

Undergraduate Thesis: The effect of temperature on germination behavior and seedling morphology in locally adapted maize landraces from Chiapas, Mexico

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## Abstract

Southern Mexico is the center of origin for maize (*Zea mays ssp mays*) and hosts an array of landraces (or traditional varieties). The diversity found within and among landraces is largely shaped by the interplay between selection and gene flow, resulting in populations that often evolve to be locally adapted to a particular environment. Mercer et al. (2008) discovered that landrace maize populations sourced from along an elevational gradient in the Mexican state of Chiapas exhibited a pattern of asymmetrical local adaption. We aimed to build upon this revelation by elucidating the role of temperature as a driving force of local adaptation in the early phases of the maize lifecycle. Using a thermogradient table, we exposed the seeds of nine landrace maize populations, three from the highlands, three from the midlands, and three from the lowlands, to temperatures the seeds would likely encounter during the planting season in Chiapas, and examined the rate of germination and percent germination. We also exposed the seeds of the nine landrace populations to periods of cold (approximately 10.0 °C) lasting zero, seven, and 21 days, followed by exposure to favorable conditions (25.0 °C), in order to test the effects of cold periods on seed germination and seedling morphology. Interestingly, elevational group was often not a significant predictor of fitness measures such as the percent germination and percentage of normal seedlings, indicating that patterns of local adaption in response to temperature may not be discernible in the early lifecycle at the temperatures we tested. One exception to this pattern occurred at the lowest temperature, 10.0 °C, where the highland populations exhibited the highest percent germination and germination rate, followed by midland populations, and lowland populations, and a pattern of local adaption was clear. By contrast, in the rest of the temperature treatments, the midland populations generally performed best, although not always significantly. This suggests that the evolutionary advantages imparted by

local adaptation and enjoyed by highland populations in highland environment, described by Mercer et al. (2008), may not be present in the seed or seedling stage of the lifecycle in temperatures above 10.0 °C. Thus, the ecological relevance of local adaption driven by temperature in the seed and seedling phase of the lifecycle may be limited to early planting situations in the cool, highland environment.

## **Contents**

Abstract.....	2
Introduction.....	5
Objectives and Hypotheses.....	13
Materials and Methods.....	14
Results and Discussion .....	20
Conclusion.....	33
Acknowledgements.....	36
Literature Cited.....	36
Tables.....	40
Figures.....	43

## Introduction:

Crop genetic resources are the most crucial foundational element in our agricultural system. The development of breeding programs, which are dependent upon crop genetic resources, has allowed scientists to greatly improve global human well-being (Burke et al. 2009). Crop genetic resources promise to be a key tool in the quest to adapt our agriculture to the unprecedented challenge of a rapidly changing climate. Preserving crop genetic resources is vitally important because they are the most significant source of the novel alleles needed to contend with pests, equip plants with stress tolerance, and raise and stabilize yields (Burke et al., 2009). Plant breeders frequently use traits from genetically diverse populations, such as landraces, and introgress them into elite lines by through a breeding technique known as backcrossing (Tanksley and McCouch, 2006). Consequently, the value of preserving these genetic resources was recognized nearly 90 years ago by Nikolai Vavilov and others (Brush, 1997). Their preservation is a major focus of organizations for agricultural advancement, such as the Consultative Group on International Agriculture Research (CGIAR), the UN Food and Agriculture Organization, as well as numerous NGO's and governmental agencies (e.g., the USDA).

Crop genetic resources are either conserved *ex situ*, in gene banks, or *in situ*, in the fields of the farmers as they grow the crop and maintain their seed stocks (Rice et al., 2006). Efforts to preserve crop genetic resources *ex situ* have achieved significant successes, including the incorporation of the International Board for Plant Genetic Resources (IBPGR) circa 1974 and the construction of a network of gene banks that preserve lines of the world's most important crops (Altieri and Merrick, 1987). *Ex situ* preservation is fundamentally limited, however, by the nature of the process itself. Although great strides have been made in the collection of

accessions, due to the application of population genetics modeling and the shift in focus from preserving phenotypic diversity to preserving allelic diversity, the fact remains that accessions can never fully capture the genetic diversity present in the populations that the *ex situ* accessions are representing (Rice et al. 2006). Furthermore, the length of time between “grow outs”, or regeneration of the seed stock, can be many years depending on the storage method. If cold storage is used, the relatively long length of time in between grow outs creates a situation where the populations are extremely stable and have little opportunity to evolve (Roberts, 1975, found in Rice et al. 2006). Accessions are never static, however, and “genetic shift” can occur due to founder effects, random genetic drift, and selection in the new environment (Rice et al. 2006). Finally, the success of *ex situ* conservation efforts has been tempered by funding limitations and confusion arising from the overlapping jurisdictions of NGO’s, governments, and intergovernmental agencies (Altieri and Merrick, 1987). Thus, *ex situ* preservation of crop genetic resources is fundamentally limited and must be supplanted with *in situ* preservation.

*In situ* preservation allows farmers to preserve large and more diverse gene pools. The populations being preserved are able to respond to evolutionary forces such as selection and gene flow. These forces may help increase, decrease, or maintain the fitness of the population in response to changing environmental factors (Mercer and Perales, 2010). In the case of pearl millet landraces in the Sahel, *in situ* conservation allowed landraces to evolve in response to changing climatic conditions, namely decreased rainfall patterns (Vigouroux, et al. 20011). Landrace varieties that were planted yearly and were able to evolve, were found to be more drought tolerant than landraces which were collected in the 1970’s, regenerated and planted (Vigouroux et al. 2011). This yield stability in the face of a changing environment was maintained through the processes of selection and evolution, and was a direct benefit to Sahelian

farmers. Therefore, *in situ* conservation serves the dual purpose of increasing genetic diversity and providing much need yield stability to some of the world's most economically vulnerable famers.

Nota bene, *in situ* preservation is not a panacea and is not advisable in systems where genetic diversity is being severely depleted or where populations risk extinction (Mercer and Perales, 2010). Crop varieties can be lost or abandoned due myriad environmental, social, or economic factors (Smale et al. 2003). Consequently, many of the world's preeminent crop genetic resource conservation scholars argue for the application of both *in situ* and *ex situ* conservation methods simultaneously, since they are complementary (Brush, 1991, Altieri and Merrick, 1987). Yet both methods of crop genetic resource preservation are limited by a paucity of information regarding the accessions and populations being preserved (Hoisington et al., 1999, Tanksley and McCouch, 2006). The task of evaluating the millions of accessions for even the most basic agronomic traits must be undertaken, since it is the foundational step for further genetic work and ultimately, improved breeding programs with access to and understanding of the full range of genetic diversity in their arsenal. My work seeks to append this body of knowledge as it relates to landrace maize from Mexico by examining response of early lifecycle traits to temperature.

Landrace varieties are a particularly rich source of genetic material, when compared with elite or creolized varieties (Van Heerwaarden et al. 2009, Eagles and Lothrop, 1994). A landrace can be defined as “a dynamic population of a cultivated plant that has historical origin, distinct identity, and lacks formal crop improvement, as well as being genetically diverse, locally adapted, and associated with traditional farming systems” (Camacho Villa et al. 2005). They are often found in crop centers of origin and are marked by “a high yield stability and intermediate

yield level under a low agricultural input system” (Zeven, 1998). Since they are able to withstand environmental stress, they are often used in areas with low access to agricultural inputs such as pesticides and fertilizers (Altieri and Merrick, 1987). Numerous maize landraces can be found in Mexico (Wellhausen et al. 1952) and in Chiapas, “fifteen local varieties are recognized” (Bellon and Brush, 1994). The prevalence of many landraces is due to the heterogeneity the mountainous environment in which they are grown, as well as the varied uses farmers cultivate them for: “storage, tortilla or feed” (Bellon and Brush, 1994).

Chiapas, Mexico is extremely mountainous; and the endemic landraces are locally adapted (Mercer et al. 2008). Patterns of local adaptation emerge in metapopulations that occupy a heterogeneous environment where divergent forces of natural selection act upon and produce locally adapted populations (Kawecki and Ebert, 2004). Local adaptation is an evolutionary phenomenon that occurs when local populations maintain higher fitness than foreign populations in their home environment (Kawecki and Ebert, 2004). Local adaptation in plants is studied using common garden or reciprocal planting studies in which populations originating from different environments or locations are brought together for comparison (Kawecki and Ebert, 2004). Elevational gradients are often sites for local adaptation because they are home to extreme environmental differentiation, such as very distinctive temperature, UV light, or moisture regimes, within a spatial area small enough to host a metapopulation (Gimenec-Benavides et al. 2007). It is essential for the world’s biologists and agronomists to gain a better understanding of the process of local adaption in order to understand its effect on crop genetic diversity and its ability to yield valuable agronomic traits such as adaption to temperature or UV environments (Redden, 2013).

Within crop landraces in Chiapas, an interesting and asymmetrical pattern of local adaption was found amongst 21 maize landrace populations sourced from an elevational gradient (Mercer et al. 2008). Highland landraces were four times more likely to produce good quality seed in the highland common garden than when they were grown in the midland common garden. Lowland landraces produced good quality seed in both the midland and highland common gardens, but produced 25% less seed mass than midland or highland races in the highland garden. The mass of good seed, multiplied by the likelihood of producing good seed per plant (defined as seed from ears with no more than 50% rotten seed) was used as a measure of fitness, called “adjusted fitness”. Using this metric, the highland landraces were more fit than other types in the highland common garden, and the lowland and midland landraces were more fit than the highland type in the midland common garden, a pattern indicative of local adaptation. However, the large degree by which highland types lagged behind midland and lowland types in seed production in the warmer, midland conditions versus the smaller degree by which midland and lowland populations lagged in fitness in the highland common garden made this pattern of local adaptation asymmetrical (Mercer et al. 2008).

While these overall patterns of local adaptation have been discerned, it is not clear which environmental factors were most important as selection pressures, and which traits were most significant contributors to the pattern of local adaptation observed. Our research seeks to address this gap in the knowledge by examining the role of temperature in the earliest phase of the maize lifecycle. “Common garden” studies within a lab setting can be useful because they allow the researcher to isolate an environmental variable, such as contaminated soils, soil temperature, or the presence of a pathogen, and determine if it affects fitness, thereby indicating its possible role in natural selection (Kawecki and Ebert, 2004). In our work, we have isolated temperature as the

manipulated factor effecting seeds and seedlings in order to explore its effects on early life cycle traits in locally adapted maize landraces endemic to the Southern Mexican state of Chiapas. Temperature was identified as an environmental factor of interest due to its important role in the germination and seedling phase of the maize life cycle (Fenner and Thompson, 2005) and its prominence as a key driver of divergent evolution and local adaptation (Berry and Bjorkman, 1980).

The optimal temperature for maize germination is between 26 °C and 29 °C (Riley, 1981). As temperature increases above this range, protein synthesis and the specific activities of enzymes are often hindered in the embryo, although there is significant genetic variation for this sensitivity (Riley, 1981). Furthermore, many studies on the effect of cold temperatures in maize have shown a strong, negative relationship between low temperatures and germination rate, emergence time, and germination uniformity (Miedema, 1982). The influence of seed genetics on germination response to temperature can be seen clearly in Eagles and Hardacre (1979). They created full-sibling and S1 (male parent selfed) families using highland tropical maize populations. They found that populations with highland germplasm exhibited significant variation in percent germination, percent emergence, and time to emergence at 10 ° C, especially when compared with hybrids from temperate regions (Eagles and Hardacre, 1979). Full-sibling families germinated and emerged at the highest rate and in the shortest time period, followed closely by S1 families. The crosses between corn of US, Canadian, or French origin germinated and emerged at the lowest rates and emerged an average of seven days after the Full-sibling families (Eagles and Hardacre, 1979). The authors concluded that maternal affects played a larger role in determining germination and emergence time than paternal affects (Eagles and Hardacre, 1979). Eagles and Brooking (1981) also found that at 11 ° C, 15/5 ° C, and 15/10 ° C,

several populations of highland Mexican maize emerged significantly faster than the Corn Belt Dent cultivars.

The relationship between local adaptation, temperature and emergence was further explored in a study examining early lifecycle characteristics of highland tropical, lowland tropical, northern latitude flint, and Corn Belt dent maize hybrids at 16/6 °C, 25/20 °C, and 35/30°C (Hardacre and Eagles, 1989). Highland hybrids exhibited “faster emergence, faster growth and leaf expansion rates, higher net assimilation rates, higher chlorophyll concentrations ... and higher dry weight portioning” at the coolest temperature (6/16°C ) while Corn Belt Dent hybrids exhibited superior growth at the warmer temperatures (Hardacre and Eagles, 1989). No hybrid was able to maintain productivity at all temperatures, illustrating the limits of phenotypic plasticity (Hardacre and Eagles, 1989). Thus, there is a substantial body of evidence supporting the assertion that response to temperature in the early maize lifecycle varies according to genetic differences and that locally adapted Mexican landraces are interesting candidates in which to study this aspect of genetic diversity.

Focusing on these early life cycle traits is essential in order to elucidate evolutionary processes, since the seedling stage is often the most sensitive stage in the plant lifecycle and maladaptation in this phase can create a demographic bottleneck in the population (Shimono and Kudo, 2003). Germination response to temperature is an especially important trait to characterize, since this the timing of germination is often temperature dependent and will effect the environment that the plant will experience for the remainder of the growing season (Shimono and Kudo, 2003). Previous work has been done to explore the role of temperature as a selective agent in the germination and seedling phase of the lifecycle. For *Silene ciliata*, a perennial alpine plant found in the Northern Mediterranean Basin, a reciprocal sowing experiment demonstrated

that populations had higher germination rates and seedling survival rates at the center of their elevational range than at the boundaries (Gimenez-Benavides et al. 2007). Since seedling survival is an essential component of lifetime fitness, it is an indication of local adaptation (Gimenez-Benavides et al. 2007). Evidence of patterns of local adaptation in the seed and seedling phases of the lifecycle has also been found in wild barley (*Hordeum spontaneum*) as well as two *Senecio* subspecies in Sicily (Volis et al. 2002, Ross et al. 2012).

The evidence marshaled clearly shows that there is a lacuna in the literature in regards to the germination behavior and early lifecycle traits of maize landraces sourced from along an elevational gradient in response to temperature. Maize seeds and seedlings are prone to respond to temperature. Thus, patterns of local adaptation at these phases in the lifecycle have been found in maize and other species. A great deal of work has been done to explore response to temperature in the early lifecycle in highland landraces from Mexico, but they have generally been compared with varieties from the US. Our work aims to determine how maize landraces from the highlands, midlands, and lowlands compare to *one another*. In this work, we hope to begin to elucidate the phases in the maize lifecycle that are most responsible for producing the pattern of local adaptation observed in Mercer et al. (2008), as well as the environmental influences that are most important in shaping this pattern. Furthermore, our work aids in the immense task of screening diverse germplasm from all over the globe for traits that could be utilized by maize farmers worldwide. Finally, this work illustrates the value of protecting crop genetic resources both *in situ* and *ex situ* for generations to come by describing the genetic variation within the populations for germination behavior and seedling morphological response to temperature.

