Climate Change Impacts on a Native Endemic Thistle, *Cirsium pitcheri*

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Abstract

Two experiments were conducted to assess the germination, growth, and survival of *Cirsium pitcheri* Torr. ex Eaton T & G in the Chicagoland region. The first experiment involved growing seeds from various native populations to determine tolerance for the current Chicagoland climate. The second experiment tested three of the populations from the first experiment to determine tolerance for the future Chicagoland climate predicted by climate change models. The three populations were selected to test large versus small populations and three populations along a latitudinal gradient, as well as examine populations that did well in the first experiment versus a population that did poorly. The first experiment revealed that large, northern populations had the highest germination rates and the low inbreeding coefficients were the best predictor of high rates of germination and survival. The results from the climate change experiment showed that temperature, not precipitation, was the important factor for future growth. All populations performed worse at the 2095 temperature than the control or 2030 temperature for all four measured growth metrics: length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight. No comparisons of the increased precipitation versus decreased precipitation treatments revealed a significant difference, suggesting that predicted changes in precipitation amount will not be a factor affecting growth or survival. Phenotypic plasticity did not have enough of an impact to prevent significant biomass losses under temperature stress, indicating that a combination of high genetic diversity and low inbreeding coefficients appears to be the best mechanism for tolerating climate change.

Introduction

Climate change has become a serious concern for the protection of species with narrow distributions or particular climate restrictions. As climates shift, these at-risk populations may be unable to tolerate new conditions or find ecological bridges to new areas of suitable climate (Williams et al. 2007) and will get phased out as new steady-state species assemblages arise (Chapin et al. 2004). Prior research has tended to focus on the risks to species at significant altitude and the polar ends of their ranges (Pauli et al. 2003, Gimenez-Benavides et al. 2007) due to the frequent inability of those populations to find a new habitat that replicates the one that was lost. However, plants associated with narrow habitat specificity, such as shoreline ecosystems around the Great Lakes, can face similar challenges. Rate of plant migration may not match the rate of climate change, leaving species dependent on rare events of long-distance outlier dispersal to find new suitable habitats (Pitelka 1997). Additionally, human impacts that currently threaten shoreline communities (development, recreation, management practices) can also interrupt natural migration routes and prevent movement of species impacted by climate change. If migration is not possible, because of barriers to dispersal or the desire to preserve the species in its current position, the focus then shifts to the ability of the population to tolerate the new environmental conditions. Some plants may attempt to adapt to their new climate, though the number of generations required may exceed the rate of predicted climate change (Etterson and Shaw 2001). Other plants may find themselves unchallenged by the change in environment. For dune plants at the southern end of their range, the possibility exists that their distribution has been limited by the boundary of their habitat, not the extent of their tolerances. Therefore, it is important for conservation efforts focused on the Chicagoland region to determine whether plants of concern have the ability to survive the climate conditions they are predicted to encounter in their current location.

Population survival is directly related to the impact climate change has on plant life stages: germination, growth, and reproduction. Unfortunately, very little research has focused on the effect of climate change on any aspect of plant germination. The predicted lengthening of the growing season (Jentsch et al. 2007) means that seedlings will emerge earlier in the year, but the effect this change will have on the germination rate is unknown. Temporal shifts brought about by climate change are also predicted to have a major impact on reproduction. Research has already uncovered mistimed mating in a range of bird species (Schiegg et al. 2002) and reports of early flowering (Parmesan 2006) raise concerns regarding plant-pollinator asynchronicity, though factors like long flowering times and multiple generalist pollinators have the potential to ameliorate some of those concerns (Keddy and Keddy 1984). Potentially more compelling for Great Lakes dune plants is the effect climate change will have on the interplay between growth and reproduction. Increasing temperature is reported to increase aboveground biomass (Walker et al. 2006), while increasing CO_2 has been shown to increase belowground biomass (Ziska 2003). Aside from shortening generation time for perennial plants with size-dependent flowering cues (D'Ulisse and Maun 1996), this increased growth rate also has the potential to improve the germination rate. Additional maternal biomass increases seed weight (Lalonde and Roitberg 1989), which leads to a larger proportion of viable seeds produced (Maun et al. 1996). An added, though unexpected, boon to plant growth and reproduction could come from climate-influenced increases to deer herbivory.

Increased atmospheric CO_2 is expected to lower nitrogen content in leaves (Ayres 1993), forcing additional browsing to provide the same level of dietary nitrogen. However, deer herbivory has been shown to lead to increases in both seed production and subsequent seedling survival (Paige and Witham 1987). While these studies combine to suggest a range of benefits provided by climate change, they fail to include the water stress predicted for the Chicagoland region; in fact, some of the results are qualified as only being valid with sufficient water availability. Determining the impact of drought on predicted climate change effects is vital to forecasting the future health of local populations.

Also important to population survival is the interplay of climate change and local genetic diversity. Geographically proximate populations can exhibit a range of genetic health (Hamze and Jolls 2000), even in species that are relatively genetically depauperate (Loveless and Hamrick 1988). Unfortunately for the populations at the low end of the range, genetic diversity is predicted to be a critical factor in surviving the effects of climate change, especially potential reductions to reproductive output (Bawa and Dayanandan 1998). Inbred individuals are less likely to respond to temporal shifts in reproduction due to climate change (Schiegg et al. 2002), which is particularly dangerous for annual and monocarpic perennial plants. However, populations that are actively being managed have outlets to improve both genetic diversity and inbreeding levels in order to resist harmful effects from climate change. Gene flow between populations mitigates the loss of genetic diversity (Lowe et al. 2005), and managers have several mechanisms to choose from to artificially boost gene flow: pollen transfer, addition of donor seed to the seed bank, even transplanting individual seedlings from genetically healthy populations. Identifying populations with high genetic diversity could provide a helpful start for any efforts intended to improve the genetic stock of at-risk populations.

Potentially significant for populations with low genetic diversity is their capacity for phenotypic plasticity, the ability of an organism to alter its phenotype in response to changes in the environment. It is well established that plants are able to alter their biomass allocation between root and shoots in response to environmental signals (Bloom et al. 1985, Garnier 1991), increasing the ability of individuals to cope with climate change. However, plasticity can also manifest in other ways; plants recovering from herbivory produce more aboveground biomass than ungrazed plants, but this improvement is paired with a loss in belowground biomass (Phillips and Maun 1996, Rowland and Maun 2001). Unfortunately for plants in dune communities, plasticity may have little effect on quick-growing or short-lived species (Atkin et al. 2006) and can actually be a hindrance to individuals in highly dynamic environmental conditions (Magyar et al. 2007). It is also important to remember that a plastic response is not the same as fitness (Dybdahl and Kane 2005, Chun et al. 2007) and that long term population survival requires more than a few individuals managing to struggle through a tough growing season.

This study examined the germination and early growth rates for vegetative plants of *Cirsium pitcheri*, taken from populations around the Great Lakes and subjected to the current and predicted future climate conditions for Illinois Beach State Park. The objective was to determine which extant populations were the best donor candidates to supplement the existing Illinois Beach State Park restoration with additional seed in order to mitigate the effects of climate change.

Materials and Methods

Model Organism

C. pitcheri is endemic to beaches and sand dunes in the Western Great Lakes, primarily Lakes Huron and Michigan (Guire and Voss 1963). It is a monocarpic perennial that persists in a vegetative rosette for 5-10 years before flowering once and dying (Loveless 1984). Because *C. pitcheri* does not propagate vegetatively, it must rely on ecesis to maintain population persistence (Hamze and Jolls 2000). Flowering has been reported to begin in May and continue until July (Hamze and Jolls 2000) or begin in June and persist until late August (D'Ulisse 1995). Seed dispersal begins in July with wind-dispersed pappi carrying the achenes as far away as 4 m from the parent plant (Keddy and Keddy 1984, Loveless 1984). Seeds are generally believed to germinate the following spring, with only a small percentage of viable seeds persisting in a seed bank. The mechanisms behind breaking dormancy are not well established; hypotheses include cold shock from harsh winters and simple physical abrasion of the seed coat (Chen and Maun 1998).

The status of *C. pitcheri* in both the United States and Canada is “threatened” (Keddy 1987, Harrison 1988). Proposed reasons for population decline include shoreline development and use of sandy beaches for recreation (Keddy and Keddy 1984), infestation by plume moth (Keddy and Keddy 1984), fluctuation in lake level and chance events (Bowles et al. 1993), and browsing by white-tailed deer (Phillips and Maun 1996).

