rate
and Timing of Bluebunch Wheatgrass

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ABSTRACT

Semi-arid rangeland degradation is a reoccurring issue that is seen throughout the world. In the Great Basin of North America, seeds sown in the fall to restore degraded sagebrush (*Artemisia* spp.) steppe plant communities may experience high mortality in winter due to germinated seed being exposed to freezing, drought, fungal pathogens, and other biotic and abiotic stressors. Delaying germination until early spring when conditions are more suitable for plant growth may increase seedling survival. We evaluated the use of abscisic acid (ABA) to delay germination of *Pseudoroegneria spicata* (Pursh) Á. Löve (perennial native grass), by investigating potential delays in germination of uncoated seeds, and seeds coated with 25% ABA at 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, and 6.0 g/100 g^1 of seed. We conducted seed trials to assess the influence of temperature and ABA levels on germination, varying from 5-25 °C at 5 °C intervals and developed quadratic thermal accumulation regression models for each treatment. Further, we applied the models to four-years of historic soil moisture and temperature data across six sagebrush steppe sites to predict seed germination timing. Germination percentage remained similar across all temperatures except at 25 °C, where the treatments 2 and 6 g BioNik 100 g^{-1} seed had lower values (<70% germination). All ABA concentrations delayed germination, with the greatest delays at 5-10 °C. For example, the time required for 50% of the seeds to germinate

at 5 °C was increased by 16 - 46 d, depending on the amount of ABA applied. The synchrony of germination decreased with increasing ABA application rates. Seed germination models indicated that the majority of untreated seed germinated 5-11 weeks after an October 15th simulated planting date. In contrast, seeds treated with ABA delayed germination to late winter or early spring. Our research indicates that *P. spicata* may germinate directly after seeding, thus exposing the seedlings to the harsh environmental conditions of winter. Seed germination models predict ABA seed coatings may delay germination of *P. spicata* during years where the seed would otherwise germinate during the winter months, until conditions are more suitable for plant survival and growth.

INTRODUCTION

Disturbance and inter-annual precipitation variability cause arid and semi-arid rangelands across the world to be extremely susceptible to a decline in environmental health. (GLP 2005; Reynolds *et al.* 2007; Zhao *et al.* 2017). The sagebrush (*Artemisia* spp.)-steppe ecosystem in the Great Basin region of North America is an example of a degraded rangeland, which currently exists on only 56% of its historic range (Suring, Rowland & Wisdom 2005; Hardegree *et al.* 2016). A prominent stressor to sagebrush communities is through the invasion and dominance of annual grasses, which increase fire frequency and perpetuates weed dominance (Dantonio & Vitousek 1992; Baker 2006; Balch *et al.* 2013). Cheatgreass (*Bromus tectorum* L.) is a dominant invasive annual weed that increases wildfire frequency by producing a dry, fine, continuous fuel layer early in the summer season (Dantonio & Vitousek 1992; Bradley *et al.* 2006; Germino, Chambers & Brown 2016). The dominance *of* cheatgrass also decreases organic carbon stored in the soil (Rau *et al.* 2011), and reduces the habitat of wildlife species such as sage-grouse (*Centrocercus urophasianus)*, that depend on the sagebrush steppe system (Knick *et al.* 2003).

33

To reduce weed dominance and stabilize soils, native and introduced perennial species are typically seeded in fall after wildfires (Richards, Chambers & Ross 1998; Crawford *et al.* 2004; Madsen *et al.* 2016). However, it has been suggested failure rates within many revegetation projects could be as high as 90% (Hardegree *et al.* 2010).

Plant recruitment may be limited by ecological processes, such as freezing and thawing of the seedbed, development of physical soil crusts, and pathogen attack, during the first winter the seeds are sown (James, Svejcar & Rinella 2011; Hardegree *et al.* 2016). In the Great Basin, soil water recharge occurs in fall, winter, and spring (Roundy *et al.* 2014). Wildfires in sagebrush steppe generally occur during the hot and dry summer period. Burned rangelands are typically seeded in fall before the soils begin to freeze and before soil moisture is too high to operate planting equipment (Amaranthus & Perry 1989; Beyers 2004). James et al. (2011) and Boyd and James (2013) found that 80% of fall-seeded perennial grasses, such as bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve), germinate prior to winter but only a small percentage of the seeds sown (10-15%) produced seedlings that emerged from the soil in the spring.

Seed dormancy may prevent seed germination until conditions are favorable for establishment and growth (Baskin & Baskin 2001; Finch-Savage & Leubner-Metzger 2006; Allen *et al.* 2007). Dormancy mechanisms vary across species through adaptation to the prevailing environment (Baskin & Baskin 2004; Finch-Savage & Leubner-Metzger 2006). Physiological dormancy is the most abundant form of dormancy and varies in its severity from species that require several months of stratification before germination, to species that can germinate through after-ripening in dry storage (Baskin & Baskin 2004; Baskin & Baskin 2005). Seed dormancy may be induced through the addition of the plant hormone abscisic acid (ABA) during seed maturation on the mother plant (Kucera et al. 2005). Within the seed, ABA is a

regulator of both the induction and maintenance of dormancy, which functions through a complex network of signaling pathways (Finch-Savage & Leubner-Metzger 2006; Zhao *et al.* 2011). Within the seed, it is not necessarily the concentration of ABA that effects dormancy but its relation to the concentration of gibberellins (GA) (LeonKloosterziel *et al.* 1996; Lefebvre *et al.* 2006). When the ratio of ABA concentration to GA concentration is higher, seeds are more likely to maintain some dormancy (Kermode 2005; Duclos, Altobello & Taylor 2014). Both the localization of ABA and the competency of cells to respond to the hormone play important roles in breaking dormancy as well (Finch-Savage & Leubner-Metzger 2006).

