



Salt Tolerance in *Monarda punctata* and *Monarda citriodora*

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Introduction

For many plants, environmental salinity levels play a key role in development (Boyd and Barbour 1986). Plants living close to the coast may be exposed to high salinity levels both through saltwater in the soil as well as salt contained in sea spray that is deposited on their leaves and stems. *Monarda punctata*, a member of the mint family (Lamiaceae), is a flowering plant native to the United States that can be found in sandy soils throughout eastern North American, from prairies and the shores surrounding the Great Lakes to sand dunes along the Atlantic coast (Temeles and Rankin 2000, Pennell 1919). Previous work in our lab found that plants from the Atlantic coast are more tolerant of salt spray than those from inland populations, showing that populations are locally adapted to salt spray (Kolish et al. 2019). However, we do not understand how salt tolerance of this species compares to closely related species that have a more inland distribution. In this study we compared salt tolerance in a coastal population of *M. punctata* to that of an inland population of *Monarda citriodora*, a closely related species found primarily in inland habitats. The hypothesis is the Atlantic coastal population *M. punctata* is more salt tolerant than the inland population of *M. citriodora*.

Objectives

- 1.Compare salt tolerance of an Atlantic coast population of *Monarda punctata* to that of *Monarda citriodora*, a species with primarily inland populations.
- 2.Determine if *Monarda* shows phenotypic plasticity in stomata and trichome density, two traits known to affect salt tolerance.
- 3.Determine which individuals show the greatest salt tolerance for use in future artificial selection experiments to increase salt tolerance.

Methods

Seeds of *Monarda punctata* were collected from a population in Virginia in the sand dunes along the Chesapeake Bay where the Bay enters the Atlantic Ocean (Fig. 1). Seeds from inland populations *M. citriodora* were purchased from a native plant nursery in Texas and were originally collected from Texas Hill Country (Fig. 2), an inland area in Texas with very shallow, dry soils. Approximately 10 seeds were planted in 90mm pots in germinating mix with 26-40 pots per population. Pots in each population were given a unique number and divided into control and treatment groups. Pots were organized in flats in 6 x 3 arrangement with pots in the control and treatment group in different flats. The flats were placed under fluorescent lights in a classroom at Shippensburg University and watered twice a week on Monday and Thursday. The position of pots was rotated before watering to ensure similar amount of fluorescent light and temperature. A quarter teaspoon of Osmocote fertilizer was added to the top of the soil three weeks after seeds were planted.

When plants had at least 6 true leaves, the number of true leaves and the height of the main stem were measured. Then, they were sprayed with either a control or treatment solution where the plants were placed 18cm from the spray bottle. The control spray contained deionized water and the treatment spray contained 20g/L NaCl solution. Plants were sprayed with either control or treatment solution twice a week on Monday and Thursday, and it was ensured that the spray did not enter the soil.

The treatment was stopped after each pot received four spray treatments. The height of the main stem, the total number of leaves, and the number of damaged leaves were recorded. After 2-3 days of stopping the treatment, pictures of the second and third pair of leaves from the bottom of the main stem were taken. Total leaf area and the proportion of damage on leaves was measured from these photographs using ImageJ (Fig. 2). The abaxial side of two leaves were painted with clear nail polish and casts were removed for subsequent quantification of stomata and trichome density (still ongoing).



Figure 1. Map of the United States using Google Earth with locations of where seeds for *Monarda punctata* (yellow marker with star) and *Monarda citriodora* (white marker) were collected from.

