Influence of Drought Stress on *Castilleja Coccinea*

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1. Introduction

Climate change has brought about shifts in precipitation patterns, including heightened mid-season drought and notable fluctuations in both average temperatures and extreme temperature events[1], altering the abiotic environments of midwestern plant communities.

To investigate how midwestern plant species may respond to changes in abiotic conditions and the possible implications on fitness, our study centers *Castilleja coccinea* (Orobanchaceae), a perennial hemiparasitic forb widely distributed across the Midwest and eastern United States. *Castilleja coccinea* maintains a floral color polymorphism consisting of a red and a yellow variant, with the red coloration being a result of the stress compound and pigment known as **anthocyanin**. Beyond its visual significance, foliar anthocyanin and its derivatives also serve in UV protection[2], thermal stress tolerance[3], and defense against herbivory. Yellow populations are more commonly found in prairie ecosystems, and red is more common in exposed rocky outcrops[4], with varying drought tolerances likely due to local adaptation.

Our investigation involves a drought experiment designed to evaluate how abiotic drivers such as drought differentially impact the morphological and physiological stress tolerances of the two varieties of *Castilleja coccinea* across distinct populations.

2. Research Question

How does drought stress impact both foliar anthocyanin production and growth/survival rates across red and yellow populations?

Hypothesis

Drought conditions will induce anthocyanin accumulation in *Castilleja coccinea*, with red variants conferring an advantage over yellow variants as attributed to their higher baseline levels of anthocyanin, which support physiological resilience as demonstrated by increased growth and survival rates.
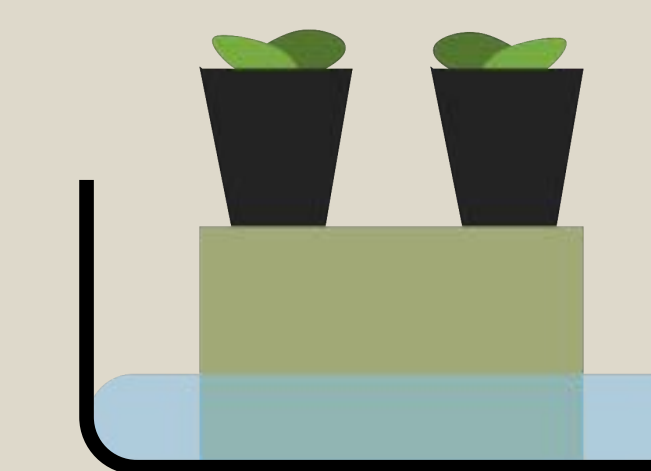
3. Methods