The effects of ABA on seeds in laboratory and agricultural experiments show that ABA can delay germination when applied exogenously.(Romagosa *et al.* 2001; Aroca *et al.* 2008; Atia *et al.* 2009; Papenfus *et al.* 2013; Hussaina *et al.* 2015). However, no study has demonstrated the effect ABA would have on seeds used for restoration efforts in rangeland settings. Research is needed to understand the rate ABA should be applied to sufficiently push germination to at least late winter or early spring when temperatures are less likely to damage plant tissue (Pearce 2001). Roundy and Madsen (2016) reported that freezing conditions in Great Basin sagebrush communities can last as long as 168 days (October to mid-March). Wet thermal accumulation models may offer the first step in determining if ABA application rates in a seed coating are sufficient to delay germination until after this freezing period. Germination timing of many non-dormant seed populations is a function of temperature accumulation when seeds are imbibed (Rawlins *et al.* 2012a). Wet thermal accumulation models predict the timing and rate of seed germination based on temperature, with progress towards germination accumulated when temperature and soil moisture are above a set threshold (Forcella *et al.* 2000; Vleeshouwers & Kropff 2000). Rawlins et al. (2012b) found that wet thermal accumulation models that were

applied to field soil moisture and temperature data could accurately predicted germination in seed bags 50-95% of the time, depending on the season the seeds were sown.

The objectives of this study were to: 1) assess the effect of ABA application rate, applied within a seed coating, on final germination percentage, seed germination timing, and the spread of when the seeds would germinate (synchrony) under different constant temperatures, 2) for each unique seed coating create thermal accumulation models that express how germination timing changes with temperature, 3) apply thermal accumulation models to field soil moisture and temperature data sets across the Great Basin to predict seed germination timing from simulated planting dates. We hypothesized that ABA seed coatings will delay seed germination with the extent of delay a function of the rate of ABA applied. We also hypothesized that models will predict that ABA coatings could provide sufficient delay to allow seed germination to occur in spring.

MATERIALS AND METHODS

Seed Coatings

To investigate ABA rates on seed germination we focused on 'Anatone' bluebunch wheatgrass, a common perennial bunchgrass in much of the Intermountain West, USA. Bluebunch wheatgrass provides quality forage for livestock and wildlife, helps suppress weeds, and is commonly seeded in restoration projects. Certified seed was purchased from Granite Seed (Lehi, UT, USA). The form of ABA used in this experiment was obtained from Valent BioSciences Corporation (Libertyville, IL, USA), under the trade name BioNik™. Seven different rates of ABA were applied to the seed at rates of 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, and 6.0 g Bionik 100 g^{-1}. A coated treatment without any ABA was not added to the study because

preliminary lab trials showed no significant difference in germination time and total germination between untreated seed and coated seed with no ABA (Badrakh, 2016).

Seeds were coated under a two-step process in a 30-cm rotary seed coater from Universal Coating Systems (Independence, OR, USA). We applied ABA during the first step of the coating process in a dilution of Agrimer SCP binder (Ashland Inc., Covington, KY, USA). In this first step, a total of 10 g of liquid was applied to the seed. In the second step, 50 g of Agrimer SCP and 175 g of calcium carbonate powder (limestone) was added slowly to the seed to cover the ABA coating and build up the size of the seed. The coated seed was then dried using a forced air dryer at 43 °C (Brace Works Automation and Electric, Lloydminster, SK, CAN).

Germination Experiment

In addition to the seven ABA treatments, untreated seeds were included in the experiment (control). Each treatment was repeated seven times. In each replicate, 25 seeds were placed in 13 x 13 cm acrylic boxes (Pioneer Plastics, Dixon KY, USA) filled with 140 g of fine sand. Before planting, the sand was watered to field capacity. Seeds were placed on the surface of the sand, and the boxes were sealed to maintain moisture levels. The study was installed as a randomized complete block split-plot design, with temperature comprising the split-plot factor. We used a range of constant temperatures to germinate seeds (5, 10, 15, 20, 25 °C). Seeds were placed in Precision Plant Growth Chambers (Thermo Fischer Scientific, Waltham, MA, USA) to maintain the different temperatures. Each block had one of each treatment, and took up an entire shelf in the growth chamber. The number of germinated seeds was counted every 1-3 d. We defined germination as the extension of the radical 2 mm from the seed. Once germinated, seeds were removed from the boxes. After each counting, the blocks were randomly placed on a new shelf within the growth chamber.

From laboratory seed germination counts, we calculated several seedling dormancy indices, final germination percentage, time to reach germination at 10% intervals from 10-90%, and germination synchrony. Final germination percentage was calculated as the ratio of the number of seeds germinated to the total number of seeds. Time to reach a certain germination percentage (Tx, i.e., time to reach 10% germination is T_{10}) was calculated as follows:

$$T = \left[\left(\frac{t_a - t_b}{n_a - n_b}\right)(N - n_b)\right] + t_b \qquad \text{(eqn 1)}$$

where: T is equal to time (days) to subpopulation germination, t_a is equal to the incubation day when subpopulation germination was reached, t_b is equal to the incubation day before subpopulation germination was reached, n_a is equal to the number of germinated seeds on the day that subpopulation germination was reached, n_b is equal to the number of germinated seeds on the day before subpopulation germination was reached, and N is equal to the number of germinated seeds equal to either 10, 20, 30, 40, 50, 60, 70, 80, or 90% of the total population (Rawlins *et al.* 2012a). Germination synchrony was calculated by subtracting T_{90} from T_{10}.

We created mixed models to first determine the significance ($p<0.05$) of ABA concentration, incubation temperature, and their interactions (unless determined to not be significant) for final germination percentage, T_{50}, and synchrony of germination. In the model, blocks were considered random, while incubation temperature and treatment were both considered fixed. We tested for differences in responses to ABA concentrations at the incubation temperatures of 5, 10, 15, 20, and 25 °C using a Tukey pairwise comparison test ($p<0.05$). The square root of T_{50} was used to normalize the data, but a transformation of total germination percentage and synchrony was not needed as indicated by viewing residuals.

Prediction of Seed Germination Timing in the Field

Linear and curvilinear regression was used to apply polynomial equations to the germination time data gathered from the experiment in the previous section. These equations, or wet thermal models, estimate germination rate (inverse of T_x) in relation to incubation temperature. Germination rate was used instead of T_x to improve model accuracy (Rawlins et al., 2012a). Models were created for all seed treatments, for each of the germination intervals described above. These models were then applied to historical soil temperature and water potential data from the Sagebrush Step Treatment and Evaluation Project (SageSTEP) (Cline 2014). We selected from the SageSTEP network six different sites to model seed germination timing, which were within Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) communities that had all been treated with prescribed burns (Moses Coulee, WA, Saddle Mountain, WA, Hart Mountain, OR, Roberts, ID, Owyhee, NV, and Onaqui, UT) (McIver & Brunson 2014). At each of the sites, hourly measurements were made at approximately 1-3 cm below the soil surface to estimate soil temperature using thermocouples and soil water potential using gypsum blocks (Delmhorst, Inc., city and state). Gypsum block resistance was converted to MPa of water potential using standard calibration curves (Rawlins *et al.* 2012b).

