**Discussion**

***Regional Differences***

If *Solanum carolinense* has locally adapted to the respective temperature regimes in TX and MN, we would expect that plants from the north would be more tolerant of cold temperatures and plants from the south would be more tolerant of hot temperatures. Rather than a clear-cut difference between north and south for hot and cold treatments, there were mixed results that support divergence between regions in ways we hadn’t anticipated.

*Sporophyte*

Plants from the south had a more stable cell membranes when exposed to an extreme cold treatment. Cold stress reduces the fluidity of the cell membrane and produces ROS that have the potential to oxidize lipids and damage the membrane (Valitova et al., 2019). The incorporation of sterols in membranes can maintain fluidity and expand temperature range for plants (Dufourc, 2008a, 2008b; Valitova et al., 2019). Conversely, saturated fatty acids can be incorporated in the cell membrane to reduce fluidity and are often associated heat tolerance (Knight & Ackerly, 2001; Zhu et al., 2018). There was no significant difference between region for HCMS for all study plants together, but there was a significant difference for plants in block A. Temperatures in the greenhouse progressively rose throughout the spring and summer leading to a block effect in both the hot and cold treatments of CMS. In block A for HCMS measurements, northern plants had a higher HCMS, but this difference degraded in the later blocks during the times when temperatures were higher. Southern plants may have the capacity to induce heat tolerance as they acclimate to warmer conditions. Block A may be the best representative measurement of baseline heat tolerance for HCMS, but the later blocks suggest that CMS is a plastic trait. The median of HCMS generally increased across blocks, while the median of CCMS decreased. While there was no significant correlation between CCMS and HCMS, these results suggest that HCMS and CCMS are inversely related. Our PCA results also showed an antagonistic relationship between the CMS variables as they were loaded in opposite directions for PC1 and PC2 in the sporophytic PCA.

HCMS was an important variable in the correlation analysis that included all plants. HCMS was weakly and positively correlated with CCHPL, CPS, and HPS. Only one of these correlations was significant when the regions were analyzed separately. HCMS was positively correlated with HPS. These results indicate that membrane structural integrity is related to photosynthetic rate in heat. Since the light reaction does occur in the thylakoid membrane within chloroplasts, the rigidity of cell membranes in heat may directly affect efficiency of energy absorption and electron transport.

There was no significant difference between northern and southern plants for net photosynthetic rate in both the hot and cold treatments. Net photosynthesis was the only sporophytic variable where the whole plant was placed in a temperature treatment and leaves were measured on the plant. The plant may compensate for temperature stress through physiological mechanisms, such as increasing transpiration. Therefore, the temperature treatments may not have stressed the plants to the extent that temperature tolerance for the northern and southern plants was distinguishable.

Plants from the north had more stable chlorophyll fluorescence in both the hot and cold treatments. Stable chlorophyll fluorescence in heat and cold for northern plants may be explained by northern plants experiencing a larger range of temperatures. Between 2018 and 2021, temperatures during the growing season (March to September) in Houston County, MN ranged from -28°C to 34°C (62°C difference), while in Collin County, TX they ranged from -7°C to 42°C (49°C difference). Since the temperate conditions of Minnesota are more variable, populations in the north may have evolved to have higher levels of temporary phenotypic plasticity, at least when it comes to chloroplast and chlorophyll stability than those in the south. Furthermore, northern plants also had significantly more variation in HCHPL than southern plants. This may suggest that there is stabilizing selection occurring in the southern region for heat tolerance in chlorophyll stability. Less variation in HCHPL in the south may contribute to the counter-gradient results we attained. If northern plants experience less heat stress selection and have greater variation, then there may be more potential to have individuals with high HCHPL.

*Gametophyte*

To test gametophytic temperature tolerance in *Solanum carolinense*, we measured pollen performance variables over a temperature gradient. The variables of interest were pollen germination and pollen tube growth rate, both of which directly impact the capacity of a pollen grain to compete with other pollen and fertilize an ovule within a flower.