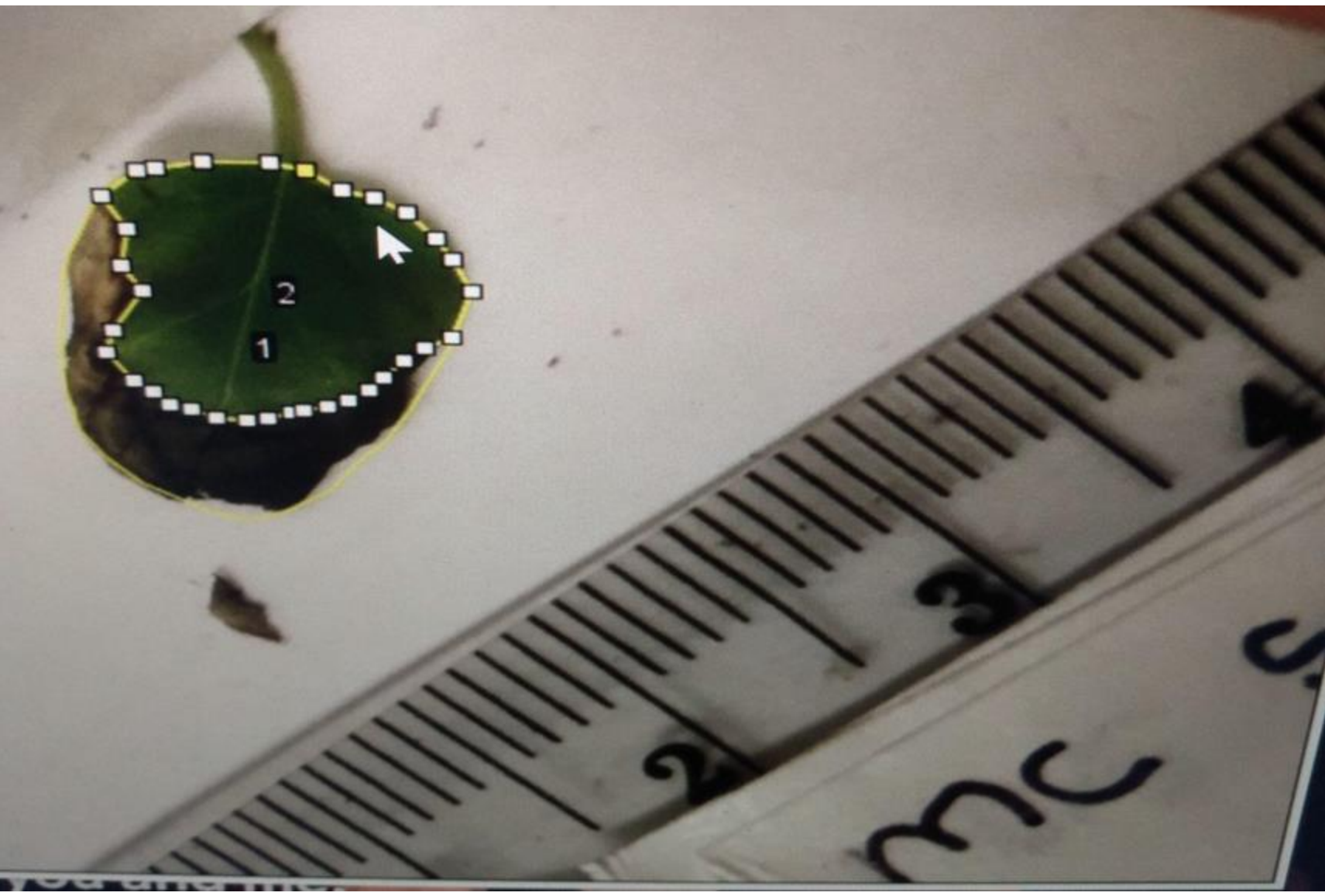


Figure 2. *Monarda citriodora* leaf from salt treatment showing outline of the entire leaf (total leaf area) and the undamaged area using ImageJ software.

Data Analysis & Results

For each dependent variable (% of leaves with damage, growth in stem length, number of new leaves produced, and total leaf area), a two-way ANOVA was run with treatment and population as independent variables with a ‘treatment x population’ interaction. A significant treatment x population interaction indicates that populations differ in their degree of salt tolerance. Both percentage of leaves showing damage and percent leaf damage on individual leaves showed the same effect, so only results for percentage of leaves showing damage are presented here.