Estimates of seed germination timing were predicted at each site over a four-year period (2011-2014) with the exception of the Moses Coulee site, which did not have enough data to be used for 2011. A simulated planting date of October 15th was set for modeling seed germination timing, which is a common time for land managers to initiate seeding projects in the Great Basin. In calculating seed germination timing, progress towards germination was determined for each individual hourly soil temperature data point starting at the planting date. Progress towards germination was calculated by dividing hourly soil temperature by the time to reach T_x at the

temperature of that data point (determined using the regression models described above). In this model, thermal progress toward germination is accumulated only for hours when soil water potential is above -1.5 Ψ. This ratio, or progress towards germination, was then converted to a percentage and accumulated until 100% was reached. At that point, we determined that T_x was reached. The process was repeated for each thermal model created. From this data we determined the time when the majority of seed for each treatment would germinate (i.e. month of the year when > 50% of the population had germinated) and then averaged the data across all sites and years. Additionally, graphs were developed that show when each treatment of seed would reach each T_x interval for each site and simulated planting year.

RESULTS

Final Germination Percentage

Mixed model analysis showed that incubation temperature (F = 5.6, *P* < 0.001), ABA concentration (F = 8.9, *P* < 0.001), and the interaction between these two factors (F = 8.0, *P* < 0.001) affected final germination percentage. Typically, germination was similar between most treatments at temperatures ranging from 5-20 °C. Germination for the lower application rates of ABA (0.25-1.0 g BioNik 100 g^{-1} seed) were slightly higher than untreated seed (Figure 2-1a). At 25 °C, the 2 and 6 g BioNik 100 g^{-1} seed treatments had lower germination (69% and 65% respectively) than the other treated and untreated seeds (average of 87%). Throughout all the temperatures, the highest final germination percentage was 97% (0.5 g BioNik 100 g^{-1} seed, 20 °C) and the lowest was 65% (6 g BioNik 100 g^{-1} seed, 25 °C).

Germination Time

Incubation temperature (F = 81.8, *P*<0.001) and ABA concentration (F = 324.38, *P* < 0.001) affected T_{50}. Typically as temperature increased, T_{50} values declined out to 15 °C and then at 25 °C, T_{50} values began to increase. Within each constant temperature, T_{50} increased with increasing concentration of ABA (Figure 2-1b). Across all incubation temperatures, there was a strong delay in germination for treated compared to untreated seed (Figure 2-1b). For example, at 5 °C, seed treated with ABA at 0.25, 0.5, 1, 2, 4, and 6 g BioNik 100 g^{-1} seed had T_{50} values that were 8.2, 10.1, 12.7, 14.3, 14.4, 19.8, and 20.0 d longer than untreated seed, respectively (Figure 1b).

Synchrony

Synchrony was affected by incubation temperature (F = 90.1, *P* < 0.001), ABA concentration (F = 109.5, *P* < 0.001), and the interaction between the two (F = 10.3, *P* < 0.001). In general, synchrony for all treatments was low at 5 °C, peaked at 10 or 15 °C, and then decreased as temperatures continued to increase (Figure 2-1c). Within each temperature regime, untreated seed typically had the most synchronous germination, ranging from 5.3 - 33.5 d (Figure 2-1c). The only exception was at 25 °C, where both the 0.25 and 0.5 g Bionik 100 g^{-1} seed were more synchronous (21.3 and 25.0 d, respectively) than the untreated seeds (33.5 d). Synchrony decreased with increasing ABA concentration. For example, at the lowest ABA application rate (0.25 g BioNik 100 g^{-1} seed) synchrony ranged from 6.4 – 21.3 d, while at the highest ABA application rate (6.0 g BioNik 100 g^{-1} seed) synchrony was between 18.1 - 68.3 d, depending on temperature (Figure 2-1c).

Prediction of Seed Germination Timing in the Field

All wet thermal accumulation models created had sufficient accuracy to predict germination time (adjusted R^2 = 0.51– 0.85). For untreated seed, the majority of the seeds were estimated to germinate during the winter period (October –February), and only 22% of the seeds were estimated to germinate in March or later (Figure 2-2). Time required for the majority of the seeds to germinate increased as ABA application rates increased (Figures 2-2). For almost all sites and planting years, the majority of ABA-coated seeds were predicted to germinate in spring or early summer (March – May) depending on the application rate of ABA applied (Figures 2-2). The majority of seeds coated with 0.25 – 2.0 g BioNik 100 g⁻¹ seed germinated 13 - 22% of the time during October – February, 52 - 57% of the time in March, and 21 - 35 % of the time in April or later (Figure 2-2). Conversely, the majority of seed coated with 4 and 6 g BioNik 100 g⁻¹ seed germinated 9% of the time during October – February, 26- 30% of the time in March, and 61- 65% of the time in April or later (Figure 2-2).

Analysis of individual sites showed high variation in germination timing for the seed treatments by site and year (Figures 2-3, 2-4). At Roberts and Owyhee, all germination for treated seed was predicted to occur by spring (March – April) (Figures 2-3, 2-4). Similar results were seen for Moses Coulee, except for 2012-2013. During this period, there were high levels of variability between treatments. The lowest ABA treatment (0.25 g BioNik 100 g⁻¹) had germination occur during the winter months (November – December), the highest ABA treatment (6 g BioNik 100 g⁻¹) was predicted to reach 20% germination during the winter (February), with the rest of the population germinating by Spring (March – April). All other treatments fell somewhere in between those two rates (Figure 2-3).At Onaqui for 2012-2013 and 2013-2014, and Hart Mountain for 2011-2012 and 2012-2013, germination of treated seed was predicted to occur by spring (March – April). During 2010-2011, for both sites, the two lowest

ABA rates (0.25 and 0.5 g BioNik 100 g^{-1}) had between 10%-30% germination occurring in winter (February), with the rest occurring by spring (March – April). For 2011-2012 at Onaqui, and 2013-2014 at Hart Mountain, the lowest five rates of ABA (0.25-2 g BioNik 100 g^{-1}) had anywhere between 10%-70% germination occurring in winter (February), with the rest occurring by spring (March – April) (Figures 2-3, 2-4).In Saddle Mountain, during 2011-2012 and 2013-2014, the lower rates of ABA (0.25-1 g BioNik 100 g^{-1}) had 10%-40% germination occurring in winter (February), with the rest occurring by spring (March – April). For 2012-2013, all germination across all treated seed was predicted to occur in winter (November – February). During 2010-2011, much more variation within treatments was seen. For the lowest five rates of ABA (0.25-2 g BioNik 100 g^{-1}), germination was predicted to occur in winter (November – February). For the two highest rates of ABA (4 and 6 g BioNik 100 g^{-1}), germination was predicted to occur between mid-winter and spring (December – March) (Figure 2-3).