Pollen germination was higher in pollen grains from the northern plants than those in the south for both Tmax and Topt. This means that pollen from the north have a higher propensity to produce pollen tubes at high temperatures than their southern counterparts. The distinct difference between north and south suggests that there is sensitivity to high temperatures and likely an adaptive response occurring in the populations of the south. Rutley et al. (2022) proposed the two-baskets model categorizing pollen, which states that there are high-ROS (reactive oxygen species) and low-ROS subpopulations of pollen within anthers of flowering species. The low-ROS pollen have a lower metabolic rate than high-ROS pollen due to partial dehydration during development. The two subpopulations of pollen are adaptive as they allow for asynchrony in pollen germination, permitting some pollen to remain dormant in a stressful environment and grow pollen tubes later in more favorable conditions. Keller et al. (2018) found that *Solanum lycopersicum* (tomato) pollen had two responses during heat stress – direct and delayed translation. Luria et al. (2019) later showed that *Solanum lycopersicum* has pollen that fall in the low-ROS and high-ROS groups, supporting the two-basket model in a species closely related to *Solanum carolinense*. We hypothesis that *Solanum carolinense* populations in the south have higher proportions of low-ROS to high-ROS pollen grains than those in the north due to stronger selection from increased exposure to extreme heat in the south. Low-ROS pollen that remains dormant would not be adaptive in northern populations, with little exposure to high temperature stress.

There was a significant negative correlation between Tmax and Tmin germination. This correlation was driven by plants from the south and supports the two-basket model. The negative correlation means that plants with pollen that germinate readily at high temperatures also germinate at low temperatures, while those that have a lower Tmax have a higher Tmin. Plants with a higher proportion of high-ROS pollen would germinate in any condition (extreme heat and cold stress). Plants with a higher proportion of low-ROS pollen would not germinate as freely during stressful conditions. Since plants of the south have likely evolved to have the dual pollen types, there may be more variation in pollen activity driving this correlation. Southern plants also had a correlation between Tmax gemination and Tmax PTGR, meaning that if plants have pollen that germinate at higher temperatures, they also have pollen tubes that grow faster at high temperatures. PTGR is likely influenced by metabolic rate, which is increased in high-ROS pollen. There was a positive correlation between Tmin and Tmax PTGR in the north. This pattern indicates that pollen tubes either grow fast at high temperatures or low temperatures, but not both. When north and south were combined, there were positive correlations between germination and PTGR Tmin and between germination and PTGR Tmax, indicating that the response to temperature for gemination and pollen tube growth are related.

There was no significant difference between northern and southern populations for Tmin. Pollen of *Solanum carolinense* may be constrained by a lower temperature limit for the physiological processes necessary for pollen tube growth. Pollen tube growth rate also remained constant across regions and genets for the five temperatures. Since pollen tube growth rate is constrained by the physiological processes involved in cell division, there is likely little variation upon which selection can act.

***Inter-generational adaptations***

Tanksley et al. (1981) first described the correlation between selection in the gametophyte and sporophyte when they found a correlation between allozymes expressed in both stages. Based on their findings and several studies that followed (Hedhly et al., 2005; Pedersen et al., 1987; Poudyal et al., 2019; Willing & Mascarenhas, 1984), including studies on temperature tolerance (Hedhly et al., 2005; Poudyal et al., 2019), we hypothesized that there would be a correlation between temperature tolerance in the sporophyte and the gametophyte. There were no significant correlations between any of the gametophytic and sporophytic variables when northern and southern plants were both included in the correlation analysis. However, independently, southern plants did have strong relationships between gametophytic and sporophytic traits.

In the southern plants, most correlations between the sporophyte and gametophyte were negative but, one was positive. Cold germination was positively correlated to HPS. The positive correlation indicates that as the minimum temperature of pollen germination increases, net photosynthetic rate is maintained at higher temperatures. On the other hand, hot gemination was negatively correlated to HPS, meaning that as the maximum temperature of germination increases, net photosynthetic rate decreases in heat. Because two correlations involve germination and photosynthesis in southern plants, it is possible that pollen type determines photosynthetic resilience in heat. HPS was higher in plants with pollen that germinated in increased minimum temperatures and lower maximum temperatures, characteristics which are consistent with low-ROS pollen.

