Life stage specific temperature tolerance in horsenettle (*Solanum carolinense*)

Submitted by:

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***Introduction***

Global climate change is altering local temperature regimes and affecting the ranges acceptable for biota. As local temperature regimes change, plants must either shift ranges or adapt to the local conditions (Kawecki and Ebert 2004; Wolkovich et al. 2012; Cooper et al. 2018; Moran 2020). The overarching question for this proposed study is how do plants adapt to changing temperature conditions? Adaptation is the result of natural selection acting on variation among genetically based phenotypes of the same species. As conditions change, adaption will occur if phenotypes that are more suitable for the conditions are more successful in survival and reproduction and therefore, are favored by natural selection (Kawecki and Ebert 2004).

Since angiosperms have alternation of generations, selection can occur at the gametophytic stage (haploid stage; pollen or ovule) and/or the sporophytic stage (diploid stage; zygote, seedling, or mature plant) (Walsh and Charlesworth 1992). I am interested in understanding how the set of traits associated with temperature tolerance evolve. Does selection for temperature tolerance occur in the sporophytic stage, gametophytic stage, or both?

Recognizing the underlying mechanisms for selection are particularly important for understanding adaptation of plants in new environments or a changing climate. Previous studies have shown an overlap in temperature tolerance between the sporophytic and gametophytic stages, meaning that thermotolerant plants produce relatively thermotolerant pollen (Zamir et al. 1982; Hedhly et al. 2005; Zhou et al. 2017; Poudyal et al. 2019). Furthermore, selection for tolerance traits in the gametophyte stage has led to evolutionary shifts in the sporophyte stage (Mulcahy 1979; Hedhly et al. 2005). These studies support the hypothesis that the combination of selection at the sporophytic and gametophytic life stages contribute to the evolution of temperature tolerance in angiosperms.

Almost all previous studies concerning climate adaptation have focused on crop species and overall, little attention has been paid to non-crop species. This is problematic because restoration and conservation efforts involve adaptation processes of non-crop species. Wang et al. (2012) conducted a meta-analysis in which the authors compared crop and non-crop species and found a difference between the two in physiological responses to temperature and CO2 levels. The authors went on to mention that their analysis was limited because of understudied groups of plants and necessitated further research of responses to climate change (Wang et al. 2012). Furthermore, novel tolerance mechanisms discovered in non-crop species might provide insight for crop breeding.

I am proposing to examine temperature tolerance at the sporophytic and gametophytic stages of *Solanum carolinense* (horsenettle). *Solanum carolinense* is an invasive, non-crop species, native to the southeastern United States that now spans much of temperate North America (Sylwester 1946). The pervasiveness of *Solanum carolinense* suggests that this species easily adapts to different conditions. For this study, I will compare temperature tolerance in populations of *Solanum carolinense* in Texas and Minnesota, and exploit plants from a wide range of local temperature thresholds (Fig. 1).

*Solanum carolinense* is not native to TX or MN and currently these states are on the outer reaches of the range for this species. As is common for exotic species first forming colonies, the populations in MN and TX are relatively small and are likely in the process of adapting to local conditions. This is an example of contemporary evolution, coined by Hendry and Kinnison in 1999, which is short-term evolution of traits and is common among invasive species. For exotic species, like *Solanum carolinense*, contemporary evolution may be assisting expansion as the rapid evolution of adaptive traits improves survival in novel environments (Stockwell et al. 2003; Colautti and Lau 2015). Since the climates are vastly different in TX and MN, I hypothesize that the adaptation of tolerance to extreme temperatures in these regions deviates. I expect that the northern plants are adapting to extreme cold and southern plants are adapting to extreme heat. With this proposed study, I intend to elucidate the mechanisms of contemporary evolution specific to temperature tolerance in *Solanum carolinense*.



Figure 1. Range of *Solanum carolinense* and locations for the origins of the plants that will be used in this study.

The objective of this study is to examine the relative effects of selection in the gametophytic and sporophytic stages for temperature tolerance in *Solanum carolinense.* I will test two null hypotheses that there is no difference in temperature tolerance between the northern and southern horsenettle populations for both life history stages. The expected alternative hypothesis is that southern plants will be more tolerant of high temperature stress in both gametophytic and sporophytic life stages. I also hypothesize that selection for temperature tolerance is reinforced in sporophytic and gametophytic stages due to overlap in gene expression (Tanksley et al. 1981; Willing and Mascarenhas 1984).