DISCUSSION

Our hypothesis that ABA seed coatings will delay seed germination with the extent of delay a function of the rate of ABA applied was validated through this study. Accumulation of ABA in dormant seeds decreases overtime due to after-ripening (Walkersimmons 1987; Bewley 1997; Ali-Rachedi *et al.* 2004). Seeds used for restoration projects are usually stored for a year or longer prior to use (Personal Communication, Joshua Buck, Granite Seed Inc. Lehi, UT, USA). Long storage periods can lead to decreased levels of ABA within the seeds, and quick germination once the seeds are sown. Our study provides a solution to this problem, and demonstrates how ABA application can be tailored to sites where early germination may lead to high seedling mortality.

While our hypothesis that increasing levels of ABA applied to the seed would create a greater delay in germination was proven to be correct, two ABA rates (2 and 6 g BioNik 100 g^{-1} seed), showed moderately lower total germination at the highest temperature used in the study (25 ℃). It was our observation that the lower germination for these ABA coating rates was due to the seeds spending more time prior to germination in warm moist conditions that are conducive to pathogen attack (Doohan, Brennan & Cooke 2003; Koseki & Isobe 2005).

Wet-thermal accumulation models applied to historic microclimate field data predicted that the majority of untreated seeds germinated within two to three weeks of being planted on most years and sites. The late fall/early winter germination of untreated seed would subject the seedlings to harsh environmental conditions during the winter and potentially result in high seedling mortality (James, Svejcar & Rinella 2011).

Germination models also estimated that ABA treated seeds can delay germination of fall planted seeds until spring, which is anticipated to be a more environmentally favorable condition for seedling survival and plant establishment. Roundy and Madsen (2016) reported that across 14-sagebrush steppe sites throughout the Great Basin there was an average of 58 freeze-thaw periods for the upper 1-3 cm of soil between October and March. Boyd and Lemos (2013) reported major reduction in emergence and tiller density for range grasses adapted to sagebrush steppe after exposure to only 4 days of freezing. The range of sub-zero temperatures that seedlings can tolerate can be quite narrow (Bois *et al.* 2006). Modeling of germination using field seedbed temperatures suggests that the delays induced by ABA in the laboratory was sufficient to avoid sagebrush steppe-frost periods for most years and sites. The majority of ABA treated seed was predicted to germinate by March or later. Coatings of ABA could be tailored to work on specific sites known to have shorter or longer freezing periods, or to species with limited frost tolerance.

While the results of our germination modeling generally indicated positive trends towards germination timing with an ABA seed treatment, it is unclear whether ABA seed coatings would improve establishment on all simulated planting years. We assume an early spring germination timing would be most beneficial because this would maximize the period seeds could grow before they were subjected to summer drought. With this germination timing objective, lower ABA application rates typically provide a more optimal germination timing. Cline (2017) characterized seedling root zone drying rates of sagebrush steppe communities. The soil water potential at the sites studied was above the plant permanent wilting point (-1.5 Mpa) during initial spring (March 1 – June 30) drying periods for 37.3 days at 3 cm and 87.3 days at 30 cm soil depth. This data suggest that seedlings could have sufficient time to establish on most years if germination is delayed into March.

For some of the modeled sites and years, primarily at the Owhyee site, the untreated seed did not start to germinate until the spring. While for many sites the addition of ABA may be beneficial, for sites where soil temperature and moisture are low during the fall and winter months, the addition of synthetic ABA may not aid in the establishment of seedling populations. In these instances an extended delay in germination with ABA until late spring or early summer, may result in the seedling not having sufficient moisture for plant establishment.

Since it may be difficult to predict what the precipitation and temperature will be for a given year, ABA may also be useful in creating a bet-hedging strategy for seed germination. The synchrony of a seed population is greatly decreased once ABA is added. This could lead to seeds germinating throughout the late winter/early spring months. Varying levels of dormancy within a seed population is a strategy used by plants to mitigate against environmental risks (Venable & Brown 1988; Hierro *et al.* 2009; Lewandrowski *et al.* 2016). It is probable that the risk of seeding failures could be reduced by expanding the envelope that seeds germinate under to

increase the likelihood that some seeds will germinate within a window that is more favorable for plant establishment and survival.

CONCLUSION

Current seeding practices need to be altered in order to increase success (James & Svejcar 2010; James, Svejcar & Rinella 2011). Species used in rangeland seeding practices germinate quickly after a fall planting and then are subjected to multiple stressors during winter that can cause mortality, such as freezing, drought, fungal pathogens, and other biotic and abiotic factors (Cline, Roundy & Christensen 2017b; Cline, Roundy & Christensen 2017a). Modeled estimates of seed germination timing in this study predict that ABA seed coatings has the potential to be a conservative strategy, which overcomes some of these environmental barriers by delaying seed germination until spring when conditions are more favorable for plant establishment. The application of ABA to seeds may also improve restoration efforts by spreading the period seeds germinate, which may increase the likelihood of seeds germinating during periods of the year with favorable growing conditions. Experiments need to be conducted to verify the results of this research in the field and determine how delaying seed germination with ABA coatings impacts plant establishment and survival.