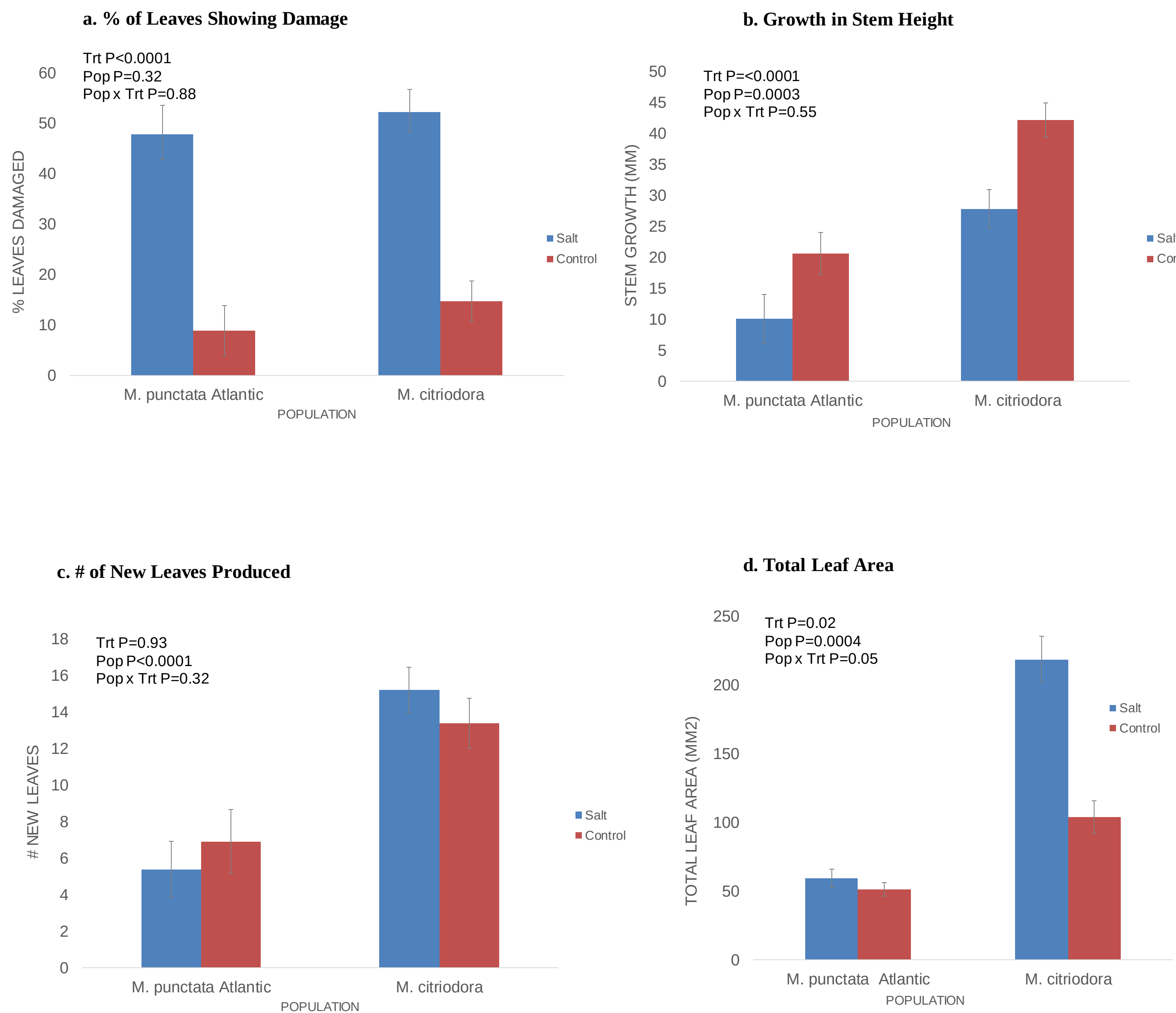


Figure 3a-d. Average plant traits in salt spray and control treatments for coastal *Monarda punctata* and inland *Monarda citriodora* populations. a) percentage of leaves showing damage, B) change in stem height after two weeks, c) number of new leaves, and d) total leaf area. Error bars represent one standard error of the mean.