## **Objectives and Hypotheses:**

We explored the early lifecycle characteristics of maize landraces sourced from an elevational gradient using temperatures that paralleled the temperature gradient present during the planting season in Chiapas, Mexico. We used nine landrace populations, three each from the lowlands, midlands, and highlands, which Mercer et al. (2008) demonstrated to be locally adapted. We designed the experiment to allow us to isolate temperature treatment, in order to better understand the role of temperature as a selective agent in the early maize lifecycle.

Specifically, our objectives were to:

- 1.) Determine how the temperatures that these landrace populations could experience during planting season along and elevational gradient effect the rate of germination and total germination percentages for the each of the nine landrace populations.
- 2.) Examine the effect of cold (10 ° C) periods of zero, seven, or 21 days on germination and seedling morphology, using AOSA standards to assess seedling normality. (AOSA, 2009)

I hypothesize that temperature will act as a directional selective agent on these populations and that it will affect populations differentially according to their home elevation. I predict that a pattern of local adaption will be observed, where the highland populations exhibit the highest levels of germination, the fastest rate of germination, and the fewest abnormal seedlings in the environment most similar to their home environment—the coldest temperatures. I predict that lowland populations will perform best in the warmest temperatures and midland populations will perform best at intermediate temperatures. If I observe this pattern, I will be able to conclude that there is evidence for local adaptation driven by temperature in the early phase of the maize lifecycle for these Mexican landrace maize populations. If this pattern is not

present, early lifecycle traits may be more plastic in their response to temperature, adaptation may be more important at later points in the lifecycle, or that temperature may not be a significant driver of local adaptation.

## **Materials and Methods:**

### *Seed Material*

In 2009, nine landrace maize population were collected from rural farmers whose farms were located at three elevations in Chiapas. Three populations were from the lowlands (~600m above sea level), three were from the midlands (~1500masl), two were from the highlands (~2000 masl) and one compilation of three populations from the highlands was used due to low seed numbers in each of the three populations. During the summer of 2010, these landrace populations were regenerated in a common garden in the highlands at ~2060 masl in Teopisca, Mexico. The seeds were collected, dried on the ear, and hand-shelled. Seeds with obvious damage sustained in transit or due to granivory or disease were removed. In germination experiments, these seeds were blocked according to seed shape (i.e., round or flat) in order to standardize germination rates, since flat seeds germinate at a faster rate than round seeds (Moreno-Martinez et al. 1998). Prior to use in the experiments, all seeds were sterilized using a 1% bleach solution agitated soak for 60 seconds followed by four, five-second rinses in distilled water. This was done in an effort to control ectophytic pathogens.

### *Experiment 1: The Effect of a Thermogradient on Germination Behavior*

In order to elucidate the differential effect of temperature on germination rate and total percent germination for nine maize landrace populations, we performed a germination

experiment using a thermogradient table. The seeds were sterilized and placed into 10cm diameter Petri dishes at a rate of ten per dish. The seeds rested atop two blue blotters (Anchor Paper Company, St. Paul, MN) wetted with distilled water and were covered with one wetted blotter for the first three days of the experiment to provide moisture on both sides of the seed during imbibition. The Petri dishes were checked daily for 25 days for germinated and dead seeds, which were removed on the day that they were recorded. Germination was defined as the breaching of the testa by both the radicle (or multiple secondary roots) and the plumule (Bewley, 1997). Distilled water was added to Petri dishes periodically to ensure that water availability was not a limiting factor for germination.

The seeds from the nine landrace maize populations were exposed to one of six constant temperatures, 10.0 °C, 14.5 °C, 19.5 °C, 23.0 °C, 27.5 °C, and 31.5 °C on a thermogradient table at the Seed Laboratory in Kottman Hall room 321 (see Table 1 for full description of temperatures used). The temperature range was designed to emulate the range of temperatures the seeds would likely encounter across the elevational transect from which they were collected, during the peak planting season (May to Mid-June) in Chiapas. Thus, the temperature range spanned from a temperature 1 °C warmer than the lowest average air temperature the seeds would experience in a highland environment, to a temperature about 3 °C cooler than the highest average temperature that the seeds would experience in a lowland environment. These averages were gleaned from Bioclim data containing the 30-year averages from 1971 to 2000 for representative locations in the highlands, the midlands, and the lowlands (<http://worldclim.org/bioclim>).

A split block design was used with the temperature bands on the thermogradient table as the main plots and maize populations as subplots randomized within each temperature. The

experiment was performed with four blocks, three of which used flat seeds and one of which used round seeds (roughly reflecting the proportion of each seed shape found within the populations). Thus, we confounded seed type with positional blocks to account for nuisance variation due to both. Each block covered half of the table and the experiment was run twice to attain four blocks.

We performed an analysis of variance using Proc GLM in SAS. First, we investigated the effects of block, temperature treatment, elevational group (Elv), and population within elevational group, as well as their interactions on cumulative germination at day four, seven, ten, and 25 (the final day of the experiment). We selected these days to provide us with “snapshots” of what might be occurring in the field at the very beginning stages of germination and emergence, the middle stages, and the final stages. Note that day 25 is almost certainly less ecologically relevant than days four, seven, and ten due to the disadvantages the seedlings would likely have encountered at this point in their life due to the increased likelihood that they would be outcompeted in the race to access precious resources.

Block and block by temperature treatment were considered random factors while the latter was also used as the error term to test the main plot effect of temperature treatment. Population within elevational group was used to test elevational group, and temperature treatment by population within elevational group was used to test the temperature treatment by elevational group interaction. Similarly, we clarified how germination curves (which elucidate rates of germination) differed across elevational groups exposed to the different temperatures and using Proc Lifetest in SAS, which performs a failure-time analysis (germination was equal to failure in our test). We also performed the same analysis by temperature to more clearly assess how elevational groups differed in their germination curves at each temperature. Finally, we

generated Wilcoxon and Log-Rank equality tests to elucidate significant differences between the curves at the beginning and end of the experiment, respectively.

### *Experiment 2: The Effect of Cold Periods on Seed Germination and Seedling Morphology*

We examined the effect of periods of cold (approximately 11.4 °C, with a range of 9.3 °C to 12.6 °C) prior to exposure to optimal conditions of 25 °C on total percent germination, the rate of germination, and seedling normality for nine landrace maize populations. We performed the experiment using standard and light weight rolled paper towels (Anchor Paper Co, St. Paul, MN) in a 25 °C germinator and a 11.4 °C cooler.

The three temperature treatments used included a zero day cold temperature treatment (i.e. immediate placement into the germinator set at 25 °C), a seven day cold temperature treatment at 11.4 °C followed by exposure to 25 °C (i.e., a traditional AOSA cold test, AOSA, 2002), and a 21 day cold temperature treatment at 11.4 °C followed by exposure to 25 °C. The first two treatments were represented in all four blocks and the third treatment was added to blocks three and four. As such, the zero day cold temperature treatment and the seven day cold temperature treatment were applied in a randomized complete block design with four blocks. Blocks three and four constituted a randomized complete block design for all three treatments (i.e., including the extended cold), albeit with less replication. The representative highland population which was composed of three populations was not used in blocks three and four for the zero day cold temperature treatment due to low seed availability.

All blocks and all treatments were analyzed jointly. Flat seeds were used in all blocks and all seeds were sterilized with 1% bleach solution. For all of the experimental units exposed to the zero day cold treatment, and blocks one and two of the seven day cold temperature

treatment, the bleach solution and the towels the seeds were planted on to and covered with were at room temperature. For blocks three and four of the seven day cold temperature treatment and the 21 day cold temperature treatment, the bleach solution and the towels the seeds were planted on to and covered with were chilled to  $\sim 10^{\circ}\text{C}$  to provide an imbibitional chilling shock. Ultimately, the block effect was not significant, however, so the imbibitional chilling shock did not affect the seeds greatly.

Fifty seeds were planted per towel using a planting board in order to ensure uniform distribution. The seeds were covered with a lightweight towel for moisture and stability. The towels were then rolled, placed into a clear plastic bag with up to three other towels, covered with another clear plastic bag to prevent moisture loss, and placed upright into either a germinator set at  $25^{\circ}\text{C}$ , with 12 hour day/night cycles, or a  $11.3^{\circ}\text{C}$  cooler with no light. Each towel represented one replicate. Seeds in the towels exposed to the zero day cold temperature treatment were planted seven days after the seeds exposed to the seven day cold temperature treatment so that they simultaneously occupied the  $25^{\circ}\text{C}$  germinator. The seeds exposed to the 21 day cold temperature treatment were planted at the same time as the seeds planted for the seven day cold treatment and were moved to the  $25^{\circ}\text{C}$  germinator after the other treatments were removed.

After the first seven days of cold treatment, all seeds were checked daily for germination and normal seedling morphology. The seeds were judged to be normal based on AOSA rules (AOSA, 2009 and Andy Evans, RST, Personal Communication), which require that a seedling possess both a healthy shoot and root system. Seedlings that were called abnormal were small, extremely diseased, were missing either a shoot or roots, or had an extremely damaged shoot. Once a seedling was judged to be normal or abnormal, it was removed from the towel. Seeds or

seedlings that died were either visibly infected with pathogens or the seed gave way to slight pressure with tweezers due to internal infection. Dead seeds were removed once it became obvious that the seed would not germinate. Diseased seeds that had germinated and then died were marked as abnormal, due to their small size. Some seeds germinated normally despite disease.

We performed analysis of variance tests using Proc GLM in SAS to elucidate the effect of block, temperature treatment, elevational group, population within elevational group, and their interactions on multiple categories of seed fitness. The categories of seed fitness we examined with this model included the total percentage of germinated seeds and the percentage of dead seeds. We also looked at the percentage of seeds that resulted in normal or abnormal seedlings, as well as the percentage of “unfit” seeds and seedlings (which consisted of abnormal seedlings plus dead seeds). Two percentages were used for each category, one derived from the total number of seeds in a towel (which was generally 50 but had a range of  $\pm 2$  seeds) and one derived from the average germination for each population under favorable conditions (the standard germination percentage). This latter method allowed us to use a measure standardized by “optimal germination”, thereby allowing us to examine effects of the cold treatments relative to the control. We graphed the least squared means and their standard errors to provide a visual representation of the analysis, explore general trends, and present the mean separations for significant factors and interactions. Finally, we used a Proc Lifetest within SAS which performed two failure-time tests using the germination data from days 0-25, with temperature treatment as strata. Finally, we generated Wilcoxon and Log-Rank equality tests to elucidate significant differences between the curves at the beginning and end of the experiment, respectively.

## **Results and Discussion:**

### *Experiment 1: The Effect of a Thermogradient on Germination Behavior*

Examining germination curves (percent germination over time) is extremely instructive. At the surface level, it clearly allows us to visualize the effect of temperature treatment and elevational type on the rate of germination and the total percent germination. It also allows us to surmise potential competition dynamics in the field. Finally, it provides a small window into the process of germination itself and the three phases of germination: imbibition, activation of enzyme systems, and the protrusion of the radicle and the plumule (Bewley, 1997).

### *The Effect of Temperature upon Germination Curves*

We used a Proc Lifetest failure-time analysis to investigate the percent germination for three elevational groups over time, at six temperatures. At the coldest temperature, 10.0 °C, we observed a pattern indicative of local adaption. The Wilcoxon and Log-Rank equality tests were both significant, indicating that curves significantly differed among the elevational groups at both the beginning and end of the experiment (Table 2). Although all three elevational types begin to germinate concurrently, circa day nine, the highlands germinated at the quickest rate and exhibited the highest level of total germination after 25 days, followed by the midlands, and the lowlands (Fig. 1A). This suggests that highland populations *may* have an advantage in the seed or early seedling phase of the lifecycle under extremely cold conditions. The ability to germinate at a fast rate can be an evolutionary advantage because it allows a seedling to begin to access limited resources such as water and nutrients more quickly (Fenner and Thompson, 2005). Conversely, fast germination and emergence under cold conditions could be maladaptive if the seedlings are prone to cold damage.

Sans perniciously cold conditions, however, earlier emergence would be a boon to a seedling, because it would decrease the seedlings likelihood of being blocked from light by nearby plants, especially weeds (Fenner and Thompson, 2005). *Ceteris paribus*, a faster germination rate is would lead to a correspondingly fast emergence rate, since germination is an irreversible process (Bewley, 1997). Eagles and Brookings (1981) found that maize populations sourced from the highlands *did* emerge faster than Cornbelt Dent populations under cold conditions ranging from 11-15 °C. Furthermore, Eagles and Hardacre (1989) found that populations with tropical highland germplasm emerged and grew faster (i.e. accumulated more dry weight and greater leaf area) when grown under 6/16 °C conditions than populations without highland germplasm. Our work corroborates their findings and fills a lacuna by demonstrating that highland populations also germinate more quickly under cold conditions than landrace maize sourced from other elevations. In conclusion, this body of evidence suggests that is most likely that seeds from the highland populations are adapted to contend with extremely cold conditions that would be damaging to other seedlings, since they germinate, emerge, and grow more quickly under cold conditions relative to populations from other elevations and latitudinal zones. This suggests that a pattern of local adaptation in response to temperature is discernible in the maize lifecycle under highland temperature conditions.

With increasing temperature, however, the evidence of local adaptation driven by temperature in the germination phase of the lifecycle dissipated. This indicates that there may be a range within which germination response to temperature is relatively plastic and that, while 10.0°C is outside this, range, both 14.5 °C and 19.5 °C are within it. At these two temperatures, neither the Wilcoxon, nor the Log-Rank equality tests were significant (Table 2), therefore, elevational type did not significantly affect germination response to temperature. It is interesting

to note, however, that although the difference was not significant, the midlands populations had the highest level of germination and the fastest rate of germination (Fig. 1B,C). Midland populations also reached their final germination levels much earlier (on day 18) than lowland and midland populations (on day 25). In fact, at every temperature except 10 °C, the midland populations were always the fastest to germinate (Fig. 1B-F).

With a continued increase in temperature, elevational group again became a significant predictor of germination rates. At 23.0 °C, 27.5 °C, and 31.5 °C, both the Wilcoxon and Log-Rank Equality test were significant (Table 2). The significance of the Wilcoxon and Log-Rank equality tests appear to be largely driven by the differences between the lowland and the midland populations (Fig.1D-F). The lowland populations were the slowest to germinate at all temperatures. Perhaps at the cooler temperatures, the slowness of the lowland populations could be a protective strategy to minimize the risk of exposing sensitive seedlings to damaging cold temperatures above ground. At the warmer temperatures, however, it is certainly not indicative of a pattern of local adaption and suggests that the different elevational groups may have differing life strategies (i.e. levels of resources allocation to individual seeds), they may have aged differentially, or it may be a result of seed vigor or seed quality differences.

#### *The Effect of Temperature on Germination Curves*

For each elevational group, it was extremely clear that the six different temperature treatments produced different germination curves. Wilcoxon and Log-Rank equality tests, which measure the effect of temperature on the germination curves, were significant for each of the elevational groups (Table 3). Their significance appears to be driven by the differences in germination rate observed at 10.0 °C, 14.5 °C, and 19.5 °C (Fig. 2A-C). For all of the elevational

types, the germination curves for 23.0 °C, 27.5 °C, and 31.5 °C did not appear to differ substantially from one another (Fig. 2A-C). The lower temperatures induced more noticeable and possibly adaptive responses to temperature.