Multi-population growth

Seeds were collected from individual *C. pitcheri* plants from 11 populations around the Great Lakes (Big Blowout, Howe's Prairie, Illinois Beach State Park, Kemil Road Blowout, Kenosha Dunes, Kohler-Andrae State Park, Miller Woods, Pictured Rocks National Lakeshore, Sleeping Bear Dunes, West Beach, and Wilderness State Park) and one self-pollinated plant grown in Boise, Idaho, and were stored in manila envelopes at room temperature. Five seeds were selected from each plant, weighed on a Mettler Toledo XP 105 balance, and stored individually in folded filter paper. If fewer than five seeds were collected from a plant, all the available seeds were used. One hundred seeds from the self-pollinated plant were selected, weighed, and individually stored. In total, 1222 seeds were weighed for the experiment, with an average seed weight of 7.81 ± 3.71 mg.

The seeds were randomly assigned a position in one of seven 200-plug planting trays filled with soil consisting of one part potting mix, three parts sand, and one part vermiculite. The volume of each plug was 17.4 cm^3 (2.2 cm by 2.2 cm by 3.6 cm). Over 14 days, each seed was planted 2 cm deep in its assigned position and covered with sand. Prior to planting, each seed had 1 mm of the seed coat clipped from the cotyledon end with fingernail clippers. The trays were stored in the dark at room temperature until planting was completed.

After all the seeds were planted, each tray was watered with 3 mL of H_2O per seed planted. The trays were incubated on a 20°C/10°C, 12hr/12hr day/night schedule for two months in a Percival Intellus I-36LLVL incubator with Phillips Alto 20 Watt light bulbs. The schedule was altered to 25°C/15°C, 14hr/10hr day/night schedule for two weeks to reflect the transition from spring to summer, at which point the trays were moved into the Chicago Botanic Garden greenhouse. After one month in the greenhouse, the seedlings were transplanted to a common garden at the Chicago Botanic Garden. The watering regime of 600 mL per tray once per week continued until the plants were moved to the common garden, at which point the seedlings were monitored but their environmental conditions were not managed.

The time from planting to emergence was recorded for each seedling. When a seedling produced its first juvenile leaf, the length and width of the cotyledons were recorded. When a seedling produced its first adult leaf, the length of the longest juvenile leaf was recorded. Number of adult leaves and length of longest adult leaf were recorded for all surviving plants on September 30, 2007, with length of longest adult leaf used as a proxy for total biomass (Loveless, 1984; Girdler and Radtke, 2006).

Climate change tolerance

The Kohler-Andrae population is located in Sheboygan, Wisconsin. It consists of 100 to 500 individuals but is relatively isolated – the nearest *C. pitcheri* population is over 50 miles away. The West Beach population is the same size as the Kohler-Andrae population but is located among several other populations within Indiana Dunes State Park. These two populations have been utilized as source populations for previous restoration attempts in Illinois (Bowles et al. 1993). Seeds from both populations were previously planted in the common garden at the Chicago Botanic Garden and several of the resulting plants flowered in 2007. Seed heads were harvested from the flowering plants August 1, 2007 to August 7, 2007. The seed heads were cleaned and seeds weighing at least 6 mg were selected for the climate change experiment. Two hundred fifty seeds from plants from both the Kohler-Andrae and West Beach populations were individually weighed and packaged in folded filter paper. However, there were fewer than 250 seeds from both populations that weighed at least 6 mg, so the difference was filled in with the heaviest available seeds. The West Beach seeds weighed 8.49 ± 2.04 mg and the Kohler-Andrae seeds weighed 6.60 ± 1.72 mg. To test a population that performed as well in the first experiment as West Beach but was located at the opposite end of the range, seeds were included from plants growing at Pictured Rocks National Lakeshore. The Pictured Rocks population is located on the northern coast of Michigan's Upper Peninsula. It is the only *C. pitcheri* population on the northern coast, so it is over 50 miles away from the nearest populations on the southern coast of the peninsula. Pictured Rocks is a much larger population than the other two, consisting of approximately 10,000 individuals. Two hundred fifty seeds were individually weighed and packaged in folded filter paper. The Pictured Rocks seeds weighed 11.01 ± 2.94 mg.

Climate change models for Illinois (Union of Concerned Scientists 2006) were consulted to determine the conditions Illinois will likely experience in the upcoming century. Three time points were chosen based on these predictions: present day, 2030, and 2095. Current temperature, precipitation, and daily hours of sunlight were determined for the months of April through August in the Chicagoland region (Table 1). The models consulted agreed on the predicted temperature change but not on the scale and magnitude of the precipitation change. To allow for this variation, the precipitation change was bracketed for the 2030 and 2095 conditions. For the 2030 conditions, the temperature was increased 2°C over the control temperature each month and the precipitation was modified ± 10% (Table 2). In 2095, the temperature was increased 6°C over the control temperature each month and the precipitation was modified ± 20% (Table 3).

Each population group of 250 seeds was randomly divided into five subgroups of 50 seeds, designating one subgroup for each of the five treatments. The seeds from each of the three populations were combined for each treatment, and the five groups of 150 seeds were randomly assigned positions in planting trays filled with soil consisting of one part potting mix, three parts sand, and one part vermiculite. Each tray contained 48 plugs, so every treatment required 3 trays plus an additional six plugs. The volume of each plug was 6 cm by 4 cm by 5.5 cm. Prior to planting, each seed had 1 mm of the seed coat clipped from the cotyledon end with fingernail clippers. Each seed was planted 2 cm deep in its assigned position and covered with sand.

The trays were stored in three Percival Intellus I-36LLVL incubators, which were set to the temperature prescribed by the treatment regimen (Tables 1-3). To minimize seedling etiolation, the shelves were arranged in the incubators so that the grow lights were 15 cm from the surface of the soil in the trays. The lights used in the incubators were Phillips Alto 20 Watt bulbs. Plants were watered every six days, starting on the sixth day of the experiment, with 20% of the monthly water allowance distributed at each watering event. Every 30 days, the temperature and day/night length settings on the incubators were altered according to the treatment regimen and the watering amount was adjusted accordingly. Miracle Gro® Quick Start liquid fertilizer was added to the watering event on day 90 of the experiment at a concentration of 30 mL of fertilizer in 3.8 L of water. On day 96, seedlings were transferred from their trays to larger circular plugs, 17.9 cm tall with a diameter of 4.8 cm, filled with a mixture of 50% soil (described above) and 50% sand. One seedling did not germinate until day 102, so it was not transferred to the larger plug until that day.

As the experiment progressed, seedling data were recorded for the following characteristics: date of germination, cotyledon length and width, survival to juvenile stage, length of longest juvenile leaf, survival to adult stage, length of longest adult leaf, number of adult leaves, and survival to the conclusion of the experiment (150 days). On day 153, all surviving plants were removed from their plugs. The roots were carefully rinsed to remove all soil particles. For each plant, the shoots and roots were separated with a razor blade, weighed to determine wet weight, and stored in a Lane Scientific Equipment Corporation Model 1A-R drying oven at 37.8°C for 10 days. At the conclusion of the drying period, the shoots and roots were weighed again to determine their dry weight.

Data Analysis

Germination and survival data in the climate change tolerance experiment were analyzed with Chi Square and Friedman's nonparametric analysis of variance (ANOVA). One-way ANOVA was used to analyze growth metrics in both experiments. Root-to-shoot ratios were arcsine transformed before analysis. Tukey's studentized range test (HSD) was used to determine significant differences between means at $P < 0.05$.

Results

Multi-population growth

When all planted seeds were considered, the average germination rate for the multi-population trial was 13% (Figure 1). Germination rates varied from 0% for the Illinois Beach State Park seeds to 20% for the Kemil Road Blowout and Miller Woods seeds. However, the standard deviation of the germination rate was 7.0%, so the *z*-scores for the individual populations (Table 4) revealed that all of the individual rates fell within the 95% confidence interval. This means that none of the germination rates for any of the population were significantly different from the group germination rate. However, there did appear to be a slight increase in germination rate with decreasing latitude.

Upon examination of the data, it became apparent that there was a rapid drop in germination success for seeds < 6 mg in weight. Only 10 of 437 seeds < 6 mg germinated, or 2.3%. When only the seeds ≥ 6 mg were considered, the germination rate increased to 19 ± 8.5%. Under this data manipulation, all populations except Illinois Beach State Park and Kemil Road Blowout increased their germination rates – Illinois Beach State Park still had no seeds germinate, and Kemil Road Blowout didn’t have any seeds < 6 mg in the experiment. Additionally, the Illinois Beach State Park germination rate exceeded the 95% confidence interval, indicating a significantly depressed rate of germination for the seeds harvested from that population. Also notable was the loss of any trend in germination rate with latitude when only seeds ≥ 6 mg were considered.