**Methods**

***Horsenettle collection***

Twenty-two rhizomes from individual plants were collected from Collin county, TX, where mean monthly temperatures range from 18°C to 43°C. Nineteen rhizomes from individual plants were collected from Houston county, MN with a mean monthly temperature ranging between -14°C and 29°C. We grew the rhizomes to maturity, cut back the vegetative material to the rhizome, and stored the rhizomes at 4°C, mimicking a dormant period.

***Greenhouse Experimental Design***

In January, we will remove the plants from dormancy, cut the rhizomes into 5 ramets (genetically identical sections of rhizome capable of growth), and grow each to maturity in a greenhouse, temporally staggered. Eventually, four of the ramets will be planted in narrow cone-shaped containers (Fig. 2) for sporophytic analysis and one will be placed in a larger container, intended to reach maturity for sporophytic and gametophytic data collection. To achieve temporal staggering, a ramet from eight randomly selected TX plants and eight randomly selected MN plants will initiate growth followed by the next set the following week until all ramets for 19 plants from TX and MN are out of dormancy. Once the plants reach maturity, sporophytic and gametophytic data will be collected.



Figure 2. Random block design with a leaf symbolizing a ramet. Blue indicates a ramet from a Minnesota plant and red symbolizes a ramet from a Texas plant. Red box outlines a block. Black box with circles used to represent structure that holds cone shaped containers, in which ramets will be planted.

To control for inconsistent conditions throughout the greenhouse, the plants will be arranged in a randomized block design with a MN and TX plant in each block, randomly assigned to the left or right side of the block. Ramets from the same individual will be temporally and spatially distanced from one another.

***Sporophytic tolerance***

Sporophytic tolerance will be analyzed using vegetative tissues, specifically leaves. I will measure temperature tolerance by the level of damage observed before and after temperature treatments. The parameters that will be used to quantify temperature tolerance are cell membrane stability, net photosynthesis, and leaf cooling. All parameters will be used for high temperature tolerance measures. Cell membrane stability and net photosynthesis will be used for both high and low temperature tolerance measures. These parameters are commonly used for observing physiological responses to stress in sporophytic tissues (Gajanayake 2011; Poudyal et al. 2019). These parameters were selected based on available resources and evidence in previous studies showing responses in these variables to temperature stress.

The Texas plants are expected preform better under high temperature stress than the Minnesota plants. Therefore, the cell membrane stability, net photosynthesis, and leaf cooling are expected to be higher in the Texas plants than the Minnesota plants. Conversely, the Minnesota plants are expected to perform better in cold stress conditions than the Texas plants and therefore, are expected to have a higher cell membrane stability and net photosynthesis when subjected to cold treatments.

*Cell membrane stability*

Cell membrane stability (CMS) will be measured using the conductance of a solution containing a specific quantity of leaf material following the protocols from Gajanayake et al. (2011) and Fang and To Nu (2016). The more damaged a plant is, the less stable the cell membrane is, leaking cytoplasm into the solution and raising the conductance (Gajanayake 2011). The conductance, measured in μS, will be recorded after the temperature treatment and then again after a maximum damage treatment (Fig. 3).

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Figure 3. Schematic describing protocol using conductance to measure cell membrane stability for the heat stress treatment.

For the high temperature stress treatment, 20 leaf disks from one leaf selected based on predetermined criteria will be divided between two test tubes with 10 mL of RO water. One test tube with leaf material will be subjected to a heat treatment of 20 minutes in a water bath at 50°C and the control test tube will remain at room temperature.

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Figure 4. Schematic describing protocol using the conductance to measure cell membrane stability for the cold stress treatment.

For the low temperature stress treatment, whole plants will be placed in an environmental chamber at 10°C while another group of plants will remain at room temperature for three days. After 72 hours, one leaf from each plant will be selected based on predetermined criteria and cut into the test tubes as described in the high-temperature stress treatment. This protocol is modified from Zhao et al. (2005).

For the maximum damage condition, the test tubes for both the treatment and control groups will be placed in a water bath at 98°C for 30 minutes, followed by a conductance measurement. The ratio of conductance after the treatment to after maximum damage will then be used to quantify cell membrane stability.