The fact that temperature influenced germination rate is not surprising, given the voluminous literature on the subject (Riley, 1981, Miedema, 1982, Fenner and Thompson, 2005). What is interesting, however, is that this pattern suggests that there may be a distinct biological threshold, in between 19.5 °C and 23.0 °C, where temperature begins to affect the seeds in a more substantial way. This is likely due to a slowdown in the reaction rate for at least one enzyme that is important in the germination process. Furthermore, our previous analysis indicates that this slowdown in enzymatic activity does not affect the elevational types in a significantly different way at 14.5 °C and 19.5 °C (Fig. 1 B,C), suggesting that the enzymes involved may be fairly equally represented in all elevational types. At 10.0 °C, however, it could be that the three elevational types produced different enzymes or different levels of enzymes according to their type, since we observed significant differences in the germination rate at this temperature (Fig.1 A). Although their results were not statistically significant, Turner et al. (1994) documented a pattern of local adaptation in the thermotolerance of the enzyme glutathione reductase, an antioxidant. Glutathione reductase functioned best at lower temperatures in highland maize cultivars and functioned best at higher temperatures in lowland maize cultivars. Our study is hardly conclusive on this matter however, as the differential germination rates observed at 10.0 °C could be the result of a multitude of factors (e.g. the level of water uptake that the seeds are capable of at a given temperature), but it is promising.

A fascinating avenue for further research would lie in continuing to expose the seeds to even more extreme temperatures and observing their germination behavior. Although this

approach would surely lose all semblance of ecological relevance, it would be a very interesting way to screen for phenological traits involved in thermotolerance and adaption. In the context of the more realistic scenario of global climate change, which is expected to warm Chiapas by approximately 3 °C in the next 40 years (Christenson et al. 2007), it appears that the germination rate and level for all the elevational types will increase slightly or largely be unchanged. Our study cannot comment on multitude of other effects of climate change (e.g. changes in the precipitation regime) or on the effect of climate change at other phases in the maize lifecycle. It is good to know however, that increases in temperature *alone* are unlikely to substantively affect the populations adversely during the germination phase, *ceteris paribus*. Our results indicate, however, that the pattern of local adaptation observed in the germination phase of the lifecycle may become obsolete with an increase in temperature of only a few degrees. Therefore, it is possible that highland populations may risk abandonment by smallholder farmers in Chiapas if their evolutionary and yield advantages (their cold tolerance) are rendered useless.

#### *ANOVA Analysis of Factors Effecting Germination Curves at Days Four, Seven, Ten and 25*

Using Proc GLM in SAS, we analyzed the effects of block, temperature treatment, block by temperature treatment, elevational group (Elv), and population within elevational group, as well as their interactions, on the cumulative germination observed on days four, seven, ten, and 25 (the final day of observation). For each of the time periods examined, an ANOVA analysis indicated that temperature treatment was significant (Table 4). Tukey-Kramer comparisons of the effect of temperature, showed an interesting pattern. On day four, germination levels at , 10.0 °C and 14.5 °C grouped together, 19.5 °C was solitary, 23.0 °C and 27.5 °C grouped together, as well as 27.5 °C and 31.5 °C (Fig. 3A). On day seven, 23.0 °C, 27.5 °C, and 31.5 °C grouped together, followed by 19.5 °C and 23 °C, while 14.5 °C and 10.0 °C were significantly different from all

other temperatures (Fig. 3B). On day ten, 19.5 °C, 23 °C, 27.5 °C, and 31.5 °C grouped together, while 14.5 °C and 10.0 °C remained solitary (Fig. 3C). Finally, on day 25, 14.5 °C through 31.5 °C grouped together, while 10.0 °C was again, solitary (Fig. 3D). Thus, over time the range of temperature in which no significant difference in cumulative germination level could be observed expanded. Even at day 25, however, that temperature range still did not include 10.0°C. These results are indicative of plasticity of germination in response to temperature, which increased over time but was never present at 10.0 °C. Of course, this pattern, whereby seeds exposed to 14.5 °C and 31.5 °C have similar rates of survival after 25 days, would likely not be observed in the field, given the advantages which seeds that have germinated earlier can accrue.

On days four and seven, population within elevational group was significant according to the ANOVA analysis (Table 4). A Tukey-Kramer comparison illustrated that this was likely largely due to one lowland population performing quite well, while the two others had the lowest germination of all populations (Fig. 5A). The midland and highland populations largely grouped as separate elevational groups, with midlands exhibiting the highest average germination on this day. On day seven, variation among populations from all elevational groups seemed to cause the significant population within elevational group effect (Fig. 5B). After day seven, variation within elevational group according to population was not significant (Table 4).

On days ten and 25, the ANOVA analysis showed that elevational group and the elevational group by temperature treatment interaction were both significant (Table 4). On day ten, the midland populations had the greatest number of seeds germinated, followed by the highland and lowland populations (Fig. 6A). A Tukey-Kramer analysis indicated that midland mean germination was significantly greater than that of the highlands and lowlands, but the highlands and lowlands did not differ. On day 25, the same ranking held, however the highlands

were not significantly different from the midlands, but both were significantly different from the lowlands (Fig. 6 B). In regards to the temperature treatment by elevational group interaction on days ten and 25, it appears to be driven largely by the behavior of all of the elevational groups under 10.0 °C conditions (Fig. 6 A,B). As temperature increased beyond 10.0 °C, the differences in cumulative germination between the three elevational types decreased (Fig. 6 A,B).

#### *Experiment 2: The Effect of Cold Periods on Seed Germination and Seedling Morphology*

Prior to the protrusion of the radicle and the plumule, the seeds were going through the previous two phases of germination: imbibition and activation of enzyme systems (Bewley, 1997). Evidence of this imbibition period, can be seen clearly when examining the effect of the zero day cold temperature treatment, the seven day cold temperature treatment, and 21 day cold temperature treatment on germination using Proc Lifetest (Fig. 7). Seeds exposed to zero days of cold treatment (i.e. immediately placed in the 25 °C germinator) required a period of at least two to three days during which imbibition and activation of the enzymes occurred before visible germination. In contrast, seeds already exposed to seven or 21 days of the cold treatment, had obviously already undergone these processes, as evidenced by their substantially faster rate of germination upon placement in the 25 °C germinator (Fig. 7). Some seeds exposed to these two cold treatments had even germinated in the 11.4 °C conditions, albeit at a slower rate. Since these seeds were two year old crop seeds, it's not likely that they had a significant level of dormancy (Gepts, 2004). Therefore, the germination rate observed was likely controlled almost exclusively by the different temperature treatments, rather than dormancy breaking mechanisms. A test of the equality of the curves by treatment using the Wilcoxon and Log-Rank statistics showed that the curves were significantly different in the early stages of the experiment as well as the later (Table 5).

### Percent Germination:

A SAS GLM analysis indicated that temperature treatment, population within elevational group, and temperature treatment by elevational group had significant effects ( $P \leq 0.05$ ) on percent germination (Table 6). Elevational group was nearly significant ( $P \leq 0.1009$ ) (Table 6). According to a Tukey-Kramer comparison, the seeds experiencing the zero day cold temperature treatment exhibited a significantly higher percent germination, than the seeds experiencing the seven day cold temperature treatment and the 21 day cold temperature treatment. The seven cold temperature treatment and the 21 day cold temperature treatment did not differ significantly from each other (Fig. 8). Although not statistically significant, it is interesting to examine the interactions between temperature treatment and elevational group. Across each of the temperature treatments, midland populations exhibited the highest levels of germination, followed by lowland and highland populations (Fig. 9). The percent germination levels of lowland and highland groups decreased with periods of cold temperature, with the seeds exposed to the 21 day extended cold period exhibiting the lowest levels of percent germination (Fig. 9). In contrast, the percent germination of the midland populations did not appear to decrease substantially with exposure to cold (Fig. 9).

### Percent Dead:

We defined percent dead, as the percentage of a seeds which died *and* did not germinate. Temperature treatment, population within elevational group, and the temperature treatment by elevational group interaction were all significant ( $P \leq 0.05$ ) (Table 6). Increased exposure to cold significantly increased seed mortality (Fig. 10), however, there was also a significant treatment

by elevational group interaction. Midland populations were significantly less likely to die before germination across all treatments (Fig. 11).

#### Percent Normal:

We defined the percent normal as the number of normal seedlings divided by the total number of seeds planted per population. Both temperature treatment and population within elevational group had significant effects on the percent normal ( $P \leq 0.001$ ) (Table 6). A Tukey-Kramer comparison indicated that while the zero day cold temperature treatment was conducive to the highest levels of normal seedlings within the populations, it did not differ significantly from the seven day cold temperature treatment. The 21 day cold temperature treatment, however, caused to a significant drop in the level of normal seedlings within the populations (Fig. 12). Thus, we can conclude that while cold period of seven days did not affect seedling normality, there is a point in between seven and 21 days in the cold where levels of seedling normality begin to decline significantly. The significance of population within elevational group seemed to be driven by variability between populations in all elevational groups (Fig.13). Although the differences were not statistically significant, midland populations performed at higher levels than both the highland and lowland populations across temperature treatments (Fig.14).

The percent normal gives us an indication of the effects of the three temperature treatments on the likely fitness levels of the populations during their seedling phase. The measure is a good addendum to percent germination, since it allows us to peer into the immediate next phase of the lifecycle and elucidate how many seeds would likely survive beyond the V1-2 leaf phase. Our results indicate that cold temperatures for seven days or less would not likely adversely affect the fitness of germinating seeds in the field. Since the

elevational group effect was insignificant (Table 6), our results suggest that the elevational groups would be equally likely to survive to the next phase of the lifecycle if in the field under the temperature conditions tested (Fig. 14).

#### Standardized Percent Normal:

By examining the total percent normal for each population divided by the percent germination observed in 25 °C conditions (i.e. the population's standard germination, AOSA, 1981), we can see the effect of treatment more clearly. For example, if a population had 80% germination in the standard germination test and 79% germination under cold conditions, the decrease in germination would be much less than if the population had 100% germination in the standard germination test and 79% germination under cold conditions. *Nota bene*, in making predictions concerning the likelihood of seedling survival to the next phase of life in the field, the percent normal is a more instructive metric than the standardized percent normal.

Overall, since germination was fairly high for most populations (Fig. 9, at the zero day cold temperature treatment), the differences between the total percent normal and the standardized percent normal were small. It is interesting to note that only temperature treatment was still significant ( $P \leq 0.001$ ), while population within elevational groups was only *nearly* significant using this metric ( $P \leq 0.0631$ ) (Table 6). In contrast with the percent normal analysis, in which elevational group was not significant, elevational group was nearly significant in the analysis of the standardized percent normal metric ( $P \leq 0.0874$ ) (Table 6). This suggests that the variation in percent normal due to elevation was increased by standardizing the data. Clearly, temperature treatment was the largest source of variation for the percent normal and the standardized percent normal in our seedling morphology experiment.

The interaction between temperature treatment and elevational group was not significant, nevertheless, we observed interesting trends. Namely, midland populations trended towards slightly outperforming both lowland and highland populations when exposed to the seven day cold temperature treatment and especially when exposed to the 21 day cold temperature treatment (Fig. 15). We would have expected that the longer the cold waiting period, the better highland populations would perform in comparison with the other populations, since highland populations are most likely to encounter cold periods *in situ*. This did not happen, however, and it was the midland populations that were least affected by the cold. The lowland populations do exhibit some elements of a pattern of local adaption, since clearly normality decreased with increasing time in the cold and they were most fit sans cold treatment. Although they were slightly more fit than midland populations under the warm treatment, they were not significantly so and thus, we cannot say that they show a complete pattern of local adaption in this phase of the lifecycle.

#### Percent Abnormal:

Examining the level of seedling abnormality is useful because it denotes the proportion of seeds which were able to germinate but would still be incapable of reaching the next stage of life. It is therefore indicative of seedling response to environment. In contrast, examination of the dead seeds only allows us to look at the ungerminated seeds response to the environment. Thus, we can subdivide our understanding of each population's response to environment into two categories: seed (dead or germinated) and seedling (normal or abnormal).

Temperature treatment was the only significant source of variation for percent abnormality ( $P \leq 0.001$ ), suggesting that populations from all elevations tend to be equally

abnormal across conditions. A Tukey-Kramer analysis indicates that seedlings experiencing the seven day cold temperature treatment produced the lowest percent abnormality, followed by those in the zero day temperature treatment, and finally, those in the 21 day cold temperature treatment (Fig. 16). All treatments were significantly different from one another. This illustrates that the seven day cold temperature treatment may be beneficial for the seedlings in some way, because it decreased levels of abnormality. This pattern can also be seen clearly when examining the interaction of treatments and elevational group, which was not significant (Figure 17). The pattern is especially evident in the midland populations, but is also present among the highland populations. For lowland populations, the seven day cold temperature treatment did not greatly affect levels of abnormality, but the 21 day cold temperature treatment produced a large increase in the percent abnormality. The pattern we observed, whereby the percentage of abnormal seedlings decreased after the seven day cold temperature treatment, may be the result of lower disease levels after this treatment. Conversely, since the percent abnormal also included those seedlings that germinated and then died, this result could be caused by the fact that seeds that might have germinated and been called abnormal when exposed to the zero degree temperature treatment, were unable to germinate when exposed to the seven day temperature treatment. Since the percentage of dead seeds increased with exposure to cold temperature, this seems likely (Fig. 10).

#### Standardized Percent Abnormal:

Using this metric, again, only the temperature treatment is significant ( $P \leq 0.001$ ) but population within altitude is nearly significant ( $P \leq 0.0633$ ) (Table 6). This metric suggests that there may be some populations which are more likely to produce abnormal seedlings.

## Percent Unfit

When analyzing the results from an evolutionary perspective, the two most interesting results are the percent normal, which could also be called the percent “fit”, and the percentage of “unfit” seeds and seedlings. We defined the percent unfit as the percentage of abnormal seedlings plus the percentage of dead seeds. It gives an indication of what percentage of the population is unlikely to survive past the seedling stage under the conditions imposed. For the percent unfit, both temperature treatment and population within elevational group were significant ( $P \leq 0.001$ ) (Table 6). The 21 day cold temperature treatment produced the highest levels of unfit seeds and seedlings, followed by the seven day cold temperature treatment, and the zero day cold temperature treatment, which were not significantly different from one another but were both significantly less than the 21 day cold temperature treatment, as shown by a Tukey-Kramer comparison (Fig. 18).

The fact that the 21 day cold temperature treatment produced more “unfit” seeds and seedlings mirrors the low germination levels observed at 10.0 °C in the thermogradient experiment (Fig. 1A). The fact that the seven day cold temperature treatment and zero day cold treatment were not significantly different from one another may indicate better tolerance of cold in landrace populations than US cultivars (Hardacre and Eagles, 1989). For population with elevational group, lowland populations were the most variable while midland and highland populations largely grouped together. In general, midlands populations were the most fit and highlands were the least fit (Fig. 19). Although the temperature treatment by elevational group interaction was not significant, midland populations showed fewer declines in fitness, while the fitness of highland and lowland populations declined more substantially with increasing time spent in the cold temperature (Fig 20).

## **Conclusions:**

In conclusion, a clear pattern can be seen throughout the analysis, whereby midland populations were most fit across temperature treatments. The exception, of course, was the percent germination levels and rates of germination observed at 10.0 °C in the thermogradient experiment, which did follow a pattern of local adaptation. Nevertheless, the preponderance of our work suggests that the evolutionary advantage in thermotolerance enjoyed by highland populations in highland environment, described by Mercer et al. (2008), may only be present in the seed or seedling stage of the lifecycle at temperatures very low temperatures, such as those experienced early in the planting season in the highlands.