Similarly, the rate for planted seeds surviving through the growing season increased when only seeds ≥ 6 mg were considered. The survival rate for all seeds in the multi-population experiment was 5.4 ± 3.5% (Figure 2). Of the 437 seeds < 6 mg planted, only three seeds (0.69%) survived to the end of the season. When only seeds ≥ 6 mg were considered, the average survival rate increased to 8.4 ± 4.3%. This manipulation of the data revealed that, despite their failure to produce any seedlings that survived the entire growing season, the survival rates of the Illinois Beach State Park and Miller Woods populations were not significantly worse than the average rate of survival (Table 5). In addition, the rate of survival did not show any trend related to latitude when seeds < 6 mg were removed from consideration.

While establishing a threshold for seed weight was important for increased rates of germination and survival, individual seed weights were only weakly correlated to metrics of plant growth. There was a slightly positive correlation between seed weight and length of longest adult leaf (Figure 3) and a slightly negative correlation between seed weight and number of adult leaves (Figure 4). However, R^2 values of 0.0015 and 0.0058, respectively, indicated that the predictive power of the lines of best fit was almost nonexistent.

The chosen growth metrics, length of longest adult leaf and number of adult leaves, failed to uncover any population that grew significantly better in the Chicagoland climate (Table 6). The Sleeping Bear Dunes population was the closest to having a significantly longer longest adult leaf and the Kohler-Andrae population was the closest to having a significantly shorter longest adult leaf, but even those populations fell within the range of random chance. The Big Blowout population had the largest number of adult leaves but was not significantly above the mean. The only significant difference was that the Kohler-Andrae population had fewer leaves than the other populations.

Climate change tolerance

The germination rate for all seeds was 13.3% (100 seeds out of 750 seeds planted). When the germination rate was broken down by population, the rate varied from 22% for the Pictured Rocks population to 5.6% for the Kohler-Andrae population (Figure 5). This difference between the populations was highly significant ($\chi^2 = 25.46$, d.f. = 2), indicating that the seeds from the Pictured Rocks population germinated more often than the seeds from the West Beach and Kohler-Andrae populations. When the germination rate was broken down by treatment, the rate varied from 16.7% for the control treatment to 11.3% for the 2030 dry treatment (Figure 6). The differences in germination rate between the treatments were not significant ($\chi^2 = 3.00$, d.f. = 4), indicating that none of the treatments were better or worse for rate of seed germination.

The pattern established for the rate of germination held when examining the rate of survivorship. The survival rate for the entire experiment was 10.5% (79 seedlings out of 750 seeds planted). When the survival rate was broken down by population, the rate varied from 18% for the Pictured Rocks population to 4% for the Kohler-Andrae population (Figure 5). The difference between the populations was highly significant ($\chi^2 = 23.57$, d.f. = 2), indicating that seeds planted from the Pictured Rocks population germinated and survived the first growing season more often than seeds from the West Beach and Kohler-Andrae populations. When the survival rate was broken down by treatment, the rate varied from 14.7% for the control treatment to 6.7% for the 2095 Dry treatment (Figure 6). However, the difference between the treatments was not significant ($\chi^2 = 4.81$, d.f. = 4), indicating the treatment did not impact the rate of survival for seeds planted in the experiment.

Four metrics were analyzed to determine the effect of the treatments on plant growth: length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight. Treatment effects were significant for all metrics ($p = 0.000$), but further inspection of the results indicated that growth in the 2095 treatment conditions was worse than the control and 2030 conditions (Figure 7). Pairwise comparison probabilities supported this, revealing significantly depressed growth in the 2095 Wet and Dry treatments for all but two comparisons: number of adult leaves between the 2030 Wet and 2095 Wet treatments and dry root weight between the 2030 Wet and 2095 Dry treatments. When the treatments were grouped by temperature, the treatment effect was significant for all four growth metrics ($p = 0.000$). Pairwise comparison probabilities confirmed that the control group and the 2030 grouping were not significantly different for any of the growth metrics, but the 2095 grouping was significantly worse than the control and 2030 groupings for all four of the growth metrics (Table 7).

With the temperature of the experiment shown to be the most important factor in overall plant growth, the next step was to determine if that finding held true for all three of the included populations. Dividing the temperature groupings by population confirmed that the individual populations all performed worst for every growth metric in the 2095 grouping (Figure 8). However, they did not all underperform equally. The West Beach population grew the best in the 2095 grouping across all of the growth metrics, followed by the Pictured Rocks population, and the Kohler-Andrae population did the worst (Figure 9). Further examination of the growth metrics (Table 8) showed that the West Beach population grew significantly better than the Kohler-Andrae population for every metric and the Pictured Rocks population grew significantly better than the Kohler-Andrae population in dry shoot weight and dry root weight. While the West Beach population performed better than the Pictured Rocks population in all growth metrics, the difference was never significant.

Analysis of shoot to root ratio failed to reveal any evidence that plants were partitioning biomass in response to water availability. When the treatments were grouped by precipitation, the control treatment was significantly different from both the Wet and Dry treatments (p=0.000 and p=0.004, respectively), but there was no difference between the Wet and Dry conditions (p=0.917). Grouping the treatments by temperature illustrated once again that growth was most influenced by the difficulty *C. pitcheri* had tolerating the warmer experimental conditions. The shoot to root ratio increased for all three populations as the temperature increased (Figure 10), indicating that root growth was depressed more severely than shoot growth as overall biomass decreased.

The average length of time for seedlings to emerge in the climate change tolerance experiment was 40.7 ± 14.1 days. The West Beach seedlings emerged significantly earlier than the Kohler-Andrae seedlings (p = 0.013), while the Pictured Rocks seedlings did not emerge significantly earlier or later than the West Beach or Kohler-Andrae seedlings (Figure 11a). When seedling emergence was broken down by treatment, it was revealed that the control seedlings germinated significantly later than the seedlings from all of the other trials (Figure 11b). Grouping the treatments by temperature showed that the control seedlings emerged significantly later than both the 2030 and 2095 seedlings but there was no difference between the 2030 and 2095 seedlings. Additionally, no seedling in any treatment emerged from the soil when the incubator temperature was below 14ºC.

Discussion

Initial attempts to reintroduce *C. pitcheri* in Illinois focused on donor populations that were predicted to be locally adapted to the Illinois climate (Bowles et al. 1993). While the restored Illinois population continues to persist, results from this study indicate that the population may face difficulty in the near future. Illinois Beach State Park was the only population to have no seeds germinate in the multi-population growth trial, though high variance in the germination rates meant that this result was not significantly worse than the mean germination rate for all included populations. Diminished recruitment of new seedlings leads to decreased population density, which, when paired with the limited seed dispersal and unknown pollen transfer distances of *C. pitcheri*, introduces the risk of inbreeding and gene loss through genetic isolation (Lowe et al. 2005). Genetic variance is predicted to be one of the most important factors in the survival of populations during periods of climate change (Bawa and Dayanandan 1998), putting populations with low genetic diversity and declining density at risk for breakdown into discrete subpopulations on the way to population collapse (Oborny et al. 2005). To provide the Illinois restoration with a better opportunity to persist and thrive in the face of climate change, an infusion of donor seed and seedlings should be implemented. The results of this study indicate that donor selection should consider factors other than local adaptation to ensure the long-term survival of the Illinois Beach State Park population.

Six of the donor populations for the Illinois Beach State Park restoration were included in the multi-population growth trial (Kohler-Andrae, Big Blowout, Howe's Prairie, Kemil Road Blowout, Miller Woods, and West Beach) and their performance helps explain some of the problems with the Illinois population. Only Kemil Road Blowout and West Beach had above-average germination and survival rates (Figures 1,2). Howe's Prairie had a below-average germination rate, but the seeds that germinated ended up having an above-average survival rate. Big Blowout and Miller Woods both squandered above-average germination rates to end up with below-average survival rates, and Kohler-Andrae was below average in both germination and survival. Clearly, the donor populations had mixed results when transplanted to the Illinois region.

However, there were a few populations in the multi-population growth trial that had better than average germination and survival rates but were not included as donor populations for the Illinois restoration. Pictured Rocks, Sleeping Bear Dunes, and Wilderness State Park were overlooked because they are located in northern Michigan and their native climate was believed to be too different from the Illinois climate for successful growth and survival. The favorable performance of the three populations in this study indicates that other factors play a significant role in the ability of donor populations to thrive in the Chicagoland climate. A partial explanation for these findings is provided by a Long Term Research in Environmental Biology (LTREB) report from the Chicago Botanic Garden (Havens 2008). All three of the Michigan populations, as well as the two above-average Indiana populations (Kemil Road Blowout and West Beach), have lower inbreeding coefficients than the other populations in the study (Table 9). These populations also tend to have higher gene diversity, but this is not as consistent and therefore does not seem to be as important to higher rates of germination and survival. Additionally, the five above-average populations vary in size from <100 individuals to >10,000 individuals, suggesting that inbreeding is not tied to population size for extant *C. pitcheri* populations and that actual F_{is} data should be examined instead of making assumptions based on number of individuals.