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FIGURES
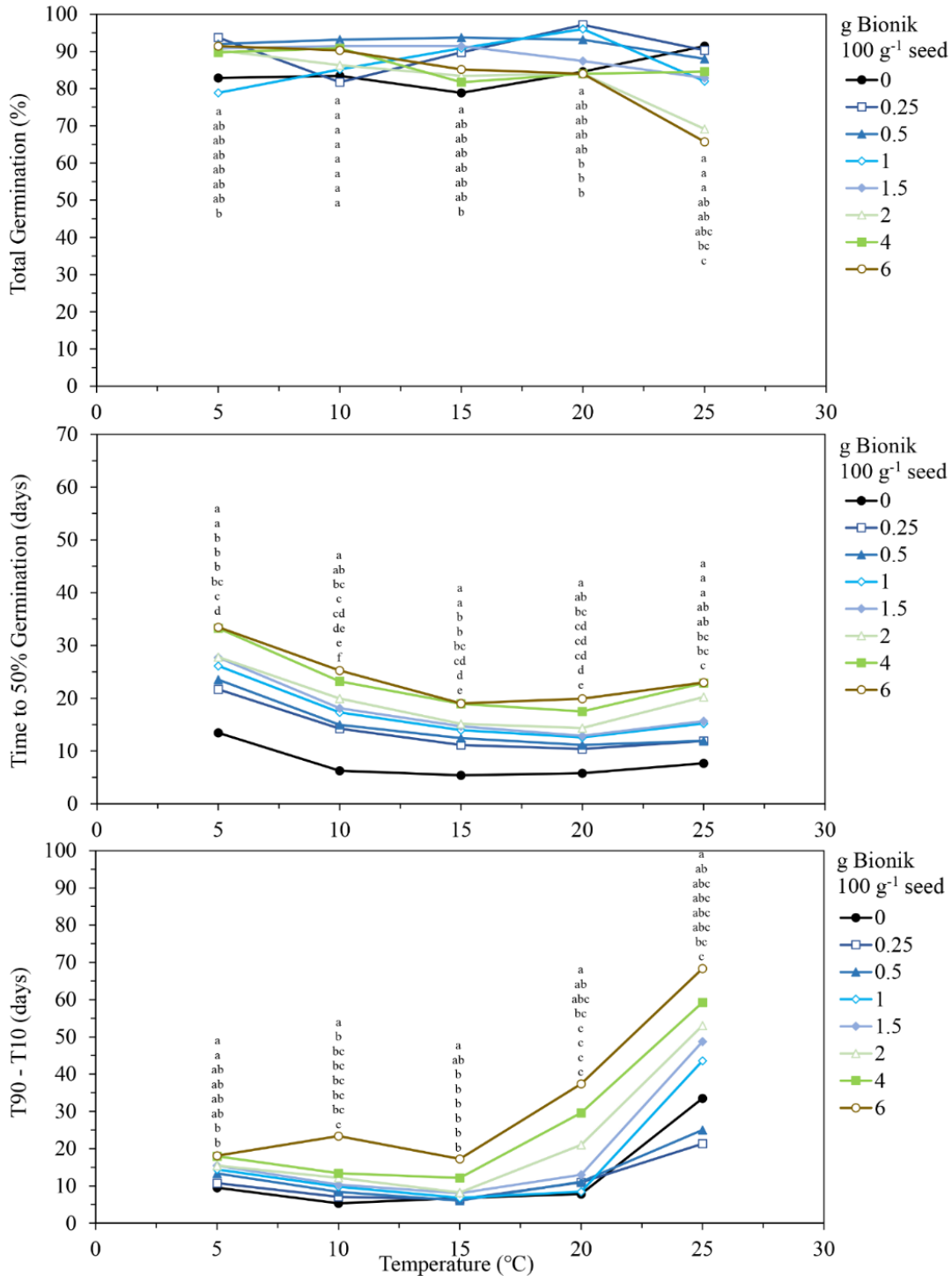
Figure 2-1. Influence of BioNik (a 25% formulation of abscisic acid) rates applied to seed on (a) final germination percentage, (b) time to reach 50% germination, and (c) germination synchrony at temperatures ranging from 5-25 °C. Values with the same incubation temperature with different letters are significantly different ($p < 0.05$) at that temperature. The letters correspond with the data points from top to bottom.
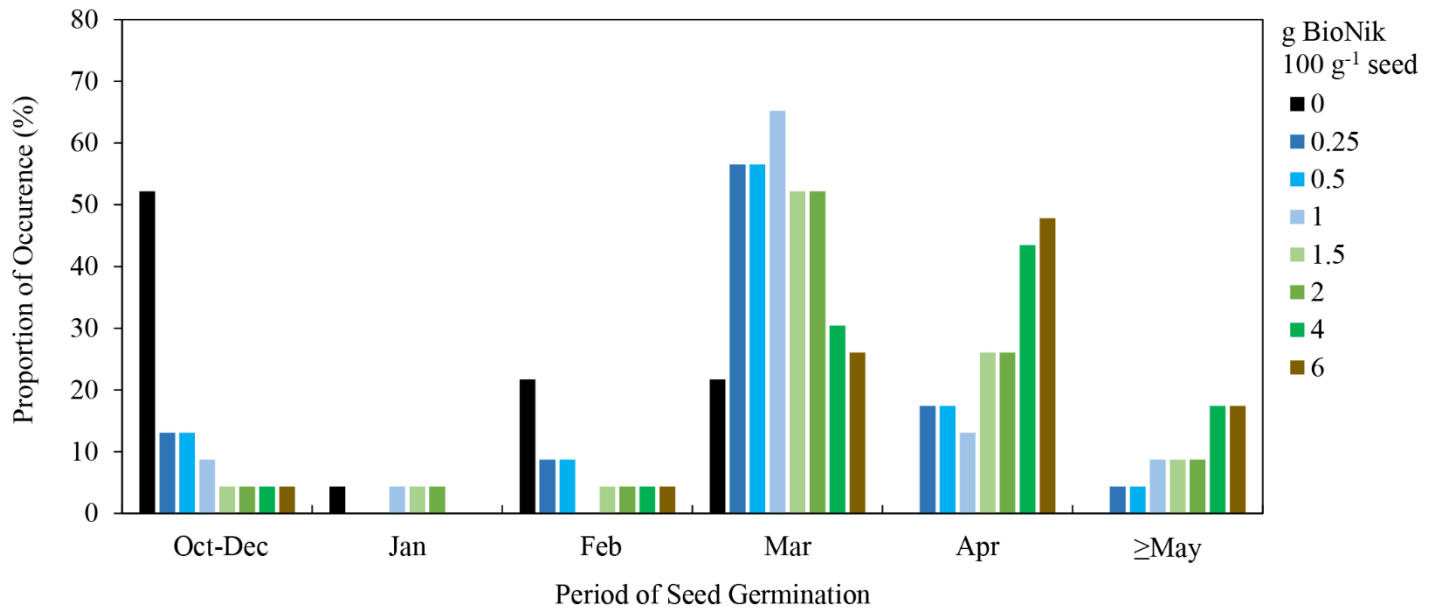
Figure 2-2. The period of the year when greater than 50% of the seed germinated. Values represent the percentage of occurrence across all sites (4 sites) and planting years (6 years) for untreated seed and seed treated with BioNik at rates ranging from 0-6 g Bionik 100 g^{-1} seed.