Chlorophyll fluorescence stability and pollen tube growth rate were also related to one another. CPTGR was negatively correlated with HCHPL and HPTGR was negatively correlated with CCHPL. These relationships suggest that cold tolerance and heat tolerance are antagonistic across stages. However, there are no positive correlations between the same variables and their counterpart in the same temperature treatment. Regardless, PTGR and chlorophyll fluorescence may both incorporate similar molecular responses to temperature in heat stress and a separate response for cold stress.

The last significant inter-generational correlation is between CCMS and cold germination. There was a negative relationship between the two, indicating that plants with decreased minimum temperature of pollen germination also had more stable cell membranes in cold stress. Often cold stress is mitigated at the cellular level by maintaining membrane fluidity. Cell membrane fluidity would also be important for pollen tube growth in cold conditions. There are likely similar mechanisms maintaining cell membranes in both stages during cold stress.

Consistency in responses to temperature stress in the sporophyte and gametophyte supports selection influencing inter-generational temperature tolerance adaptation is southern plants. Evidence of acclimation to higher temperatures in HCMS, reduced variation in HCHPL, pollen grain dormancy, and inter-generational correlations were all observed for plants from the south and could be a result of stronger selective pressures in the south.

Plants in the south experienced extremely high temperatures regularly and the maximum is much higher than temperatures in the north. The north did reach extremely low temperatures, but the plants were likely dormant during those times or covered in snow, meaning that the lower temperature limits that northern and southern plants experience might not be that different. Therefore, southern plants have greater potential for temperature-based selection to occur and thus adaptation of temperature tolerance mechanisms. The lack of coordinated response to temperature stress in the northern plants suggest that traits facilitating temperature tolerance are not important for survival. Another explanation is that horsenettle hasn’t been located in MN long enough for selection to act on the populations All populations included in this study were located toward the edge of the range for this species. Time for selective pressures to act on the populations in the north may be insufficient for local adaptation to occur. The first record of *Solanum carolinense* in Minnesota is from 1939 and in Houston County 1975 (University of Minnesota, Minnesota Biodiversity Atlas, Bell Museum - <https://bellatlas.umn.edu/collections/listtabledisplay.php>). The first record in Texas is from 1917 and the closest record of horsenettle to Collin County is from 2011 (University of Texas Austin, Billie L. Turner Plant Resources Center - <https://prc-symbiota.tacc.utexas.edu/collections/list.php>). Since horsenettle has been located in both locations for relatively similar amounts of time, the strength of temperature selection is likely driving the divergence we observed.

**Conclusion**

Our results showed evidence of local adaptation due to temperature acting as a selective pressure. At first glance, the mixed outcomes of this study do not completely support our predictions, suggesting that the response to temperature tolerance is less coordinated than we originally anticipated. However, upon recognizing that temperature might not be a strong selective pressure in the northern region, we concluded that our results support evolved responses to temperature stress in the south, but not in the north for most traits. The measurements of chlorophyll fluorescence did provide some evidence that populations from areas with larger thermal ranges, such as those in higher latitudes, have more variation and possibly more phenotypic plasticity, which is consistent with the climate variability hypothesis.

There was evidence of potential for inter-generational temperature tolerance adaptation in southern plants as well. High temperatures have likely selected for higher proportions of low-ROS pollen, which may impact sporophytic physiological processes such as photosynthesis. To fully understand the link, or lack of link, between gametophytic and sporophytic temperature tolerance, we must understand the molecular underpinnings driving these responses and how they evolve.

These results could inform restoration efforts by changing the way we think about seed sourcing and adaptive potential in a rapidly changing environment. Seeds from the south may have evolved stress responses to temperature that are lacking in northern populations. The evidence for the two-basket model in a wild species is also a novel finding that could add to our perception of the influence gametophytic traits have on a species persisting in extreme environments.

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