Bolstering the existing Illinois population with seeds from new donor populations has the potential to not only increase recruitment in the short term but also increase likelihood of population survival in the face of climate change. Plants with short generation times are predicted to have an increased opportunity to adapt to novel environmental conditions and the current understanding of *C. pitcheri* identifies width of the stem at the base of the rosette as the trigger that initiates flowering. Therefore, improved growth rate should shorten the time from germination to flowering and provide the Illinois population with more opportunities to produce tolerant offspring. While the depressed growth of all the populations at the 2095 temperature appears to be a foreboding result, the differences between the three suggest the possibility of choosing advantageous donor populations to increase the ability of the Illinois restoration to persevere. The West Beach population outperformed both the Kohler-Andrae and Pictured Rocks populations for every growth metric, indicating that the initial decision to focus on locally-adapted donor populations was not without merit. However, the multi-population growth trial and the LTREB report illustrate that not all local populations are equally good donor options. Supplementing the current Illinois restoration with seeds from the West Beach, Howe’s Prairie, Kemil Road Blowout, and possibly Big Blowout populations should minimize the effects of climate change on growth rate and prevent generational lengthening. If the Illinois population is able to meld the growth rate from the low-inbreeding coefficient Indiana populations with the germination and survival rates from the northern Michigan populations, the result will improve the current performance of the restoration and increase the survival potential of the population in the future.

Unfortunately, improvements in the genetic stock of the Illinois population have the potential to be undermined by a range of effects induced by climate change. One of the main concerns for the survival of many plant species is the development of pollinator/flowering asynchronicity arising from differential responses to climatic cues. This study revealed that an increase in average temperature of only 2ºC shortened the time until seedling emergence by over two weeks. While there is no direct evidence that this will translate into earlier flowering for *C. pitcheri*, work on a range of other species showed that flowering times were most correlated with mean temperatures in the months just before flowering (Miller-Rushing and Primack, 2008), implying that earlier flowering is a likely outcome of warmer temperatures. However, there has been no work done on *C. pitcheri* pollinators to determine temporal shifts in their life cycle due to climate change, so concerns about pollinator/flowering asynchronicity have yet to be substantiated. Also potentially troubling are two possibilities concerning flowering time separation within individual populations. The first scenario concerns individuals flowering at different times than their neighboring plants. Because the dispersal range of *C. pitcheri* pollen is unknown but is believed to be rather small, local temporal isolation could compel an increase in self-pollination and trigger accelerated inbreeding depression. The second scenario involves neighboring plants flowering together but at different times than the rest of the population. Temporal assortative mating impedes gene flow (Gerard et al., 2006) and could eventually fragment the Illinois restoration into spatially-cohesive but reproductively-distinct subpopulations. As with the asynchronicity concerns, these scenarios are still in the realm of supposition. *C. pitcheri* currently flowers for upwards of four weeks (Keddy and Keddy, 1984), but the triggers for the start and end of the flowering period are unknown. It is possible that flowering earlier could simply lengthen the flowering period, extending the window for pollination opportunities. The third climate-driven threat to the survival of the Illinois population involves the timing of precipitation events. While this study demonstrated that an average decrease in rainfall throughout the growing season is not significantly detrimental to growth or survival, research focusing on episodic precipitation has revealed that *C. pitcheri* has certain months where it is more susceptible to water stress-induced mortality (Bell, personal communication). Current IPCC predictions for Illinois foresee an overall precipitation decrease for the summer months but provide little information regarding actual rainfall in the critical months. Again, this potential pitfall could be a serious issue for the Illinois restoration but presently remains only a theoretical concern.

One more compelling concern is the absence of any evidence that phenotypic plasticity could function as a survival mechanism in response to climate stress. While the seeds from each population were not genetically identical, most of the seeds came from a small number of parent plants so the relatedness of the seedlings should be high and plasticity should be a factor. However, there was no difference in shoot weight or root weight between the Wet and Dry treatments. The slight growth advantage in the Dry treatments suggests that perhaps the Wet treatments were receiving excess precipitation and the Dry treatments were not actually water stressed. Yet the decline in shoot weight and root weight in the 2095 treatments proved that the increased temperature was a very real stress, one that the seedlings struggled to overcome. One strategy typically employed by plants to counteract heat stress is to produce less leaf tissue, thereby capturing less light energy. While the shoot weight decreased at the 2095 temperature, shoot:root ratio increased, meaning the root weight actually decreased proportionally more than the shoot weight did. This pattern of biomass allocation looks less like environmentally-responsive resource partitioning and more like struggling to endure an untenable situation. Further evidence of the negligible impact of plasticity on survival is the fact that the rank of growth metrics by population at the 2095 temperature was predicted by the number of alleles per loci in the 2008 LTREB report (Table 9). The West Beach population, with 6.0 alleles per loci, showed the most robust growth for all growth metrics, while the Kohler-Andrae population, with 1.6 alleles per loci, did the worst. Pictured Rocks, with 4.6 alleles per loci, performed between the other two populations. These results suggest that tolerance of temperature stress originated from genetic diversity and that populations with low diversity cannot rely on plasticity to survive climate change.

Despite the possibility of asynchronicity induced by climate change and no evidence that phenotypic plasticity will enable *C. pitcheri* to tolerate increased temperatures, there still exists a very real opportunity to fortify the existing Illinois restoration with new inputs from genetically-healthy donor populations. Incorporating seed and seedlings from Indiana and northern Michigan populations with low inbreeding coefficients has the potential to improve rates of both growth and survival. In addition to strengthening the restoration in the short term, this proscribed course of action has the capability to mitigate the effects of climate change on the existing population and ensure that the denizens of Illinois Beach State Park abide far into the future.

Conclusions

This study revealed that extant *C. pitcheri* populations are potentially vulnerable to the increased temperature predicted by climate change models. Like other endemic species, the range limitations imposed on *C. pitcheri* have increased the likelihood that populations at the equatorial end of the distribution will succumb to climate stress and that this loss will significantly impact the species as a whole. This vulnerability is partially explained by the fact that *C. pitcheri* is relatively young species, believed to be descended from *Cirsium canescens* after the most recent North American glacial retreat (Keddy and Keddy 1984), and individual populations often lack the genetic diversity predicted to be crucial for tolerating climate change. However, populations with low gene diversity appear to have a higher potential for survival as long as inter-population gene flow and random mating are keeping F_{is} values low. This study showed that high inbreeding coefficients correlate with lower rates of germination and survival, implying that individual plant health stems from healthy functioning of macro processes. Examining high-inbreeding and low-inbreeding populations to determine the factors that contribute to the inbreeding disparity has the potential to not only inform efforts to protect *C. pitcheri* but also could have implications for the preservation of a range of rare plants. Lastly, this study recommended combining low F_{is} northern and southern populations for the purpose of generating offspring with the high germination and survival rates of the northern populations and the locally-adapted growth rate of the southern populations. However, it is currently unknown whether this crossing would actually produce the desired offspring. Combining field testing of north x south offspring with a comprehensive genetic analysis could reveal both the fitness of the crossed offspring as well as the genes or gene complexes responsible for controlling the traits of interest. While the offspring fitness might only be applicable to *C. pitcheri*, the genetic information has the potential to be relevant to a large range of plant species.

Figures and Tables

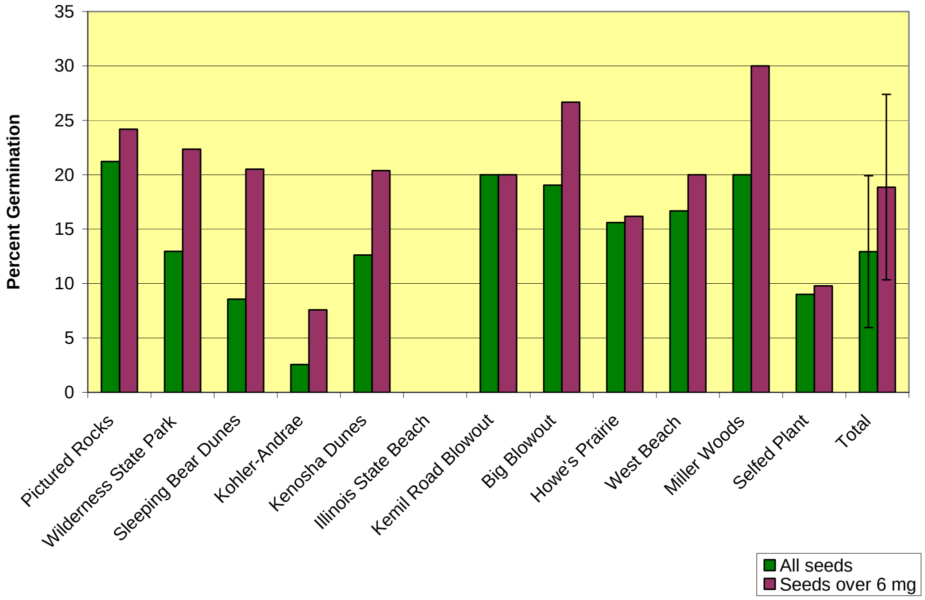


Figure 1: Rate of seed germination for all included populations in the multi-population growth experiment. Populations are organized by latitude, with the northernmost population on the left. The selfed plant is of unknown origin.