Follow up research examining the mechanisms responsible for the phenomenon we observed (differential germination rate according to elevational type at 10.0 °C) is warranted. Our work has helped identify a narrow range of temperatures (10.0 °C or below) within which this pattern can be observed, and could be foundational for further studies examining the activity and thermotolerance of enzymes or other biological processes (such as imbibition) that affect germination. Our work also suggests that a period of cold temperature (11.4 °C), followed by exposure to warmer temperatures (25 °C) does not necessarily harm seedlings after seven days but does harm seedlings after 21 days. Since temperature in the field is constantly changing, further inquiry into the effect of periods of cold temperatures is greatly needed. It is possible that periods of cold temperature, which slows the germination rate of populations from different elevational groups differentially, could impart advantages to cold adapted populations, since early germination is often advantageous from an evolutionary perspective.

The fact that midland populations outperformed the others across temperature treatments may be due to increased plasticity or other factors, such as increased vigor. Vigor in seeds could result from adaptations to factors other than temperature (tested here) or could result from hybrid vigor. On this latter point, it seems likely that midland populations might frequently cross with lowland or highland populations or farmers might mix in seed from these other sources, given the intermediate character of the midland environment. If midland crosses with these other types are successful (i.e. seeds survive and reproduce), then midland populations could acquire a wider genetic base. Some of this diversity might influence hybrid vigor. Of course, while the patterns indicated by our research may endorse this hypothesis, much more research would be needed to make this determination. What our research clearly indicates is that across these treatments, the midland populations were significantly more fit in this stage of the life cycle in an *ex situ*, common garden situation.

It is also possible that the different fitness levels could be the result of seed quality. Seed quality is partially defined as the ability of a seed to germinate rapidly and uniformly across temperatures (Ellis, 1992). The differing seed quality could be the result of the health of the maternal plant, genetics, or possibly different life strategies for the various populations. For example, perhaps midland populations produced less seed per ear or per plant than highland populations, but the seed they produced was of higher quality. It is interesting to consider seed quality in this way, as a life strategy of the plant rather than a characteristic of the seed. Further research is needed to ascertain whether midland plants always produce the highest quality seed because it is part of an evolutionary strategy or if seed quality relative to other elevational types is stochastic.

Another reason that we could be seeing increased midland seed fitness is that the seeds from different elevational groups may age differentially. Our populations were grown and collected in 2010 and they have sat for approximately 2 ½ years. It is possibly that this length of time could have reduced seed quality in lowland and highland types more quickly than the midland types, as they consist of different maize races with substantively different phenological traits. Unfortunately, the effect of ageing would be difficult for us to determine without knowing the health of the population when it was first harvested and at varying intervals afterward. This would be a fascinating follow up experiment.

The human dimension of agriculture also further truncates our ability to describe and predict the genetic changes occurring at a population level for landraces in Chiapas (Mercer and Perales, 2010). This is due to the fact that farmers are the final arbitrators of what gets planted each year. Since most farmers in Chiapas plant three seeds per hole (Mercer, personal communication), seed quality may not be highly important as a driver of selection. If only 33% of the seed a farmer planted survived, a farmer's yield could still remain unaffected. Nevertheless, these potential varying life strategies are of interest to biologists and should be of interest to agronomists as well, since high germination rates for some populations may indicate that portions of the seeds planted could be saved and eaten.

In conclusion, our research illustrates a distinct, although not significant, pattern of midlands performing better than lowland or highland populations across all temperature treatments, except for the coldest temperature used in the thermogradient experiment. At this temperature, 10.0 °C, we observed a clear and significant pattern of local adaption; whereby the seeds from the highland populations germinated at the highest level and the fastest rate, followed the midland and lowland populations. This suggests that local adaption may be observed at

temperatures at 11.4 °C and below, and that local adaption in the seedling phase in response to temperature is likely most important *in situ* in highland gardens in an early planting situations. This phenomenon also helps to explain why improved varieties, which perform well in the midland and lowland areas, do not perform well in highland environments. These interactions, and the light they can shine on the importance of early the early lifecycle in the process of local adaptation, are important to understand both from an ecological perspective as well as an agronomic perspective in order to assist farmers globally, who benefit from the *in situ* preservation of maize germplasm, and the farmers of Chiapas themselves.

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## Tables

**Table 1:** Temperatures measured on the thermogradient table throughout the course of the experiment. Temperatures were designed to emulate temperatures that maize seeds from Chiapas, Mexico would likely encounter during the planting season. Mode will be used as short hand for temperature in results and figures, but it important to keep in mind that the seeds were exposed to a range. The range was produced by the thermogradient table itself, which had a small gradient within the bands from one end of the table to the other.

Temperature Band	Range	Mode
1	7.9-12.01 °C	10.0 °C
2	14.77-14.05 °C	14.5 °C
3	20.22-18.91 °C	19.5 °C
4	24.17-22.72 °C	23.0 °C
5	27.82-26.45 °C	27.5 °C
6	31.92-30.29 °C	31.5 °C

**Table 2.** Test of equality of curves by elevational group at each of the six temperatures used in the thermogradient experiment, using the Wilcoxon and Log-Rank statistics in Proc Lifetest. Significant effects of  $P < 0.05$  are in bold.

Wilcoxon				Log-Rank		
Temperature	DF	$X^2$	$Pr > X^2$	DF	$X^2$	$Pr > X^2$
10.0 °C	2	25.3318	<b>&lt;.0001</b>	2	21.6898	<b>&lt;.0001</b>
14.5 °C	2	4.8957	0.0865	2	5.4671	0.065
19.5 °C	2	3.4488	0.1783	2	2.014	0.3653
23.0 °C	2	6.6837	<b>0.0354</b>	2	9.1838	<b>0.0101</b>
27.5 °C	2	17.6521	<b>0.0001</b>	2	18.3415	<b>0.0001</b>
31.5 °C	2	17.2745	<b>0.0002</b>	2	22.0589	<b>&lt;.0001</b>

**Table 3.** Test of equality of curves by temperature for each of the three elevational groups in the thermogradient experiment, using the Wilcoxon and Log-Rank statistics in Proc Lifetest. Significant effects of  $P < 0.05$  are in bold.

Wilcoxon				Log-Rank		
Elevational Group	DF	$X^2$	$Pr > X^2$	DF	$X^2$	$Pr > X^2$
Highland	5	511.576	<b>&lt;.0001</b>	5	627.15	<b>&lt;.0001</b>
Midland	5	533.407	<b>&lt;.0001</b>	5	673.873	<b>&lt;.0001</b>
Lowland	5	432.736	<b>&lt;.0001</b>	5	562.067	<b>&lt;.0001</b>

**Table 4.** ANOVA, generated in SAS GLM, identifying the factors affecting cumulative germination at day four, seven, ten, and 25 in the thermogradient experiment. Significance ( $P < 0.05$ ) is indicated in bold.

Day 4 Cumulative Germination				Day 7 Cumulative Germination			Day 10 Cumulative Germination			Day 25 Cumulative Germination		
Source	D F	F Value	Pr > F	D F	F Value	Pr > F	D F	F Value	Pr > F	D F	F Value	Pr > F
Block	3	50.62	<b>&lt;.0001</b>	3	12.57	<b>&lt;.0001</b>	3	20.49	<b>&lt;.0001</b>	3	13.42	<b>&lt;.0001</b>
Temperature Treatment	5	74.31	<b>&lt;.0001</b>	5	147.58	<b>&lt;.0001</b>	5	66.91	<b>&lt;.0001</b>	5	22.17	<b>&lt;.0001</b>
Block* Temperature Treatment	15	6.12	<b>&lt;.0001</b>	15	5.56	<b>&lt;.0001</b>	15	5.77	<b>&lt;.0001</b>	15	6.8	<b>&lt;.0001</b>
Elevational Group	2	3.55	0.0959	2	3.08	0.1199	2	6.42	<b>0.0323</b>	2	12.79	<b>0.0069</b>
Population (Elevational Group)	6	3.99	<b>0.001</b>	6	2.64	<b>0.0183</b>	6	1.67	0.1333	6	0.88	0.5109
Temperature Treatment* Elevational Group	10	1.06	0.4243	10	1.08	0.4043	10	4.51	<b>0.0006</b>	10	5.08	<b>0.0002</b>
Temperature Treatment* Population (Elevational Group)	30	1.02	0.4488	30	1.27	0.1764	30	0.67	0.9029	30	0.72	0.8513

**Table 5:** Test of equality of curves generated with the temperature treatments used in the seedling morphology experiment as strata, using the Wilcoxon and Log-Rank statistics in Proc Lifetest. Significant effects of  $P < 0.05$  are in bold.

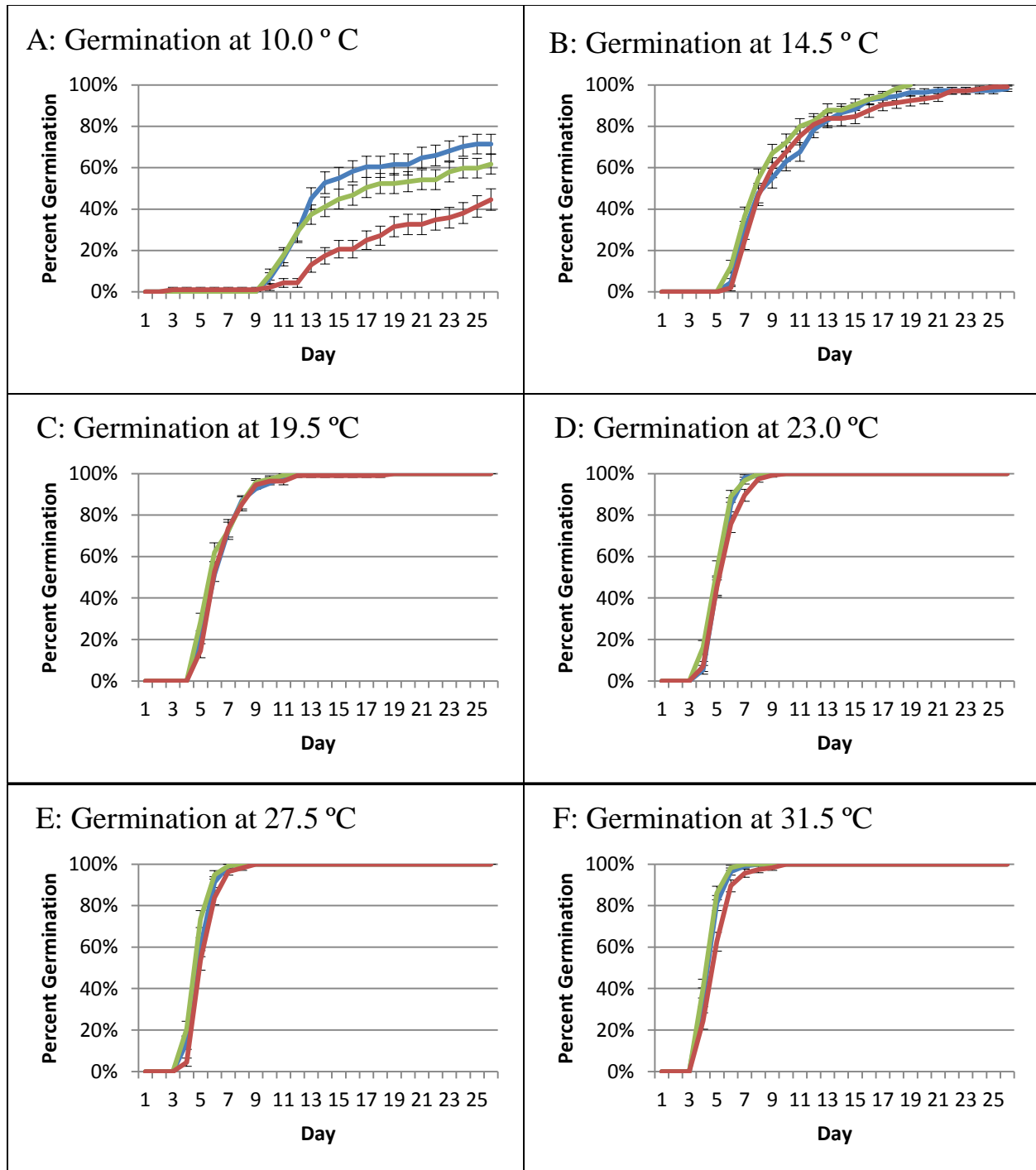
Test	DF	$X^2$	Pr > $X^2$
Log-Rank	2	2087.312	<b>&lt;.0001</b>
Wilcoxon	2	2191.869	<b>&lt;.0001</b>

**Table 6:** ANOVA, generated in SAS GLM, identifying the factors affecting the total percentage of seeds that germinated, died, were normal, abnormal, or unfit. Significance ( $P > 0.05$ ) is indicated in bold.