Greenhouse Setup

Figure 1. Greenhouse setup demonstrating a control bin and a drought bin
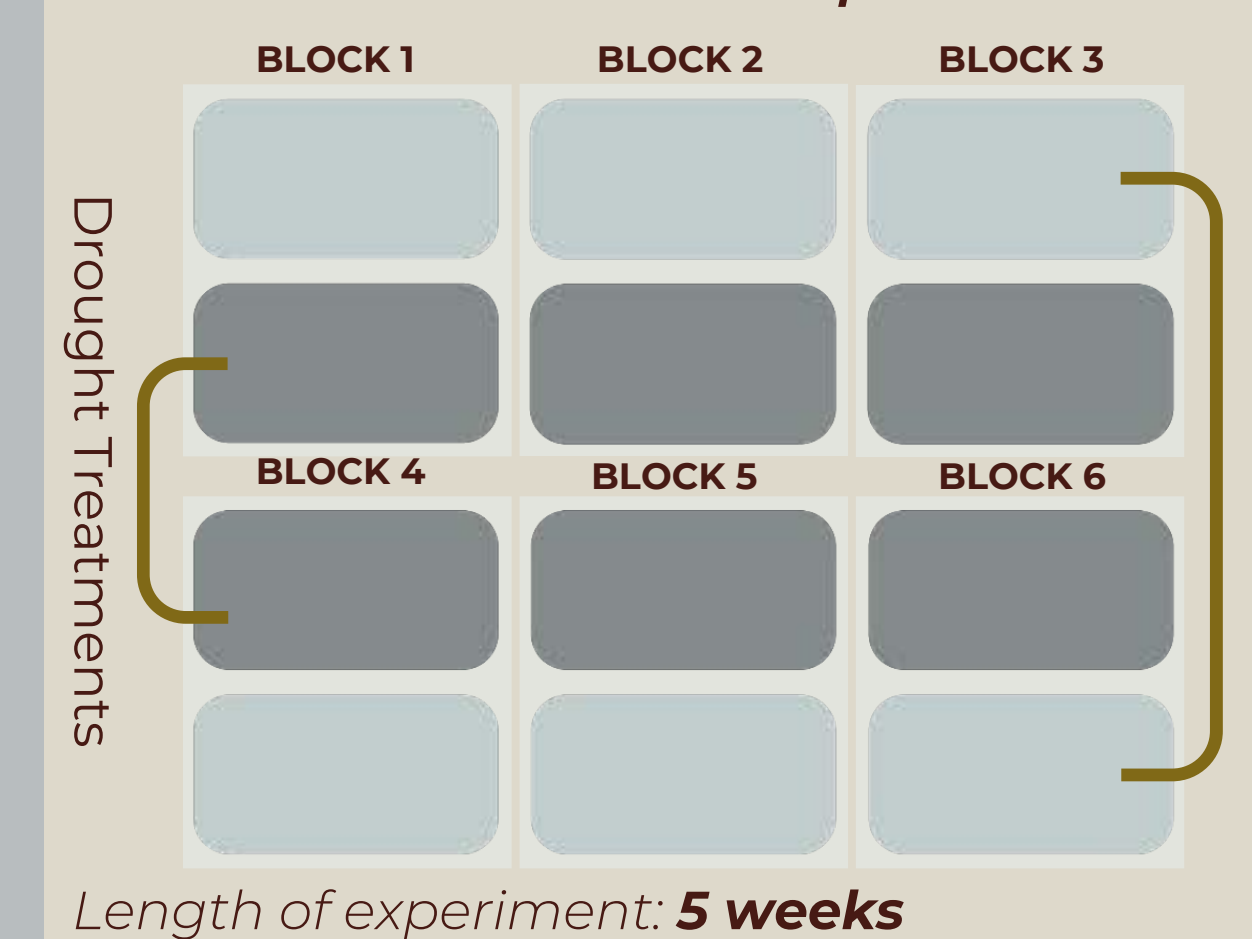
- Two red populations (R1 + R2) and two yellow populations (Y1 + Y2)
  - R1: Cook County Nature Preserve
  - R2/Y1/Y2: Prairie Moon Nursery, Winona, MN
- Two bins per block
  - 20 plants per bin with 5 plants from each population
  - Randomized positions