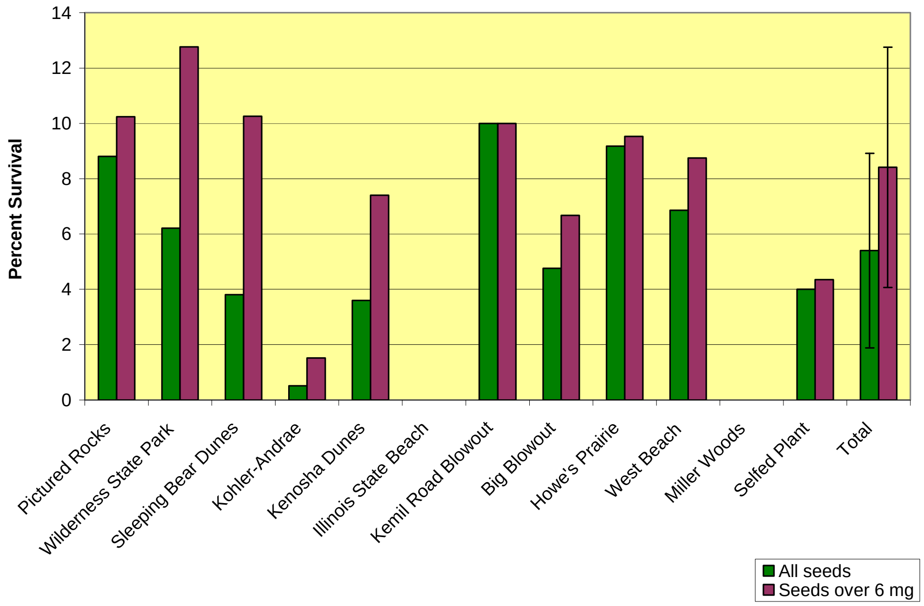


Figure 2: Rate of seed survival for all included populations in the multi-population growth experiment. Populations are organized by latitude, with the northernmost population on the left. The selfed plant is of unknown origin.

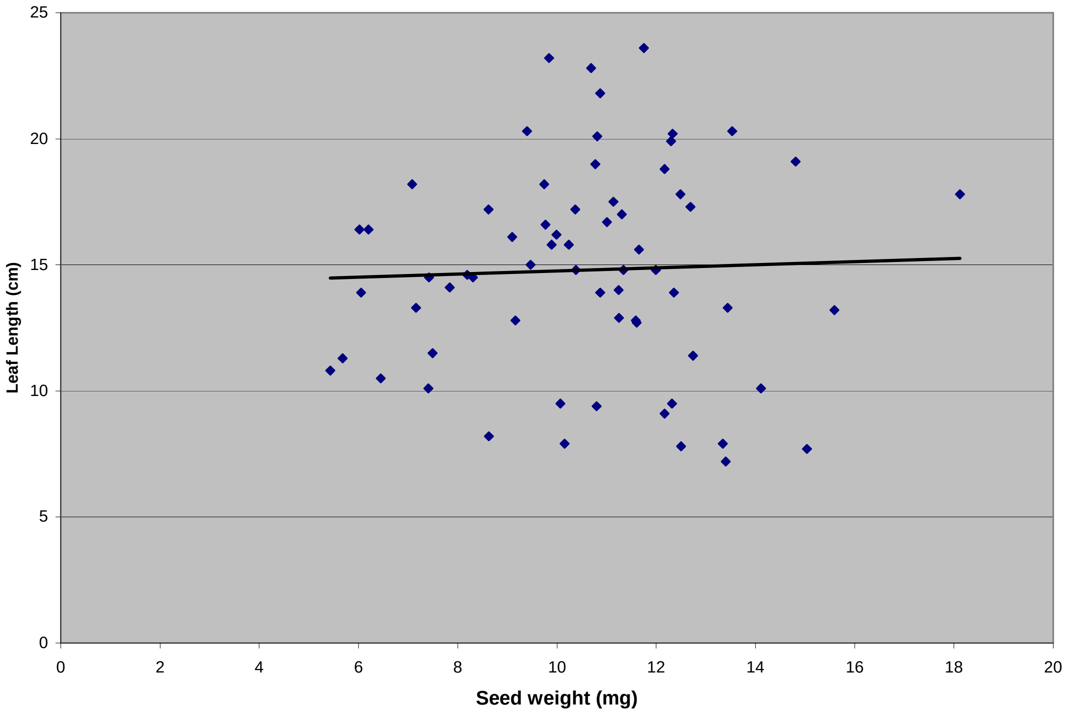


Figure 3: Length of longest adult leaf after five months of growth related to seed weight by linear regression. Slope = 0.0617, R^2 = 0.0015.

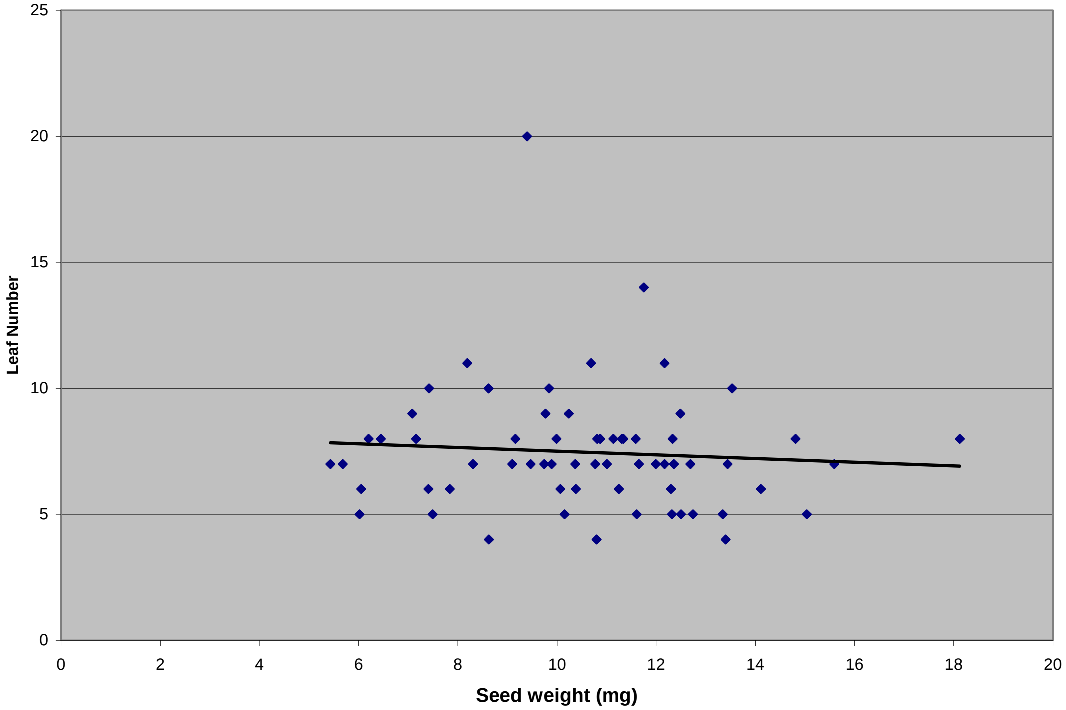


Figure 4: Number of adult leaves produced in five months of growth related to seed weight by linear regression. Slope = -0.073, R^2 = 0.0058.