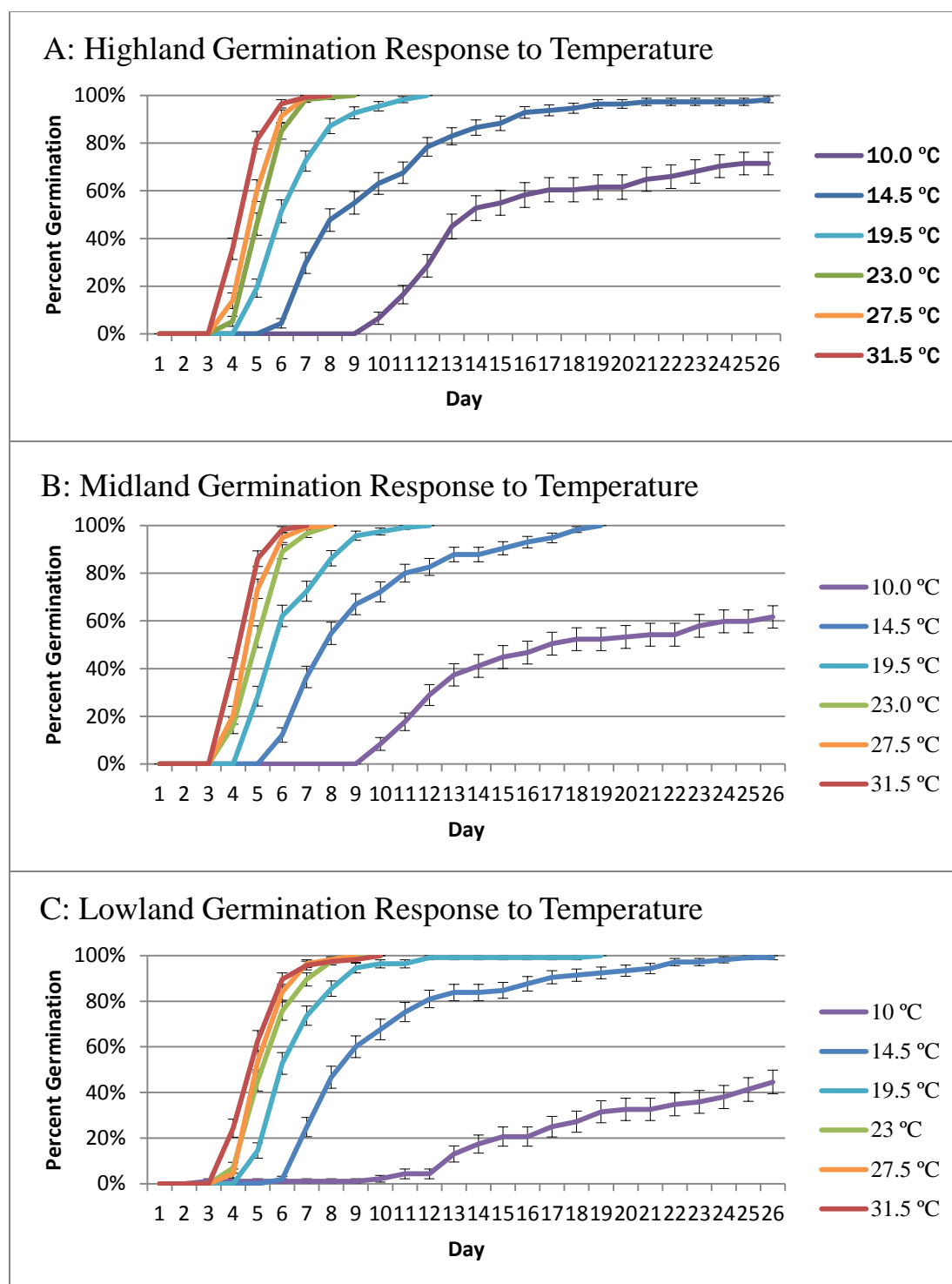
Percent Germinated				Percent Dead			Percent Normal			Percent Normal (Standardized)		
Source	D F	F	Pr > F	D F	F	Pr > F	D F	F	Pr > F	D F	F	Pr > F
Block	3	2.15	0.1037	3	2.8	<b>0.0482</b>	3	0.78	0.5117	3	0.51	0.6759
Temp_Trt	2	17.6 6	<b>&lt;.0001</b>	2	28.12	<b>&lt;.0001</b>	2	16.82	<b>&lt;.0001</b>	2	17.6 3	<b>&lt;.0001</b>
Elev Group	2	3.44	0.1009	2	2.11	0.2025	2	2.61	0.1526	2	3.76	0.0874
Population (Elev Group)	6	7.77	<b>&lt;.0001</b>	6	12.44	<b>&lt;.0001</b>	6	8.14	<b>&lt;.0001</b>	6	2.14	0.0631
Temp_Trt * Elev Group	4	4.62	<b>0.0172</b>	4	3.61	<b>0.0374</b>	4	1.83	0.1881	4	1.29	0.3291
Temp_Trt* Population (Elev Group)	12	0.93	0.5231	12	1.27	0.264	12	1.42	0.1837	1 2	1.43	0.1812

Percent Abnormal				Percent Abnormal (Standardized)			Percent Unfit		
Source	D F	F	Pr > F	D F	F	Pr > F	D F	F	Pr > F
Block	3	2.69	0.0547	3	2.7	0.054	3	0.78	0.5117
Temp_Trt	2	15.4 7	<b>&lt;.0001</b>	2	15.68	<b>&lt;.0001</b>	2	16.82	<b>&lt;.0001</b>
Elev Group	2	0.47	0.6436	2	0.52	0.6179	2	2.61	0.1526
Population (Elev Group)	6	1.71	0.1358	6	2.14	0.0633	6	8.14	<b>&lt;.0001</b>
Temp_Trt * Elev Group	4	1.28	0.3331	4	1.25	0.3407	4	1.83	0.1881
Temp_Trt* Population (Elev Group)	12	1.43	0.1803	12	1.57	0.1285	12	1.42	0.1837

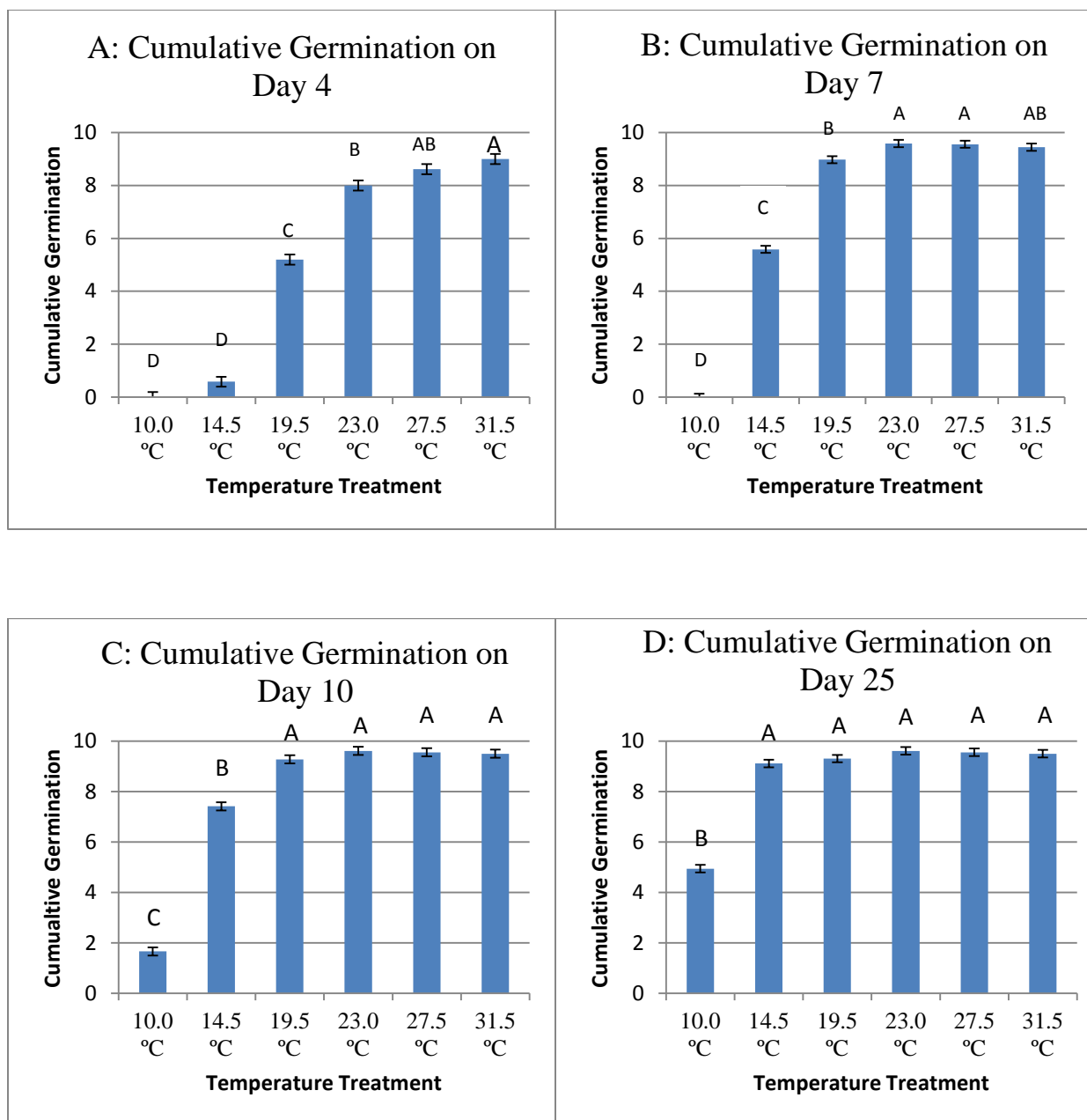
# Figures:



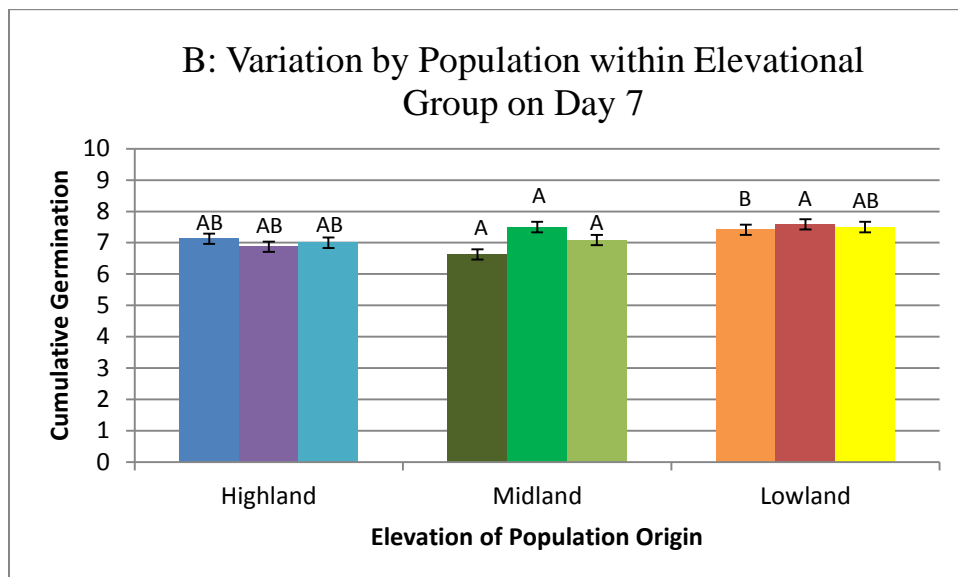
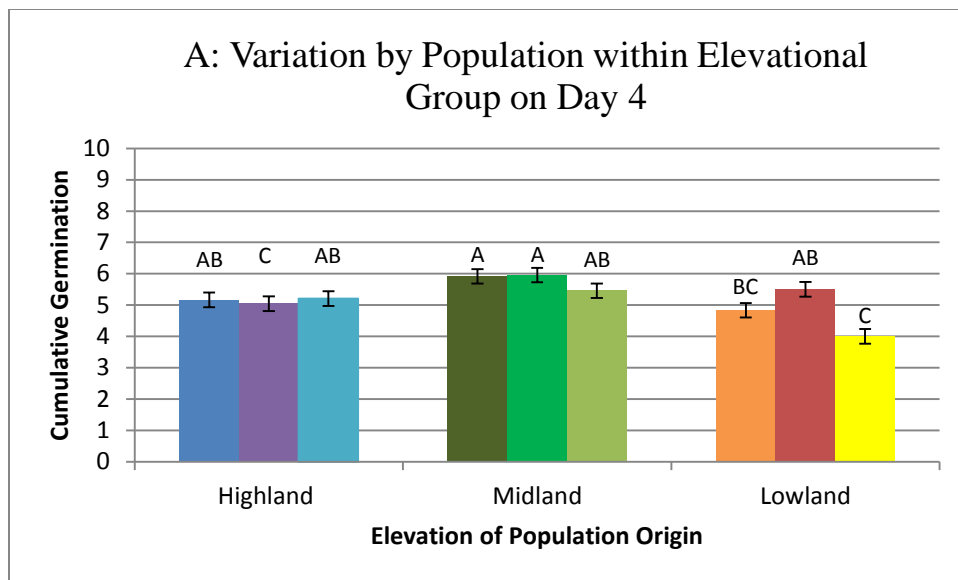
**Fig. 1** The effect of elevational type on germination of landrace maize from Chiapas, Mexico at six temperatures. Germination curves and standard error bars were produced using Proc Lifetest. Significant differences among elevational group curves at each temperature are indicated by tests in Table 2.



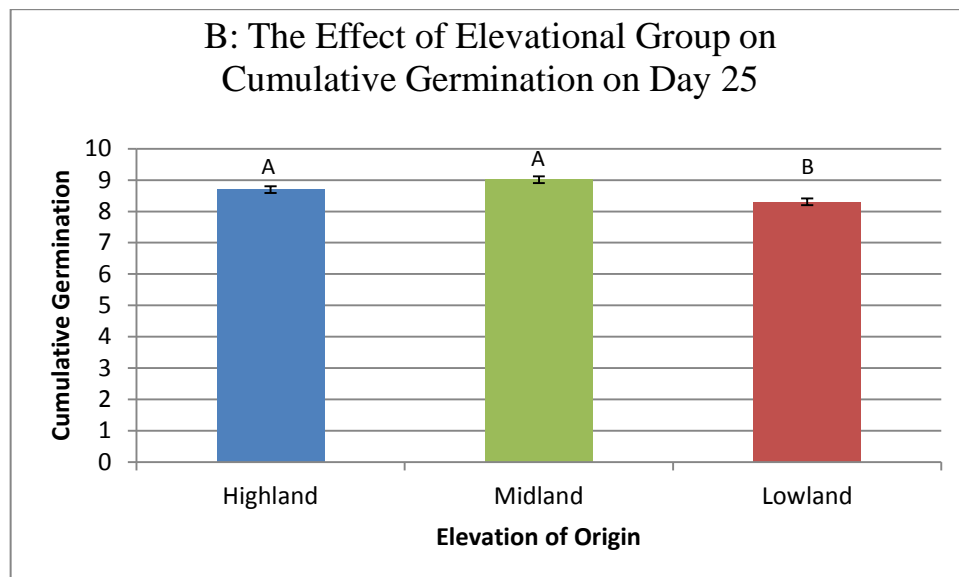
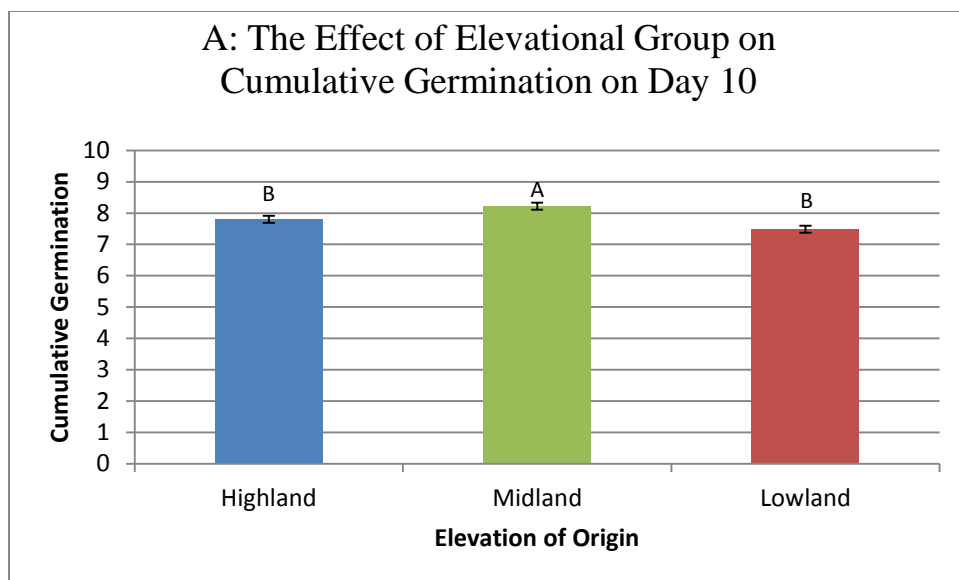
**Fig. 2** The effect of the temperature on germination rate for landrace maize from Chiapas, Mexico among highland, midland, and lowland elevational groups. Germination curves and standard error bars were produced by Proc Lifetest. Significant differences among the temperatures treatments for each elevational group are indicated by tests in Table 3.