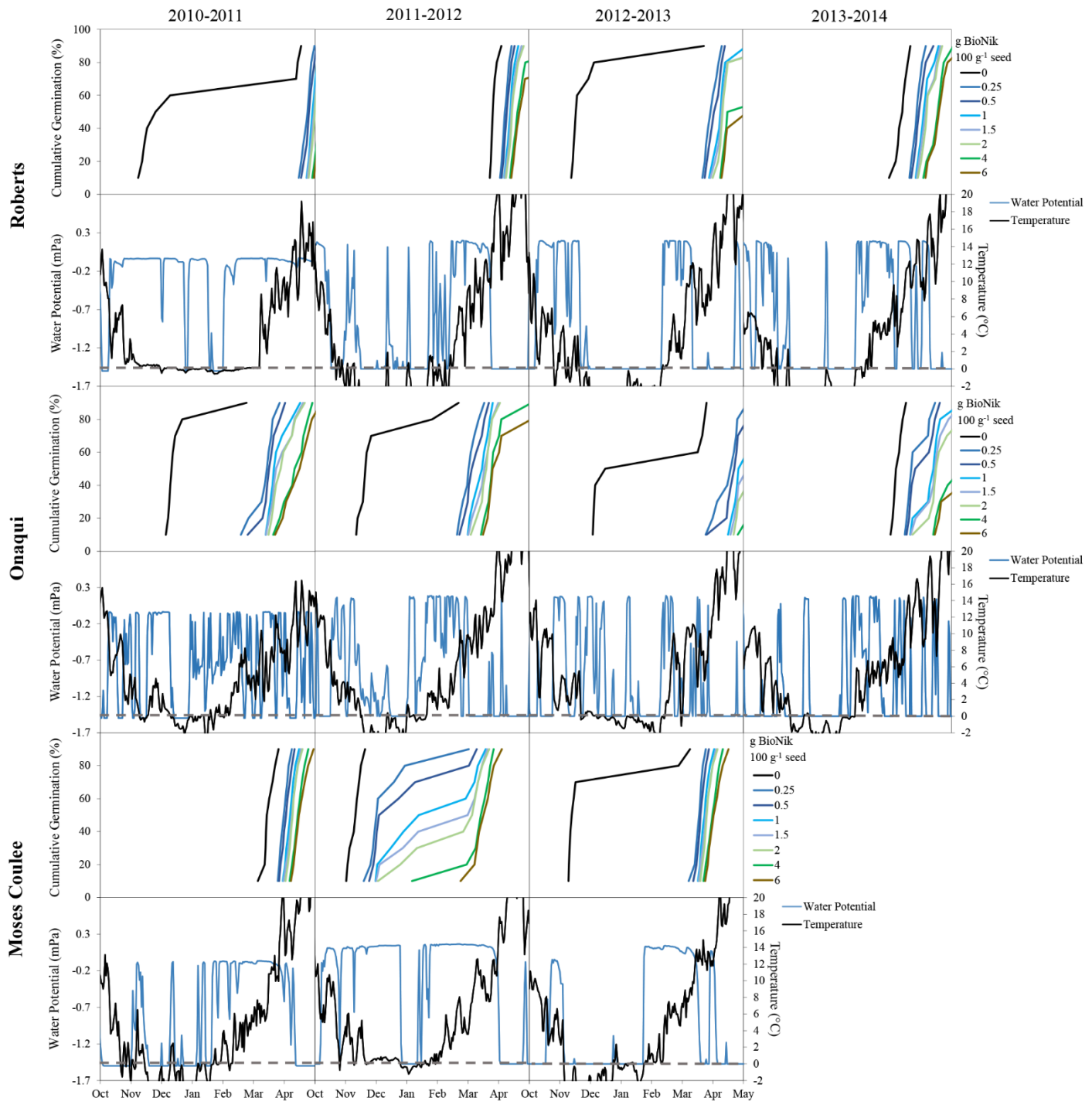
Figure 2-3. Modeled estimates of the percentage of seeds expected to germinate over time for untreated seed and seed coated with increasing rates of BioNik and soil water potential and temperature used to model seed germination timing. Simulations were run with an October 15th planting date on four separate years (2010-2013) for sites in (a) Roberts, ID, Onaqui, UT, and Moses Coulee, WA.
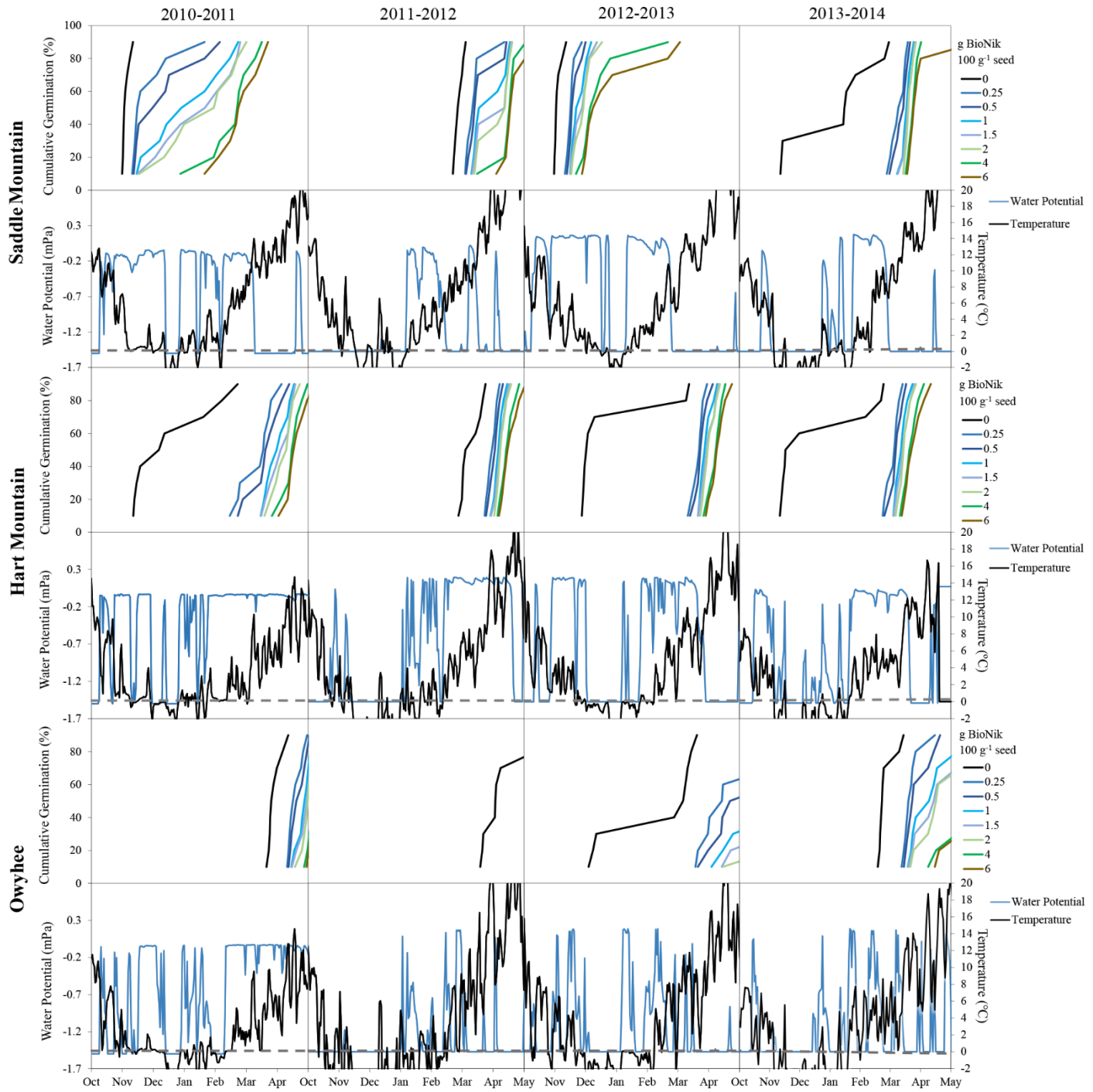
Figure 2-4. Modeled estimates of the percentage of seeds expected to germinate over time for untreated seed and seed coated with increasing rates of BioNik and Soil water potential and temperature used to model seed germination timing. Simulations were run with an October 15th planting date on four separate years (2010-2013) for sites in (a) Saddle Mountain, WA, Hart Mountain, OR, and Owyhee, NV.