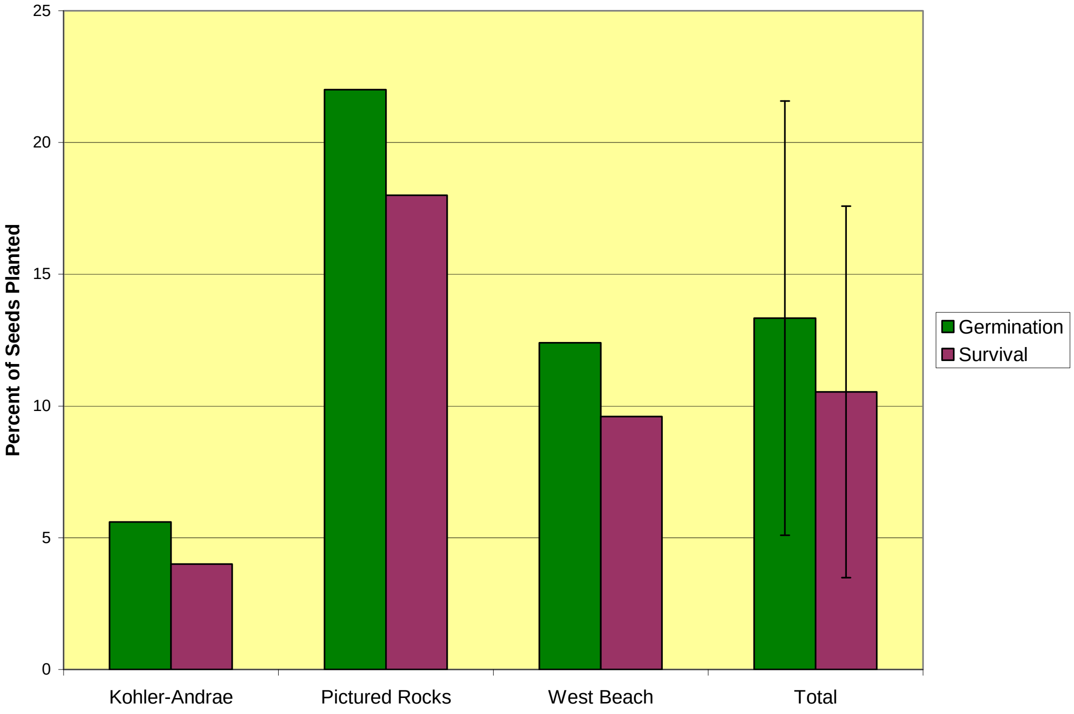


Figure 5: Rates of germination and survival in the climate change experiment when the seeds are grouped by population.

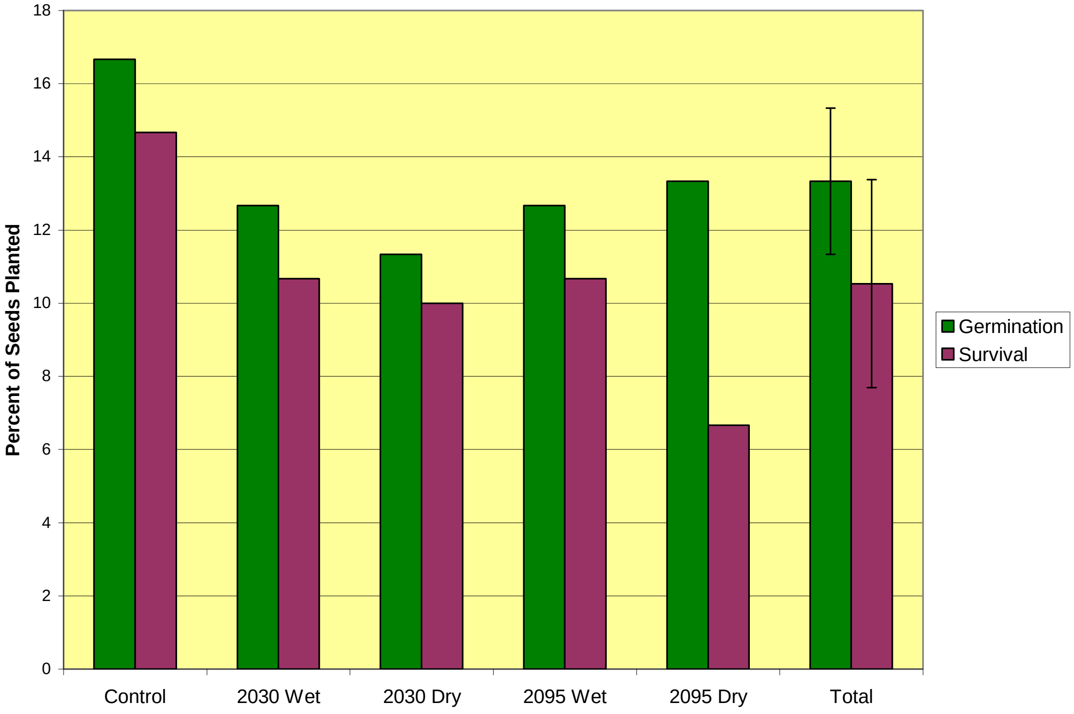


Figure 6: Rates of germination and survival in the climate change experiment when the seeds are grouped by treatment

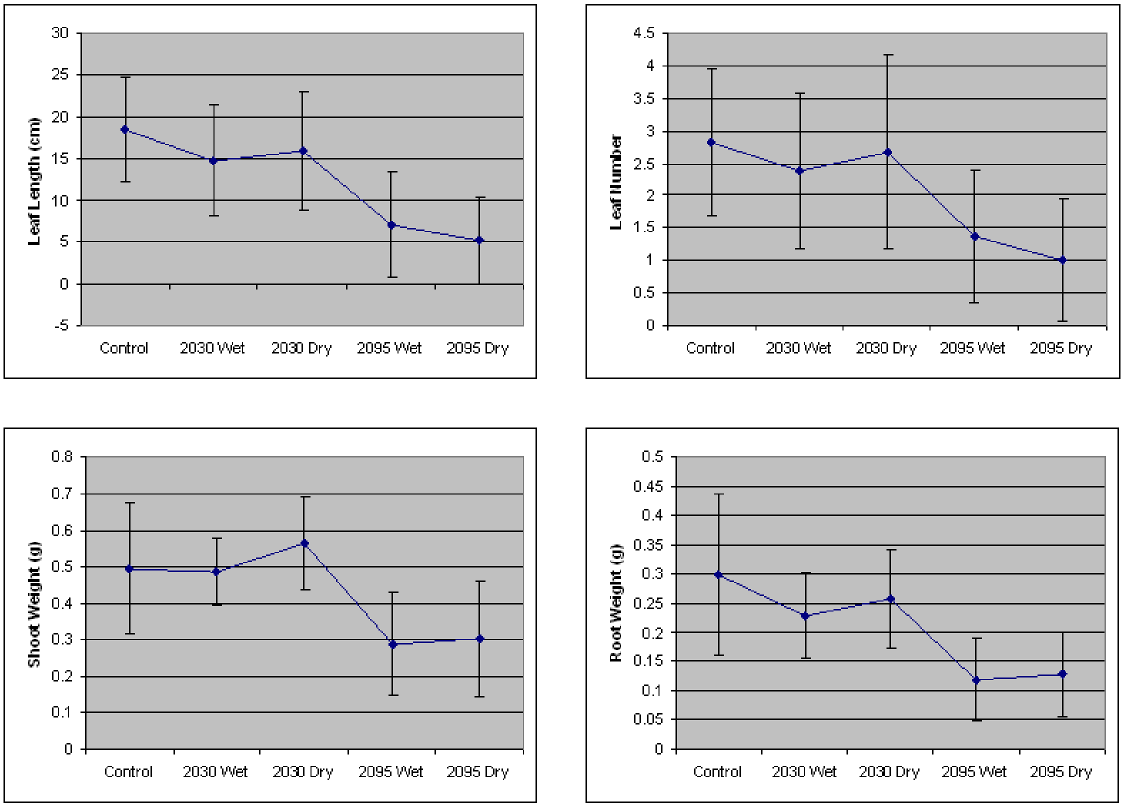


Figure 7: Growth metrics analyzed by treatment. The characteristics included are length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight.