Length of experiment: **5 weeks**

Measurements

- Rosette diameter
- Number of leaves
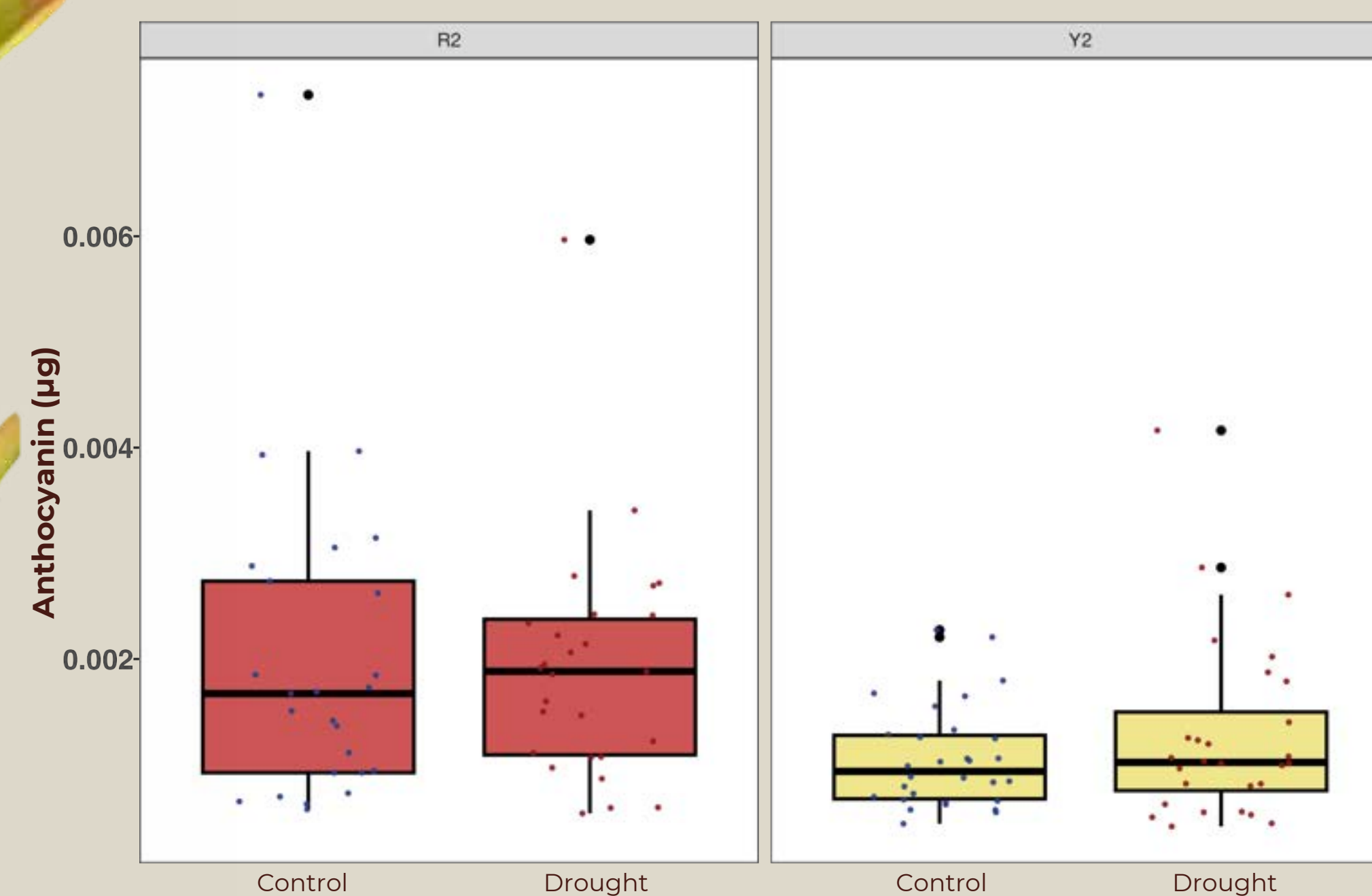- Longest leaf
- Soil moisture

4. Results

Figure 2.
Anthocyanin content (μg) is compared between drought and well-watered treatments in the R2 and Y2 populations. A Wilcoxon signed-rank test was performed and found a significant difference within the treatment (p-value: 0.0011) and control (p-value: 0.0271) groups between the red and yellow populations.