Figure 3-1-APPENDIX 1. The first or "Home" worksheet in Auto-Germ. This worksheet has step by step instructions on how to use each feature of the workbook.

Temp (°C)	Rep/Block	Plot ID	Treatment	Seeds Planted	7/19/2017	7/20/2017	7/21/2017	7/22/2017	7/24/2017	7/25/2017	7/26/2017
5	Block 1	8	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 2	18	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 3	21	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 4	29	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 5	37	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 6	48	Idaho fescue	25	0	0	0	0	0	0	0
5	Block 7	60	Idaho fescue	25	0	0	0	0	0	0	0
10	Block 1	72	Idaho fescue	25	0	0	0	0	0	0	2
10	Block 2	79	Idaho fescue	25	0	0	0	0	1	4	3
10	Block 3	85	Idaho fescue	25	0	0	0	0	0	0	3
10	Block 4	91	Idaho fescue	25	0	0	0	0	1	6	5
10	Block 5	100	Idaho fescue	25	0	0	0	0	0	2	5
10	Block 6	115	Idaho fescue	25	0	0	0	0	0	6	1
10	Block 7	119	Idaho fescue	25	0	0	0	0	0	3	3
15	Block 1	133	Idaho fescue	25	0	0	0	0	6	10	4
15	Block 2	138	Idaho fescue	25	0	0	0	0	3	2	7
15	Block 3	146	Idaho fescue	25	0	0	0	0	2	9	0
15	Block 4	155	Idaho fescue	25	0	0	0	0	8	4	4
15	Block 5	170	Idaho fescue	25	0	0	0	0	5	3	7

Figure 3-2-APPENDIX 1. A completed example of the "Data Entry" worksheet in Auto-Germ. This is where germination counting data is input from constant-temperature laboratory trials.

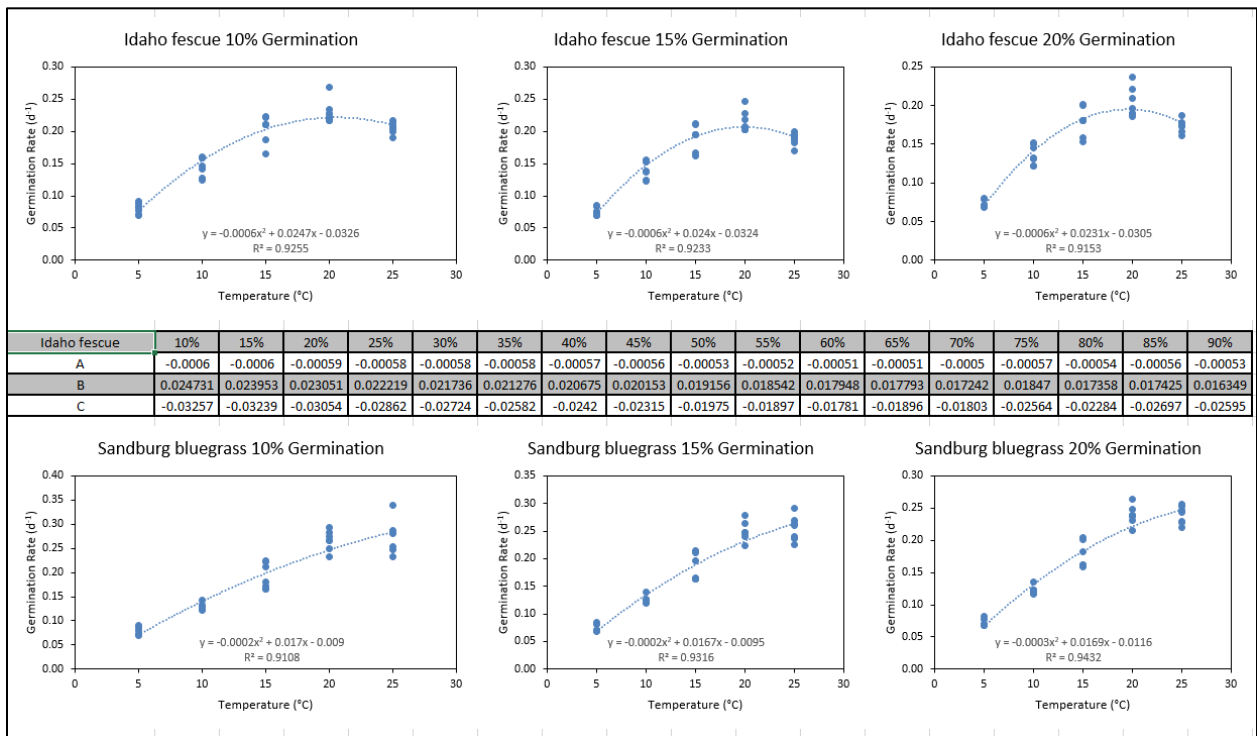| Idaho fescue	10%	15%	20%	25%	30%	35%	40%	45%	50%	55%	60%	65%	70%	75%	80%	85%	90%
A	-0.0006	-0.0006	-0.00059	-0.00058	-0.00058	-0.00058	-0.00057	-0.00056	-0.00053	-0.00052	-0.00051	-0.00051	-0.0005	-0.00057	-0.00054	-0.00056	-0.00053
B	0.024731	0.023953	0.023051	0.022219	0.021736	0.021276	0.020675	0.020153	0.019156	0.018542	0.017948	0.017793	0.017242	0.01847	0.017358	0.017425	0.016349
C	-0.03257	-0.03239	-0.03054	-0.02862	-0.02724	-0.02582	-0.0242	-0.02315	-0.01975	-0.01897	-0.01781	-0.01896	-0.01803	-0.02564	-0.02284	-0.02697	-0.02595