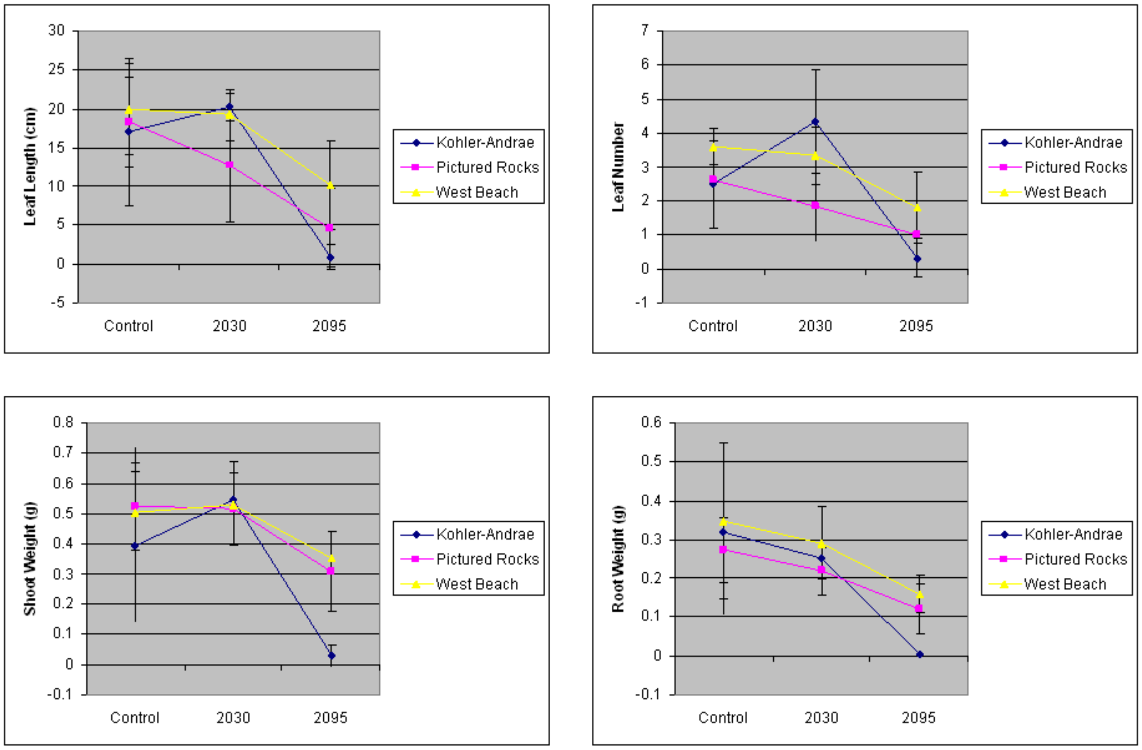


Figure 8: Growth metrics analyzed by temperature and population. The characteristics included are length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight.

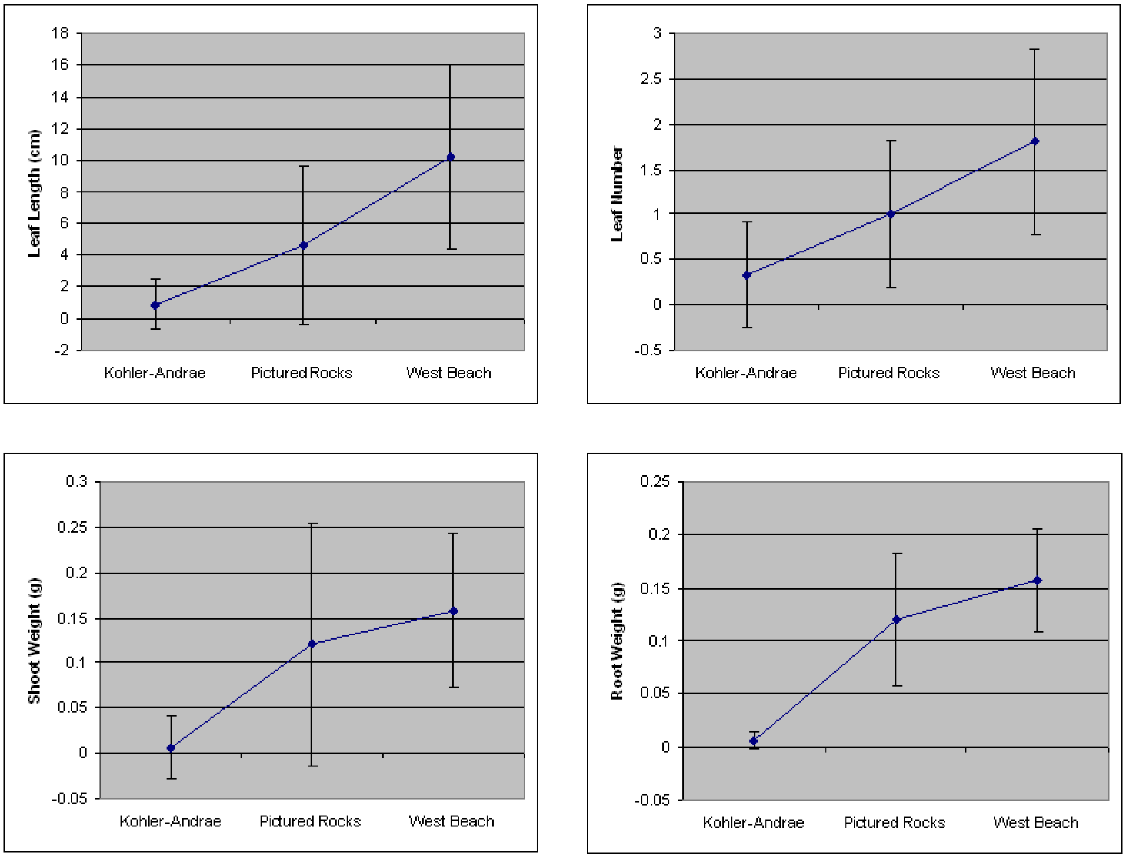


Figure 9: Growth metrics analyzed by population at the 2095 temperature. The characteristics included are length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight.

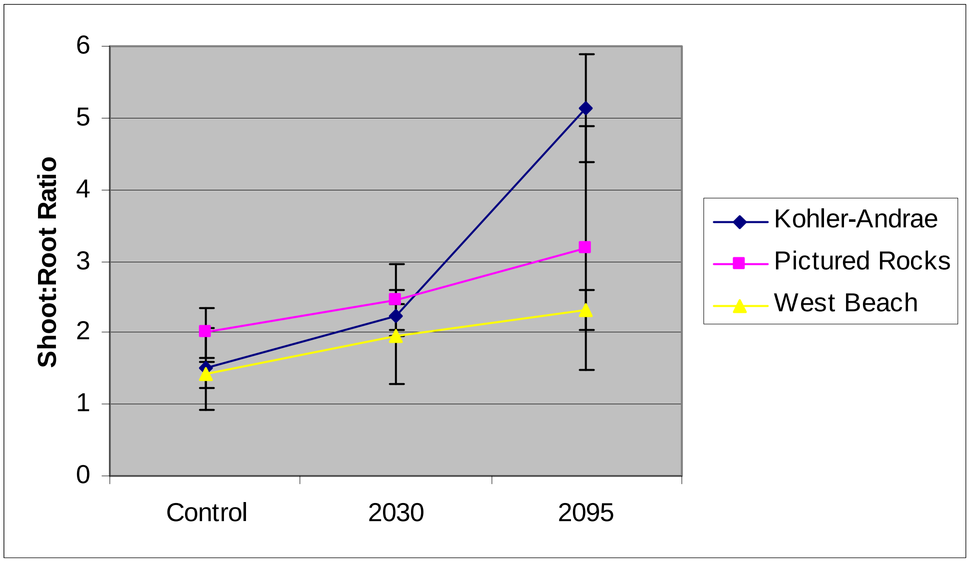


Figure 10: Shoot to root ratio analyzed by population and temperature.

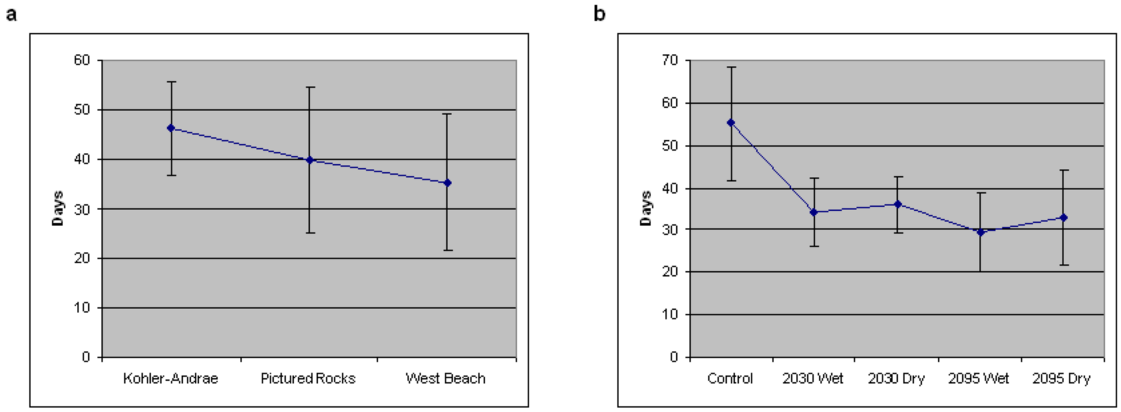


Figure 11: (a) Number of days from planting to seedling emergence by population. **(b)** Number of days from planting to seedling emergence by treatment.