Anthocyanin Extraction

Foliar **anthocyanin** content was quantified for 60 plants from the R2 population and 60 plants from the Y2 population
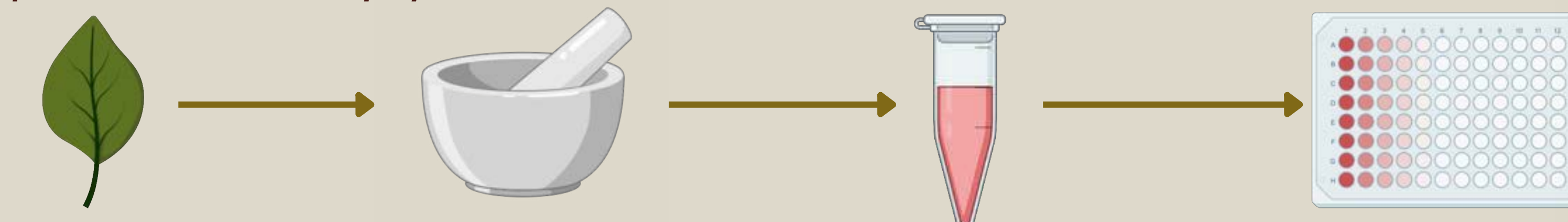
100mg of leaf material was collected from each plant

Leaf tissue was frozen using liquid nitrogen and pulverized into a fine powder

The tissue was combined with an extraction solution, placed on a heat block, and incubated overnight

Samples were pipetted into a 96 well plate and anthocyanin absorbance was measured using a spectrophotometer at 535nm and 650nm

Figure 3A. Rosette diameter (cm) — Population: R1, R2, Y1, Y2

Figure 3B. Longest Leaf (cm) — Population: R1, R2, Y1, Y2

Figure 3C. Number of Leaves — Population: R1, R2, Y1, Y2

Treatment: C / D

During week five of the experiment, the mean rosette diameter (3A), longest leaf (3B), and number of leaves (3C) of populations R1, R2, Y1, and Y2 in the drought and well-watered treatments were measured. A Wilcoxon signed-rank test found a significant difference in rosette diameter within Y1 (p-value: 0.0433) and Y2 (p-value: 0.0046), as well as a significant difference in number of leaves within R1 (p-value: 0.000853) and Y2 (p-value: 0.00167).

5. Discussion

- Under drought stress, **yellow variants exhibited smaller rosette diameters** than the control treatment groups
  - We found no significant difference in rosette diameter between treatment groups in R1 and R2
- While there was no significant relationship in anthocyanin accumulation across the drought and well-watered conditions, anthocyanin levels were significantly higher in the red populations
  - A genetic and population dynamics analysis is necessary to determine (1) the level of inbreeding within populations and its implications for the results, and (2) whether physiological mechanisms aside from anthocyanin content may confer an advantage to withstanding dry conditions
- Biomass could provide a more accurate measure of growth, and extending the sampling period + increasing sample size could yield a more precise response