Figure 3-3-APPENDIX 1. A completed example of the "Polynomial Equations" worksheet. This sheet contains the coefficient values and graphs of the second degree polynomial equations for each treatment and percent germination combination.
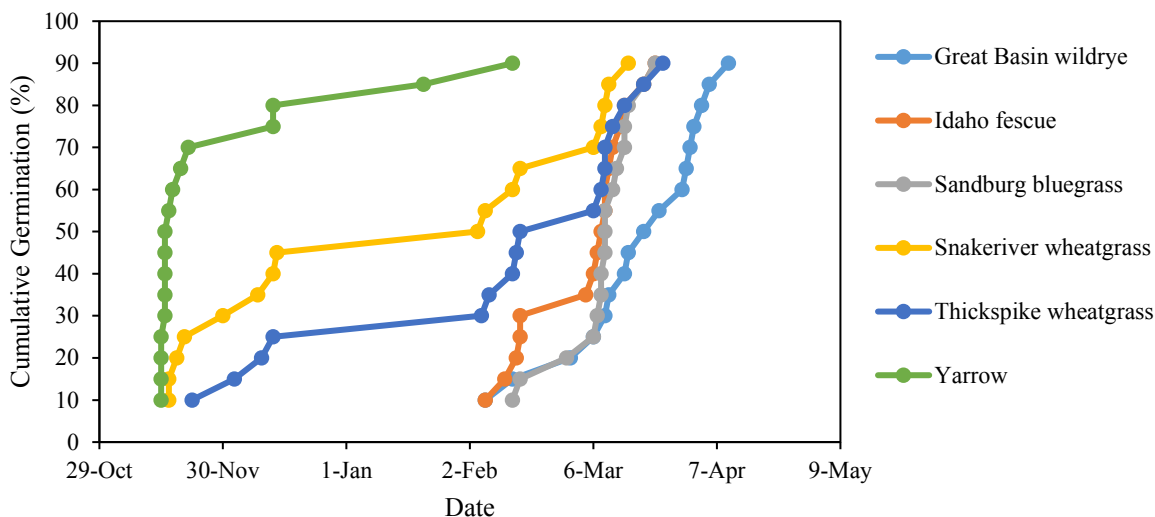
Figure 3-4-APPENDIX 1. An example of the figures found in the "Planting Date" worksheet. Shows the predicted germination times of six species common to the Great Basin of North America, based on soil temperature and water potential data from Hart Mountain, OR for the year 2010. The simulated planting date was on October 15th.
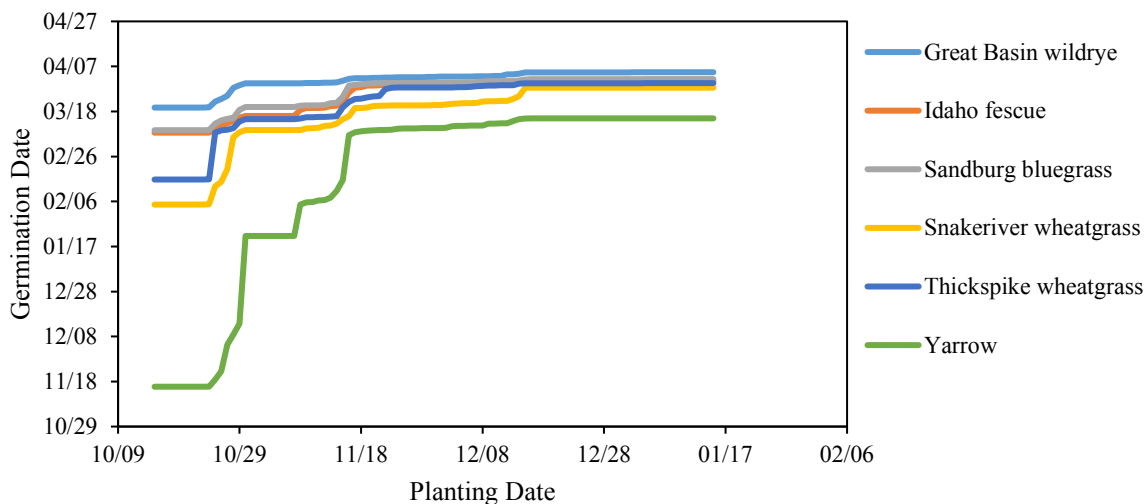

Figure 3-5-APPENDIX 1. An example of the figures found in the "% Germination" worksheet. Shows the date at which the simulated population will reach 50% germination for six species common to the Great Basin of North America, for every planting date between 10/15/2010 and 01/15/2011 at Hart Mountain, OR.