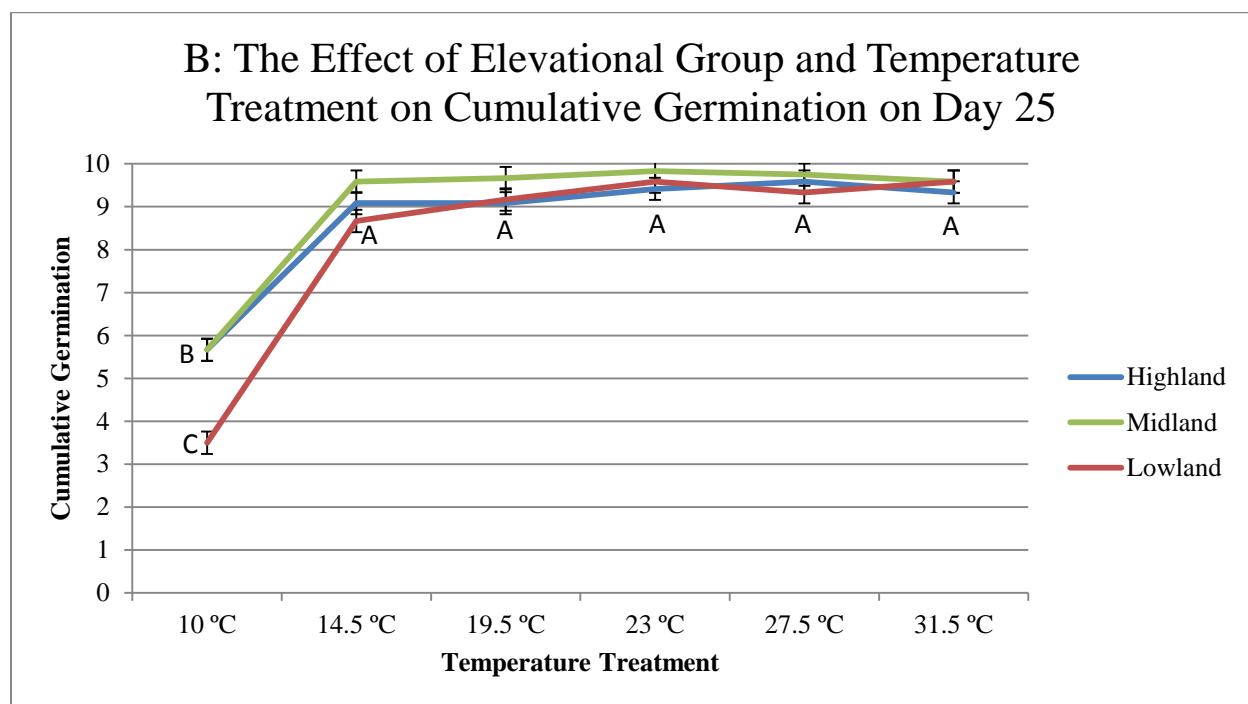
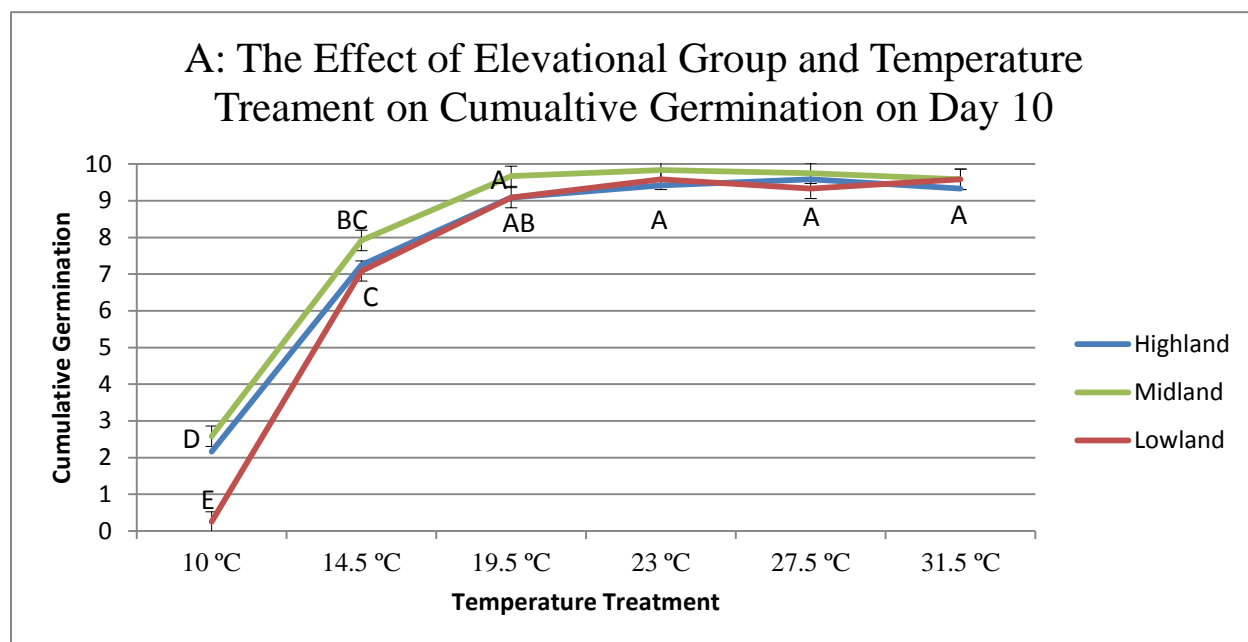
**Fig. 3** The effect of temperature treatment on cumulative germination of landrace maize from Chiapas, Mexico, from the thermogradient experiment. The ls means and standard errors were produced in a GLM SAS analysis. Significant differences between bars, elucidated via a Tukey-Kramer analysis, are indicated using letters.



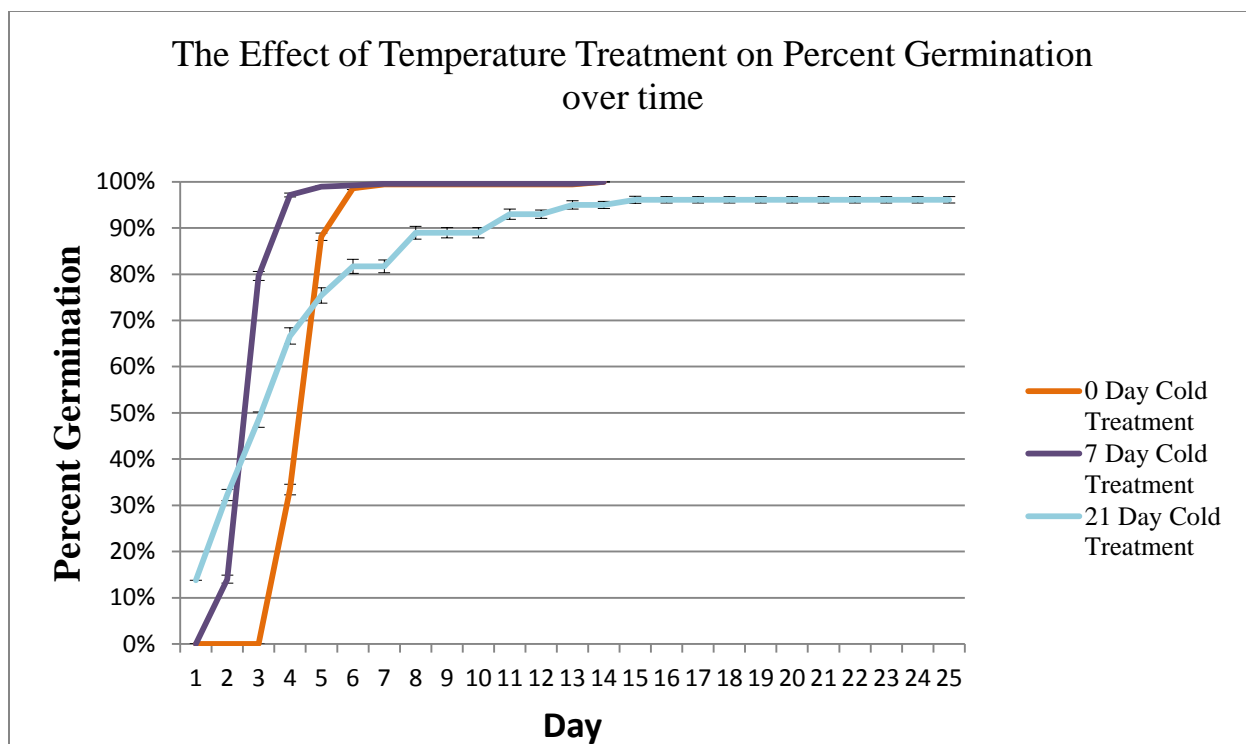
**Fig. 4** The effect of population within elevational group on cumulative germination in populations of landrace maize from Chiapas, Mexico on days four (Panel A) and seven (Panel B). Each population is shown via one bar. We used SAS GLM to generate the ls mean and standard error bars. Significant differences found in a Tukey-Kramer analysis are indicated with letters.



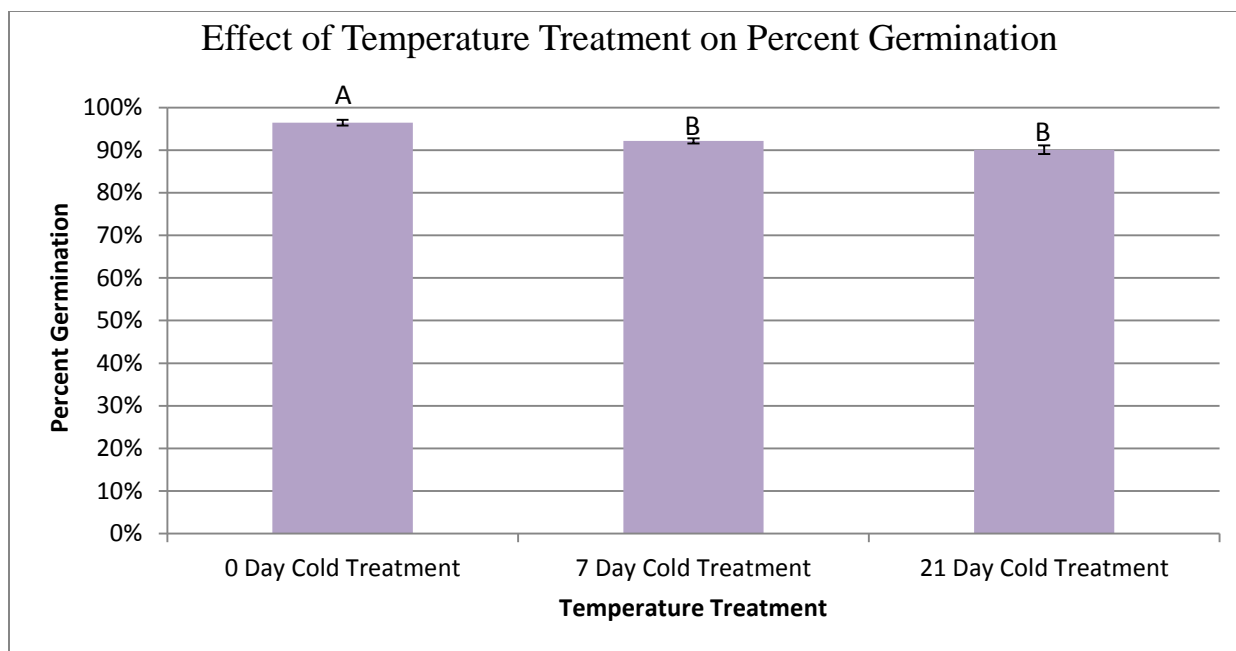
**Fig. 5** The effect of the elevation of origin (elevational group) on cumulative germination of Mexican landrace maize from Chiapas, on day ten (Panel A) and day 25 (Panel B) in the thermogradient experiment. We used SAS GLM to produce the ls means and standard error bars. Significance, according to a Tukey-Kramer comparison, is denoted with letters.



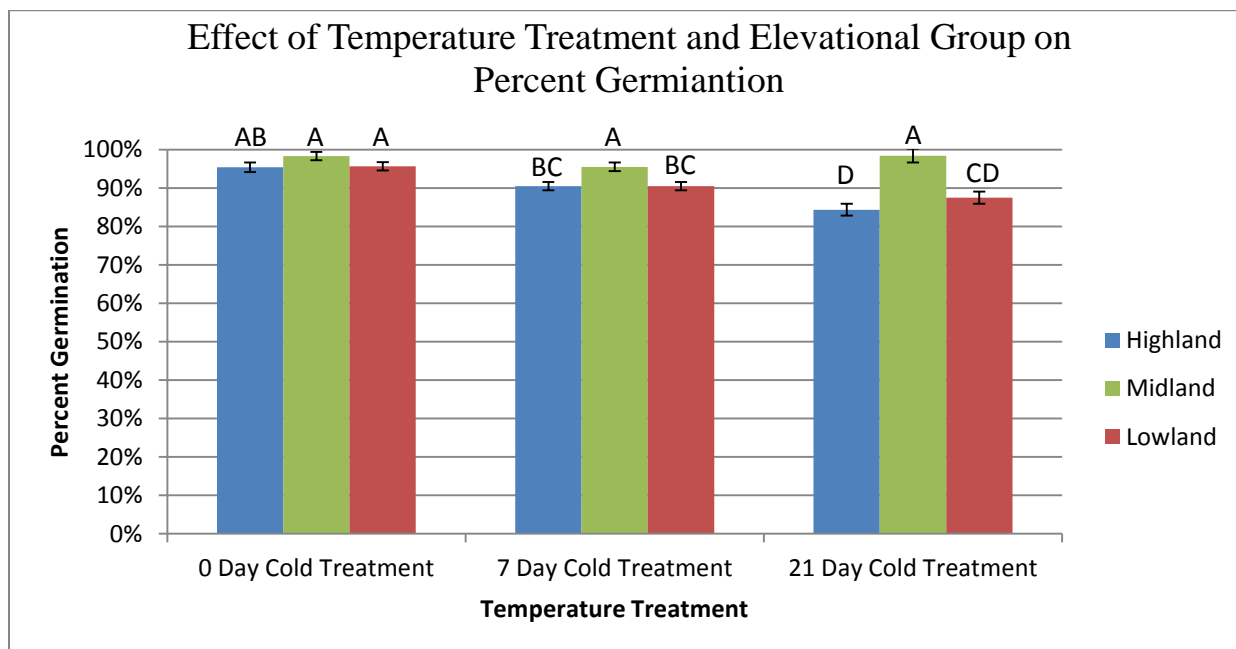
**Fig. 6** The effect of temperature treatment and the elevation of origin on cumulative germination of landrace maize from Chiapas, Mexico on day ten (Panel A) and day 25 (Panel B) of the thermogradient experiment. Proc SAS GLM was used to produce the ls means and standard error bars. Significant differences, as ascertained through a Tukey-Kramer comparison, are denoted with letters. Temperatures 23.0 °C to 31.5 °C on day ten and 14.5 °C to 31.5 °C on day 25 did not significantly differ from one another.