Future Directions

- Evaluate other anthocyanin-containing restoration target plant species to determine whether associated stress tolerance mechanisms are unique to *Castilleja coccinea* or are common across diverse taxa
- Investigate the effects of other climate change-related variables, such as varying CO_2 levels and habitat fragmentation, on plant responses
- Explore potential connections between population genetics, dynamics, and functional traits across red and yellow populations
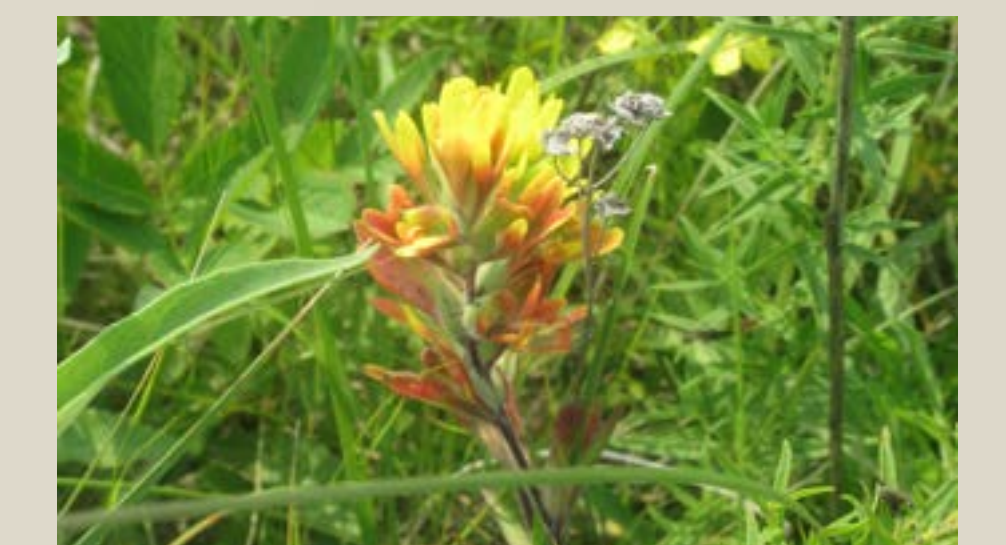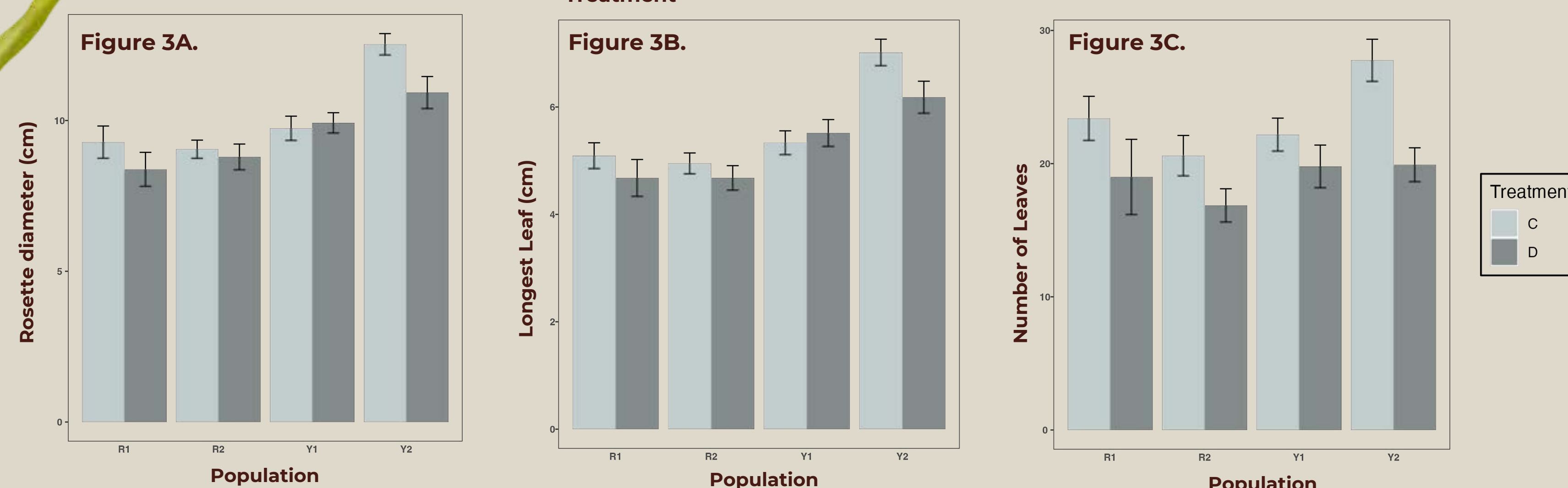

Figure 4. Yellow variant of *Castilleja coccinea* at the Fugile Waterfowl Production Area, Clay County, MN on 7/13/23

Literature Cited

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2. Quina, F., Moreira, P., Vautier-Giongo, C., Rettori, D., Rodrigues, R., Freitas, A., Silva, P. & Maçanita, A. (2009). Photochemistry of anthocyanins and their biological role in plant tissues. Pure and Applied Chemistry, 81(9), 1687-1694.
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