*Photosynthesis and leaf cooling*

Photosynthesis and leaf cooling will be measured before heat treatment, after heat treatment, and following a recovery period as described by Poudyal et al. (2019). Photosynthesis and leaf temperature will be measured using a Li-COR infrared gas analyzer after the plant is dark adapted for 45 minutes following protocol modified from Mishra et al. (2019). Photosynthesis will be reported through the quantum efficiency of photosystem II using the ratio of variable fluorescence and maximum fluorescence (Fv/Fm) in units of μmol/ m2s. Leaf cooling, or canopy temperature depression, is the difference between the leaf temperature and the ambient temperature measured in °C. Plants with higher leaf cooling in heat stress conditions have more control over temperature and are more likely to maintain normal functioning. Photosynthesis will also be measured before, during, and after the cold treatment described for the cell membrane stability cold tolerance. There is currently no evidence of leaf heating in response to cold stress and therefore, will not be included in this study.

***Gametophytic tolerance***

*Pollen tube growth rate and viable pollen*

Gametophytic tolerance will be analyzed following the protocol of Gajanayake et al. (2011). Pollen success relies on pollen tube growth through the length of the style for germination at the ovule. Therefore, pollen tube growth rate will be measured as an indication of pollen fitness. The length of pollen tubes will be recorded after 24 hours in incubation at one of five temperatures: 10°C, 15°C, 25°C, 30°C, and 40°C. We will use a light microscope to photograph the pollen tubes on the agar plates. ImageJ will be used to measure the lengths of the pollen tubes in the pictures taken. We will fit a curve to the growth rates at all temperatures to determine low, optimal, and high temperature limits for pollen tube growth. We will also count the total number of pollen grains to determine the proportion of viable pollen.

***Data Analysis***

Data analysis will be conducted in R. Variables reported as proportions will be arcsin sqrt transformed before analysis. Mixed model analysis of variance with provenance nested and genet as a random effect will be used for the analysis of each variable. Correlation analysis will be used to determine the relationships between temperature tolerance at the sporophytic and gametophytic stages.

**Expected Results**

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Figure 5. Pilot data for cell membrane stability under high temperature treatment. Experimental unit was one leaf from individual plant. Outliers identified by Grubb’s test excluded. T-test of arcsin sqrt transformed cell membrane stability proportion had p-value 0.028.

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Figure 6. Expected results for cell membrane stability under low temperature treatment.

The expected outcome for the cell membrane stability is that the southern population has a higher stability than the northern population when exposed to a high temperature treatment (Fig. 5). The opposite is expected for cell membrane stability exposed to the cold treatment (Fig. 6). This outcome would support the hypothesis that southern horsenettle populations are more tolerant of high temperatures than northern populations are more tolerant of low temperatures for the sporophytic life history stage. The null hypothesis would be supported if there is no difference in cell membrane stability between plants from the northern and southern provenances. The pilot data with individual plants as the experimental unit indicate significant differences between the northern and southern populations cell membrane stability exposed to high temperatures (Fig. 5). The pilot data is highly variable and therefore, we plan to use the average of all ramets as the experimental unit rather than one individual plant to mitigate this variation. Since the ramets are effectively clones, they will not be used as individual experimental units to avoid pseudoreplication (Hurlbert 1984).

The expected outcome for net photosynthesis is that the southern populations will have a constant net photosynthesis for the control, heat stress, and recovery treatments while, the northern population will have a constant net photosynthesis for cold stress. A constant net photosynthesis or at least higher net photosynthesis is indicative of normal functioning under stress. The expected outcome for the leaf cooling is that the southern populations will have a higher temperature difference between the ambient temperature and leaf temperature than the northern population under heat stress. A higher ΔT indicates more effective leaf cooling, reducing the heat stress. Both the net photosynthesis outcome (Fig. 7) and the leaf cooling outcome (Fig. 8) would support the hypothesis that southern populations are more tolerant of high temperature stress than the northern population.

Figure 7. Expected outcome for net photosynthesis for the control, heat stress, and recovery treatments of northern and southern horsenettle populations. The opposite is expected for the cold stress.

Figure 8. Expected outcome for leaf cooling for the control, heat stress, and recovery treatments of northern and southern horsenettle populations

***Gametophytic Tolerance***

Figure 9. Expected outcome for pollen tube growth rate or pollen viability with curves defining the minimum, maximum, and optimal pollen tube growth temperatures for northern and southern populations.

The expected outcome for the measures of gametophytic tolerance are that the southern population has a threshold of pollen tube growth and viability at increased temperatures when compared to the northern population (Fig. 9). This outcome supports the alternative hypothesis that southern populations are more tolerant of high temperature than the northern population in the gametophytic life history stage.





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