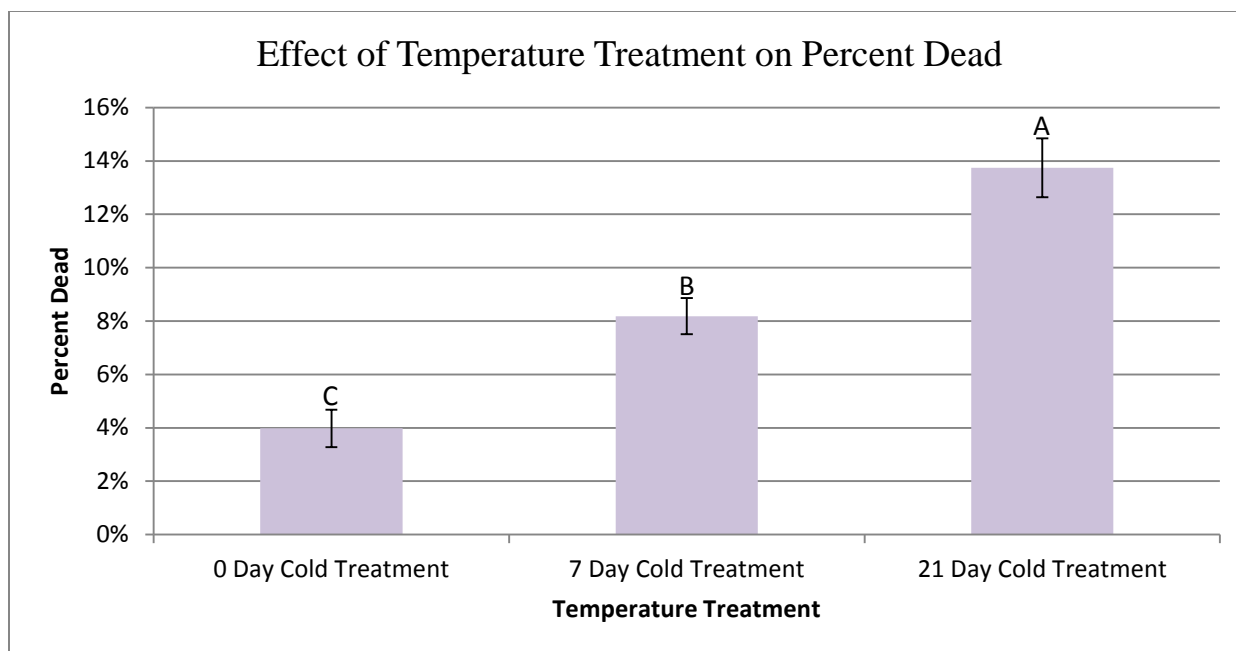
**Fig. 7** The effect of temperature treatments of zero days at 10.0 °C, seven days at 10.0 °C, and 25 days at 10.0 °C on the percent germination of landrace maize seeds from Chiapas, Mexico upon placement into a 25 °C germinator. Each series represent a different temperature treatment. Germination curves and standard error bars were produced using Proc Lifetest.



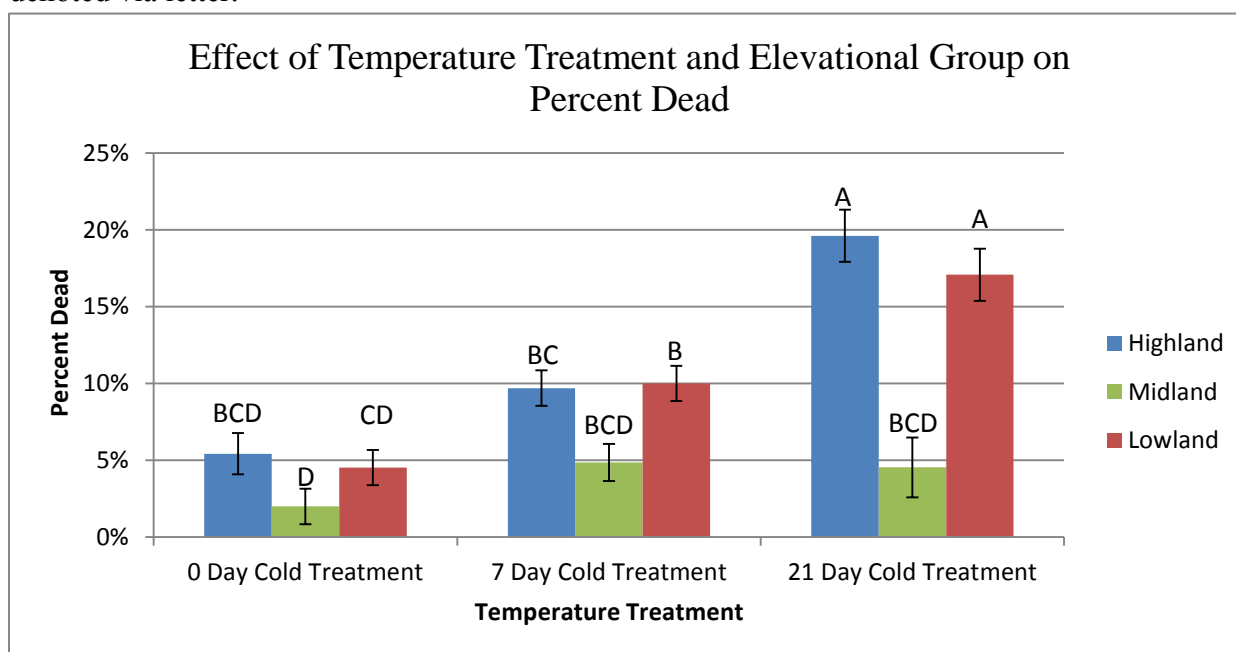
**Fig. 8** The effect of temperature treatment on final percent germination of seed lots of landrace maize from Chiapas, Mexico in the seedling morphology experiment. Each series represents a different temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



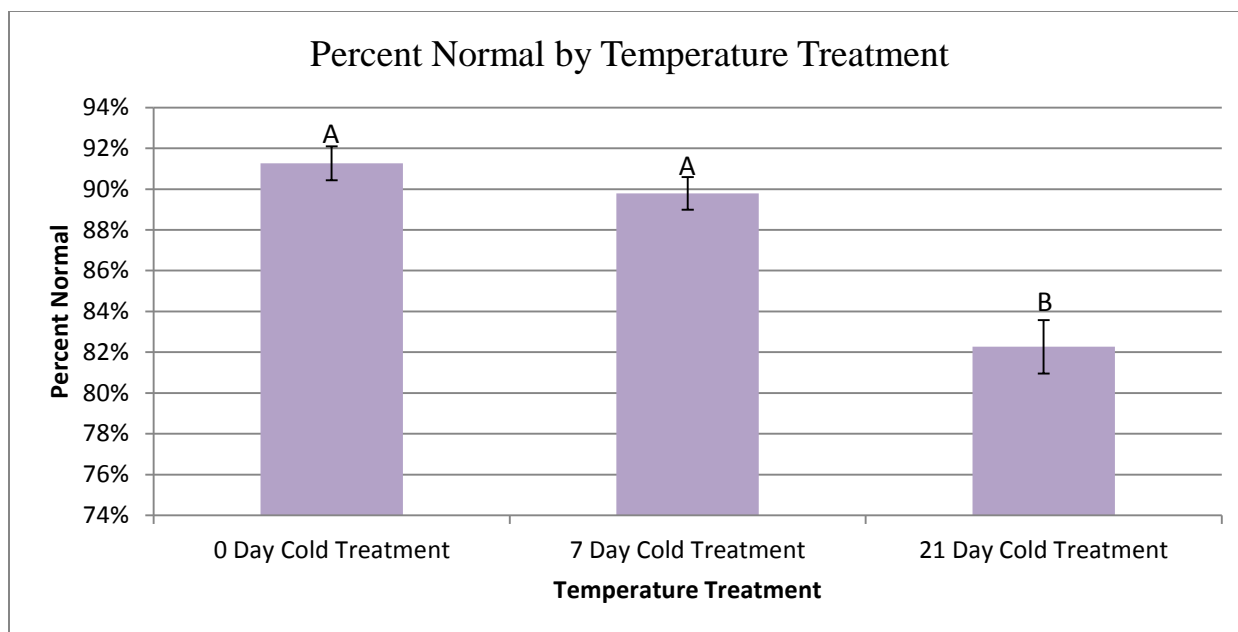
**Fig. 9** The effect of temperature treatment and elevational group on final percent germination of seed lots of landrace maize from Chiapas, Mexico in the seedling morphology experiment. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Differences were not significant.



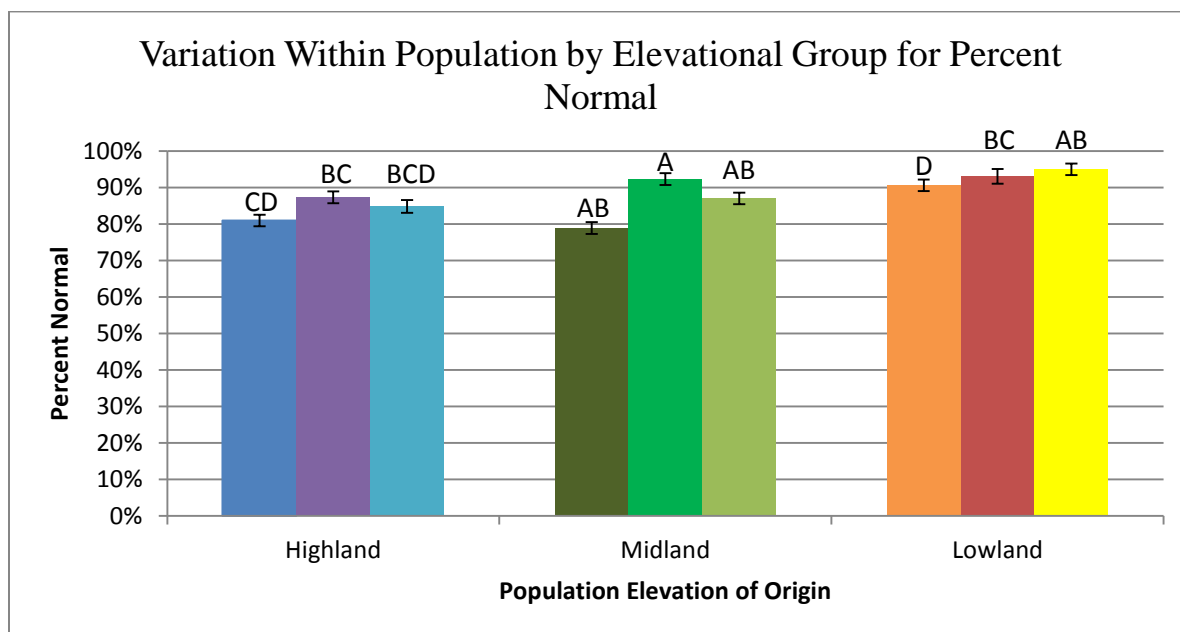
**Fig. 10** The effect of temperature treatment on the percent dead in seeds from landrace maize cultivars from Chiapas, Mexico in the seedling morphology experiment. Each series represents a different temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



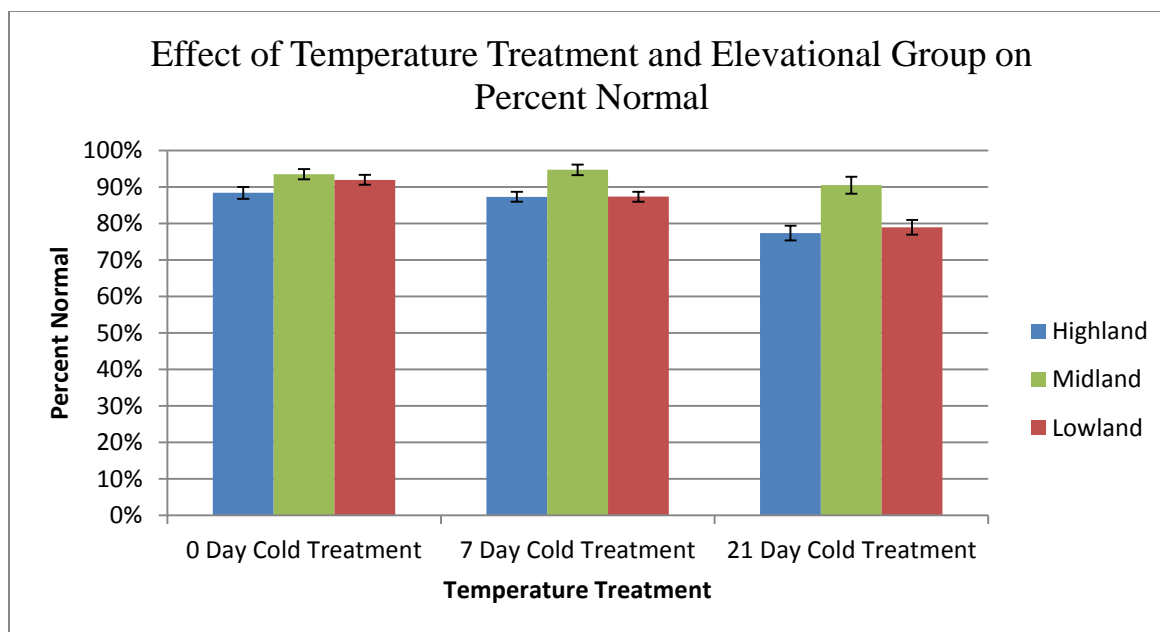
**Fig. 11** The effect of temperature treatment and elevational group on the percent dead in seeds from landrace maize cultivars from Chiapas, Mexico in the seedling morphology experiment. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



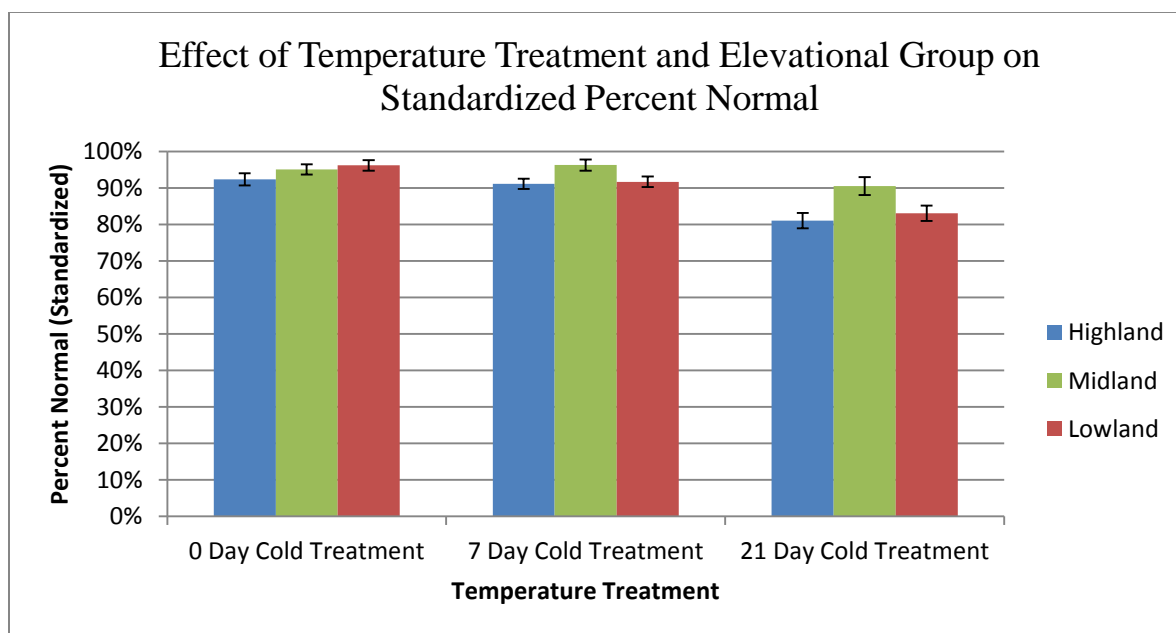
**Fig. 12** The effect of temperature treatment on the percent normal seedlings from landrace maize cultivars from Chiapas, Mexico in the seedling morphology experiment. Each series represents a different temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