	Day/night cycle (hr)	Temperature (°C)	Precipitation (in)
April	13.5/10.5	13.9/4.4	3.2
May	14.5/9.5	19.4/9.4	3.5
June	15/9	25.6/15.6	3.7
July	14.5/9.5	27.8/18.3	3.5
August	14/10	26.7/17.8	3.6

Table 1: Hours of sunlight, average temperature, and average precipitation for the Chicagoland region (1871-2007).

	Day/night cycle (hr)	Temperature (°C)	Precipitation (in)
April	13.5/10.5	15.9/6.4	3.2 ± 0.32
May	14.5/9.5	21.4/11.4	3.5 ± 0.35
June	15/9	27.6/17.6	3.7 ± 0.37
July	14.5/9.5	29.8/20.3	3.5 ± 0.35
August	14/10	28.7/19.8	3.6 ± 0.36

Table 2: Predicted hours of sunlight, average temperature, and average precipitation for the Chicagoland region in 2030. The bracketing of the precipitation is due to conflicting predictions from different models.

	Day/night cycle (hr)	Temperature (°C)	Precipitation (in)
April	13.5/10.5	19.9/10.4	3.2 ± 0.64
May	14.5/9.5	25.4/15.4	3.5 ± 0.70
June	15/9	31.6/21.6	3.7 ± 0.74
July	14.5/9.5	33.8/24.3	3.5 ± 0.70
August	14/10	32.7/23.8	3.6 ± 0.72

Table 3: Predicted hours of sunlight, average temperature, and average precipitation for the Chicagoland region in 2095. The bracketing of the precipitation is due to conflicting predictions from different models.

Population	***z*-score (all)**	***z*-score (≥ 6 mg)**
Big Blowout	0.876817	0.916521
Howe's Prairie	0.382186	-0.31239
Illinois Beach State Park	-1.85304	-2.21161
Kemil Road Blowout	1.01331	0.13449
Kenosha Dunes	-0.04543	0.177936
Kohler-Andrae	-1.48744	-1.32293
Miller Woods	1.01331	1.307537
Pictured Rocks	1.185291	0.625533
Sleeping Bear Dunes	-0.62461	0.194646
West Beach	0.535584	0.13449
Wilderness State Park	0.003403	0.409033
Selfed Plant	-0.56318	-1.06406

Table 4: *z*-scores for the germination rates of seeds in the multi-population growth trial. Column two is the *z*-score for the population when all seeds used in the experiment are considered. Column three is the *z*-score for the population when only the seeds greater than or equal to 6 mg are considered. 95% confidence interval lies within ± 1.96σ.

Population	***z*-score (all)**	***z*-score (≥ 6 mg)**
Big Blowout	-0.18177	-0.40076
Howe's Prairie	1.073232	0.256933
Illinois Beach State Park	-1.53618	-1.93538
Kemil Road Blowout	1.308079	0.366549
Kenosha Dunes	-0.51122	-0.23025
Kohler-Andrae	-1.39106	-1.5866
Miller Woods	-1.53618	-1.93538
Pictured Rocks	0.966768	0.420082
Sleeping Bear Dunes	-0.45265	0.425573
West Beach	0.415763	0.078808
Wilderness State Park	0.232272	1.003252
Selfed Plant	-0.39848	-0.93454

Table 5: *z*-scores for the survival rates of seeds in the multi-population growth trial. Column two is the *z*-score for the population when all seeds used in the experiment are considered. Column three is the *z*-score for the population when only the seeds greater than or equal to 6 mg are considered. 95% confidence interval lies within ± 1.96σ.

Population	Leaf Length *z*-score	Leaf Number *z*-score
Big Blowout	0.764662	1.66345
Howe's Prairie	0.659324	-0.04563
Kemil Road Blowout	1.192595	0.442676
Kenosha Dunes	-0.2558	-0.4729
Kohler-Andrae	-1.77002	-1.99887
Pictured Rocks	-0.78398	-0.7781
Sleeping Bear Dunes	1.423021	-0.16771
West Beach	-0.04888	0.26828
Wilderness State Park	-0.48896	0.035751
Selfed Plant	-0.69196	1.053063

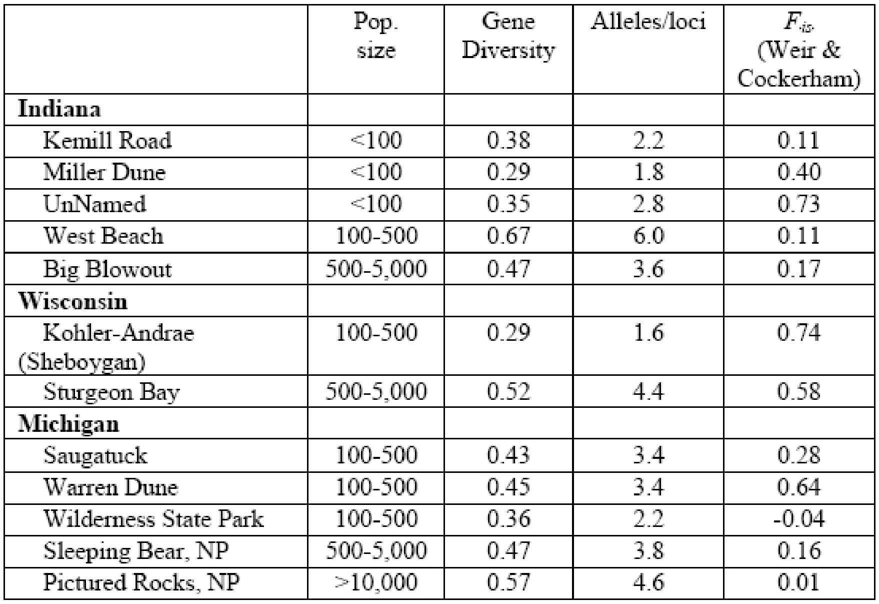
Table 6: *z*-scores for the growth metrics of plants in the multi-population growth trial. Column two is the *z*-score for the length of the longest adult leaf after five months of growth. Column three is the *z*-score for the number of adult leaves after five months of growth. 95% confidence interval lies within ± 1.96σ.

	Leaf Length	Leaf Number	Dry Shoot Weight	Dry Root Weight
Control vs. 2030	0.175	0.556	0.619	0.118
Control vs. 2095	0.000*	0.000*	0.000*	0.000*
2030 vs. 2095	0.000*	0.000*	0.000*	0.000*

Table 7: Growth metric pairwise comparison probabilities when the treatments are grouped by temperature. The characteristics included are length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight. Significant comparisons have a *p*-value ≤ 0.05 and are denoted with an (*).

	Leaf Length (cm)	**Leaf Number**	**Dry Shoot Weight (g)**	**Dry Root Weight (g)**
Kohler-Andrae	0.900 ± 1.56	0.333 ± 0.577	0.0297 ± 0.0348	0.00600 ± 0.00794
Pictured Rocks	4.63 ± 5.01	1.00 ± 0.816	0.308 ± 0.134*	0.120 ± 0.0628*
West Beach	10.2 ± 5.82*	1.80 ± 1.03*	0.354 ± 0.0858*	0.158 ± 0.0482*

Table 8: Growth metrics for all populations at the 2095 temperature. The characteristics included are length of longest adult leaf, number of adult leaves, dry shoot weight, and dry root weight. Results that are significantly greater than Kohler-Andrae have a *p*-value ≤ 0.05 and are denoted with an (*). West Beach and Pictured Rocks results were not significantly different for any of the growth metrics.



	Pop. size	Gene Diversity	Alleles/loci	F_{is} (Weir & Cockerham)
Indiana				
Kemill Road	<100	0.38	2.2	0.11
Miller Dune	<100	0.29	1.8	0.40
UnNamed	<100	0.35	2.8	0.73
West Beach	100-500	0.67	6.0	0.11
Big Blowout	500-5,000	0.47	3.6	0.17
Wisconsin				
Kohler-Andrae (Sheboygan)	100-500	0.29	1.6	0.74
Sturgeon Bay	500-5,000	0.52	4.4	0.58
Michigan				
Saugatuck	100-500	0.43	3.4	0.28
Warren Dune	100-500	0.45	3.4	0.64
Wilderness State Park	100-500	0.36	2.2	-0.04
Sleeping Bear, NP	500-5,000	0.47	3.8	0.16
Pictured Rocks, NP	>10,000	0.57	4.6	0.01

Table 9: Genetic diversity and inbreeding coefficient (F_{is}) for a subset of native populations of *C. pitcheri* in Wisconsin, Indiana, and Michigan. Reproduced from Havens 2008.

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