In both populations, salt spray caused a significant increase in the percentage of leaves that were damaged ($P<0.0001$, Fig. 3a), significantly reduced stem growth ($P<0.0001$, Fig. 3b), and significantly reduced total leaf area ($P=0.02$, Fig. 3d). However, salt spray had no impact on the number of new leaves produced ($P=0.93$, Fig. 3c). Populations showed evolved differences in stem height, the number of leaves produced, and total leaf area, with *M. citriodora* plants growing taller ($P=0.0003$, Fig. 3b), producing more leaves ($P<0.0001$, Fig. 3c), and producing larger leaves ($P=0.0004$, Fig. 3d) than *M. punctata* plants. The only trait that showed a significant Treatment x Population interaction was total leaf area ($P=0.05$), where *M. citriodora* plants experienced a greater reduction in leaf area when exposed to salt spray compared to *M. punctata* plants (Fig. 3d).

Discussion

Salt spray had a significant negative effect on both populations of *Monarda* studied, reducing the height of stems, causing a large proportion of leaves to be damaged, and reducing leaf area, all of which can reduce photosynthesis, ultimately leading to lower seed production in these populations. Interestingly, for most of the traits studied, the *M. punctata* population from the Atlantic coast showed the same degree of salt damage as the inland *M. citriodora*, suggesting that there has been little evolution of increased salt tolerance in this population despite being exposed to salt spray. However, for one trait, total leaf area, the coastal *M. punctata* population was more well adapted to salt spray than the inland *M. citriodora* population.

Previous studies in our lab found that Atlantic coast populations have greater salt tolerance than inland populations of *M. punctata* when including several populations of *M. punctata* from each location. This suggests that either this Virginia coastal population has less salt tolerance than other coastal populations studied before, or the Texas population of *M. citriodora* included in the current study is more salt tolerant than inland populations of *M. punctata* from other states previously studied. It is possible that the hot, dry environment that the *M. citriodora* population has adapted to has led to the evolution of the same traits that might increase tolerance to salt spray such as reduction in stomata density, increased succulence, and increased trichome density. Future work will examine if populations differ in stomata or trichome density or leaf succulence. We will also determine if these traits show adaptive phenotypic plasticity in response to salt spray.

Finally, some individuals showed very little effects of salt spray within both populations. We will cross pollinate those individuals showing tolerance to salt to determine if we can artificially select for increased salt tolerance in these populations. The evolution of increased salt tolerance will be imperative in the face of sea level rise as a result of global climate change.

Acknowledgments

We would like to thank the Shippensburg Undergraduate Research Experience program for providing funding for this research.

References

- Boyd R.S. and Barbour M.G. 1986. Relative salt tolerance of *Cakile edentula* (Brassicaceae) from lacustrine and marine beaches. *American Journal of Botany*. 73(2): 236-241
- Kolish, T., Bzdak, B., Pieklo, O., and Sahli, H.F. 2019. Analysis and comparison of salt tolerance of *Monarda punctata* in coastal and inland populations. Presentation at Shippensburg University Minds at Work conference. Shippensburg, PA.
- Pennell, F.W. 1919. Notes on plants of southern United States-V. *Bulletin of the Torrey Botanical Club*. 46(5): 183-187.
- Temeles E.J. and Rankin A.G. 2000. Effect of the lower lip of *Monarda didyma* on pollen removal by hummingbirds. *Canadian Journal of Botany*. 78(9): 1164-1168.