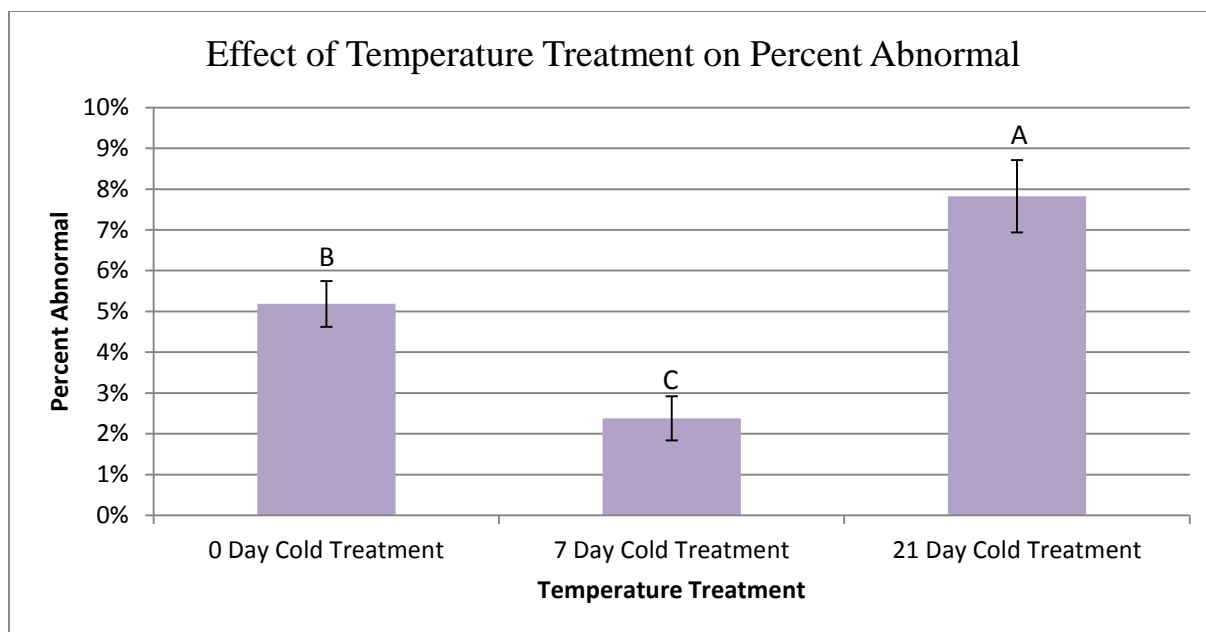
**Fig. 13** The effect of population within elevational group on percent normal in populations of landrace maize from Chiapas, Mexico. Each population is shown using one bar. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences found in a Tukey-Kramer analysis are indicated with letters.



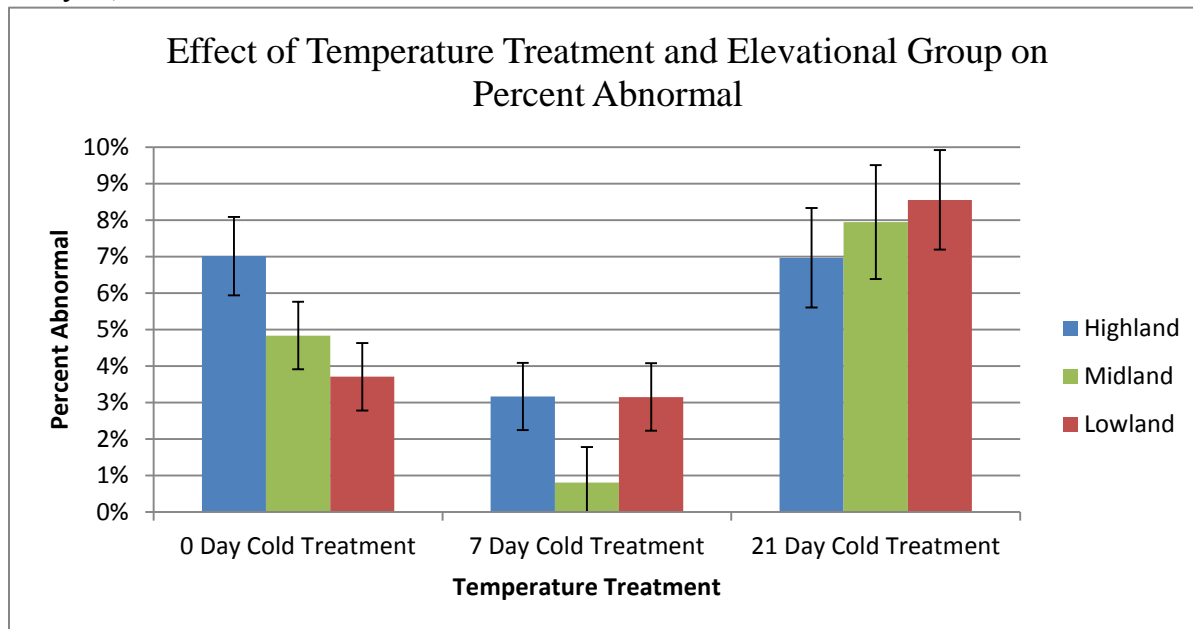
**Fig. 14** The effect of temperature treatment and elevational group on the percentage of normal seedlings in landrace maize from Chiapas, Mexico in the seedling morphology experiment. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Differences were not significant.



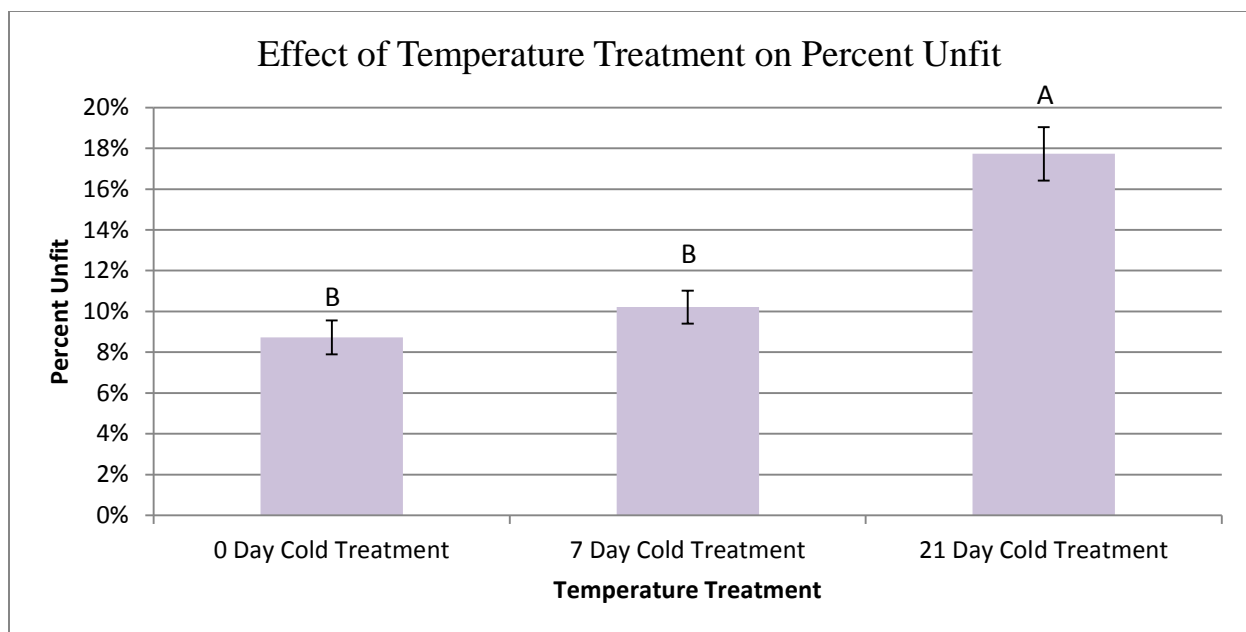
**Fig. 15** The effect of temperature treatment and elevational group on the percentage of normal seedlings over the percentage of seeds which germinated in a standard germination test for landrace maize from Chiapas, Mexico in the seedling morphology experiment. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Differences were not significant.



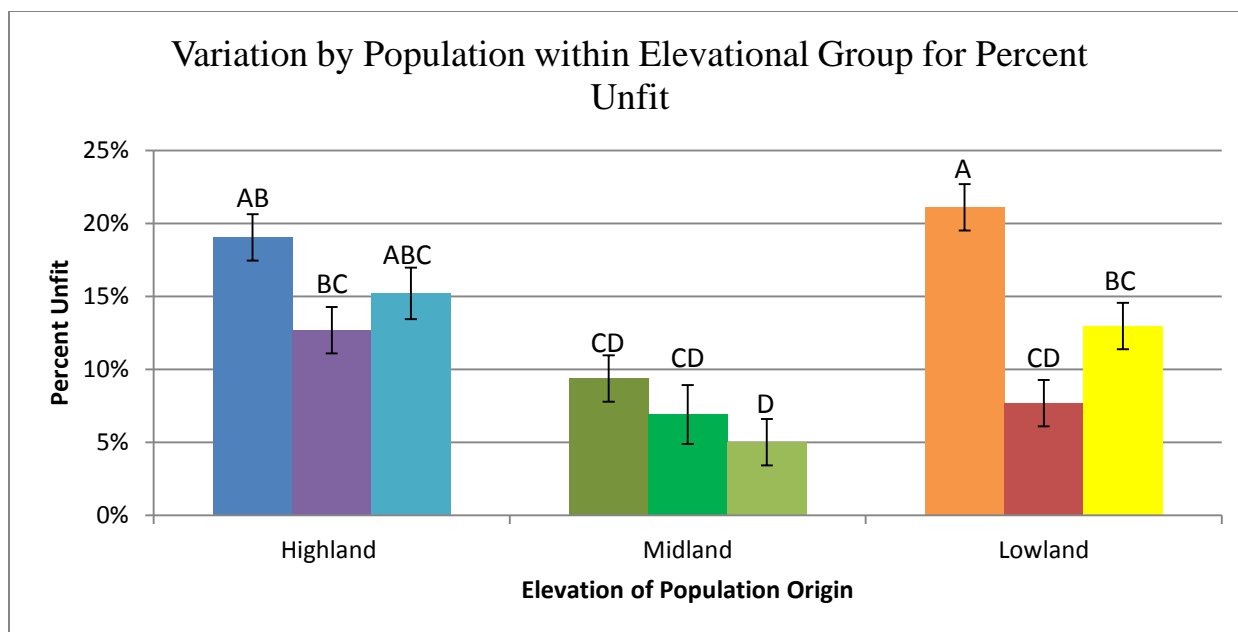
**Fig. 16** The effect of temperature treatment on the percent abnormal seedlings from landrace maize cultivars from Chiapas, Mexico in the seedling morphology experiment. Each series represents a different temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



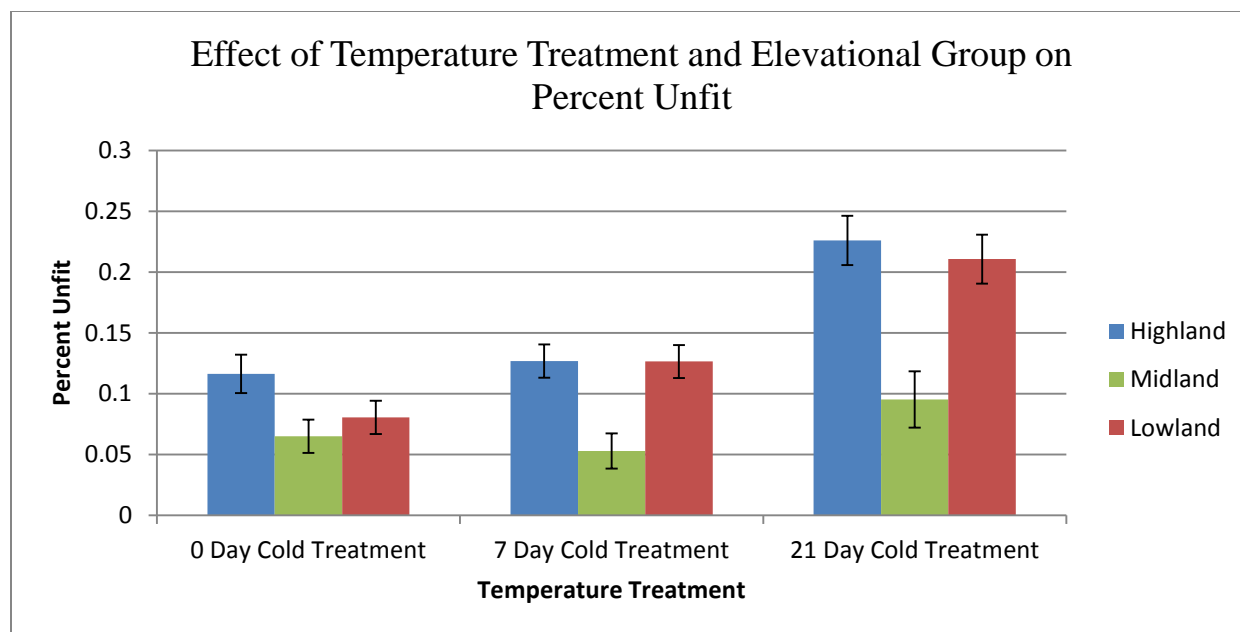
**Fig. 17** The effect of temperature treatment and elevational group on the percentage of abnormal seedlings for landrace maize from Chiapas, Mexico in the seedling morphology experiment. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Differences were not significant.



**Fig. 18** The effect of temperature treatment on the percent of “unfit” seeds and seedlings from landrace maize cultivars from Chiapas, Mexico in the seedling morphology experiment. “Unfit” seeds and seedlings either died in the course of the experiment or were abnormal, indicating that they would not survive beyond the seedling phase in the field. Each series represents a different temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences, according to a Tukey-Kramer analysis, are denoted via letter.



**Fig. 19** The effect of population within elevational group on percentage of “unfit” seeds and seedlings for landrace maize populations from Chiapas, Mexico in the seedling morphology experiment. “Unfit” seeds and seedlings either died in the course of the experiment or were abnormal, indicating that they would not survive beyond the seedling phase in the field. Each population is shown using one bar. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Significant differences found in a Tukey-Kramer analysis are indicated with letters.



**Fig. 20** The effect of temperature treatment and elevational group on the percentage of unfit seeds and seedlings for landrace maize from Chiapas, Mexico in the seedling morphology experiment. “Unfit” seeds and seedlings either died in the course of the experiment or were abnormal, indicating that they would not survive beyond the seedling phase in the field. Each series represents an elevational group at a temperature treatment. The errors bars illustrate the standard error of the LS means, produced in SAS GLM. Differences were not significant.