Role of CYP72A enzymes in *Zea mays* in response to abiotic and biotic stress

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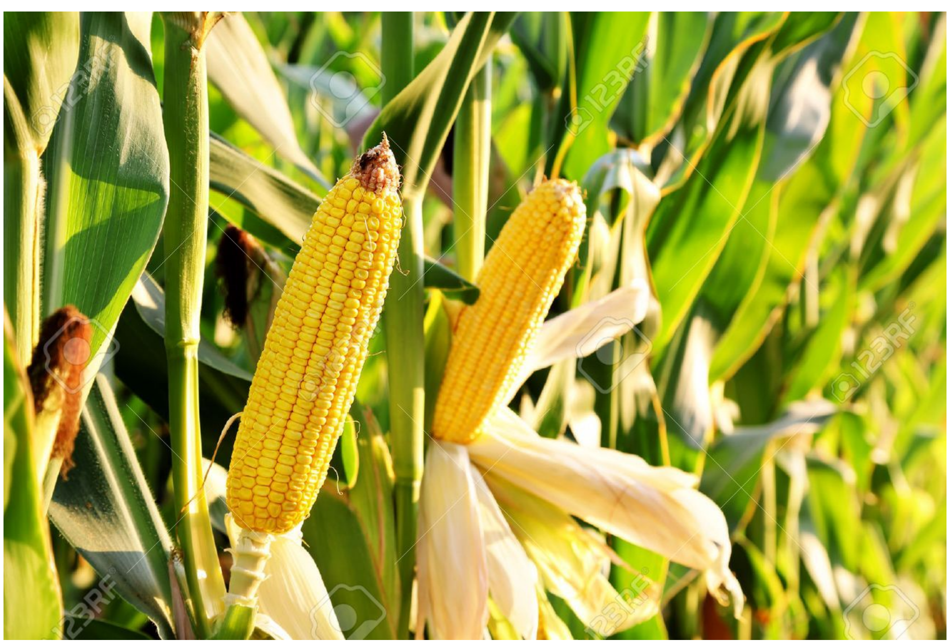
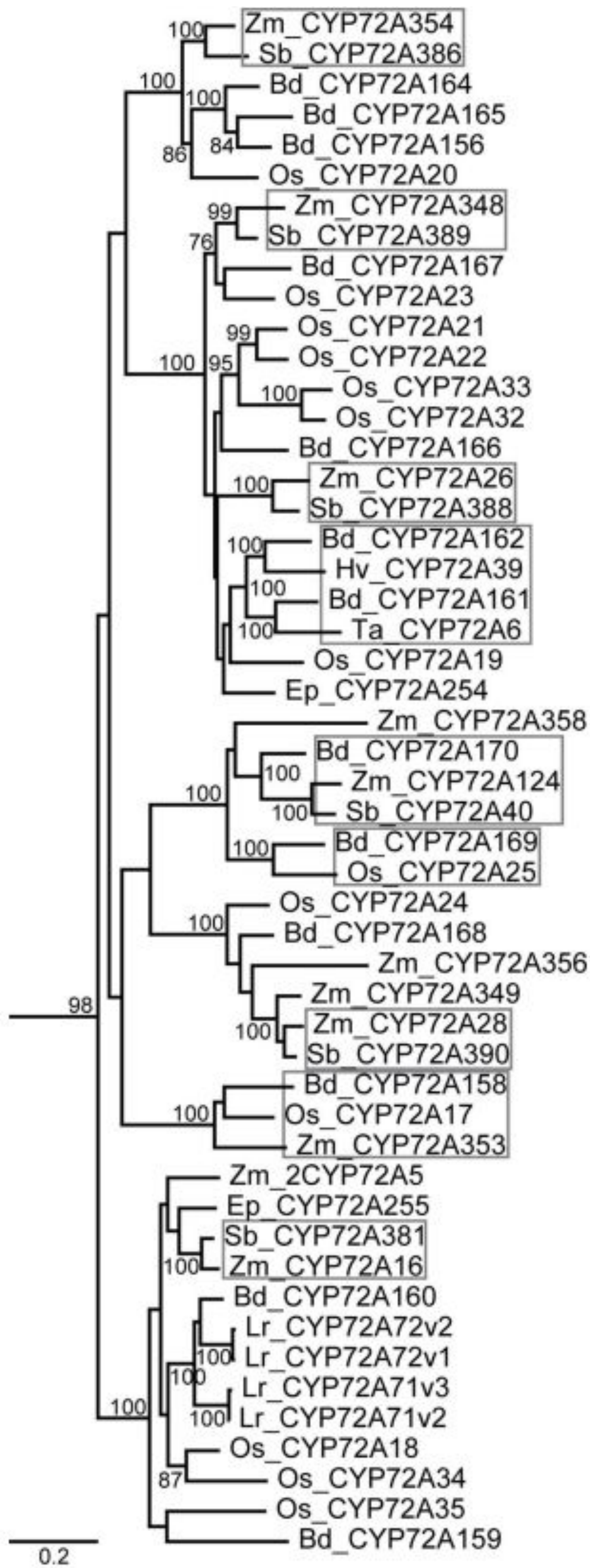


Introduction

- Anthropogenic effects, pests and disease are factors that stunt crop growth and production.
- Plants have developed chemical and physical defenses that allow them to protect themselves from predators.
- Abiotic and biotic stresses that affect plants are often combined in a real world setting which causes a biochemical response that differs from that of an individual stress.
- Cytochrome P450 (CYPs) contribute to plant defense pathways and produce different compounds that act to combat & adapt to stressors.
- Genes for CYP72A enzymes in *Zea mays* (corn) plants are activated by a combination of abiotic and biotic stresses.

Introduction (Cont.)

- Our studies aim to uncover how CYP72A enzymes help corn defend itself against biotic stress (caterpillars) after undergoing abiotic stresses (salinity, heat, and drought)
- Used beet armyworm caterpillars or *Spodoptera exigua*.
- Phylogenetic tree of the CYP72A enzymes in corn and close relatives
    - *Oryza sativa* (Asian rice)
    - *Sorghum bicolor* (great millet)
    - *Lolium rigidium* (annual ryegrass)
    - *Brachypodium distachyon* (purple false brome)
    - *Triticum aestivum* (Common wheat)
    - *Hordeum vulgare* (Barley)
    - *Echinochloa phyllopogon* (Barnyardgrass)



Methods

- Ds insertion mutants are used as genetic tools in order to modify gene expression
- Mutants: CYP72A26 (Ds436) and CYP72A349 (Ds309)
- We aim to determine the role of CYP72A enzymes in corn responses to abiotic and biotic stress.

Abiotic Stress	Biotic Stress
Salt	Caterpillars
Salt + drought	
Drought	
Heat	
Heat + drought	

- We performed a series of caterpillar choice experiments to see if caterpillars had a preference for a genotype (WT, 309, or 436).
- This would help us see if CYP72A26 and CYP72A349 enzymes are involved in defense against caterpillar herbivory.
- Statistical analysis was completed to determine if there was a statistically significant difference between feeding on the three genotypes

Experimental Design

Corn seeds planted (WT, 309, 436)

⇩

Corn grown under normal conditions for approximately 10-14 days

⇩

Abiotic stress treatment applied

⇨

Day of caterpillar choice experiment, plant tissue was cut

⇧

Plant leaf sections arranged into arenas to see which genotype tastes better to the caterpillars as seen in FIg. 1

⇧

ImageJ was used to determine the total area of caterpillar feeding

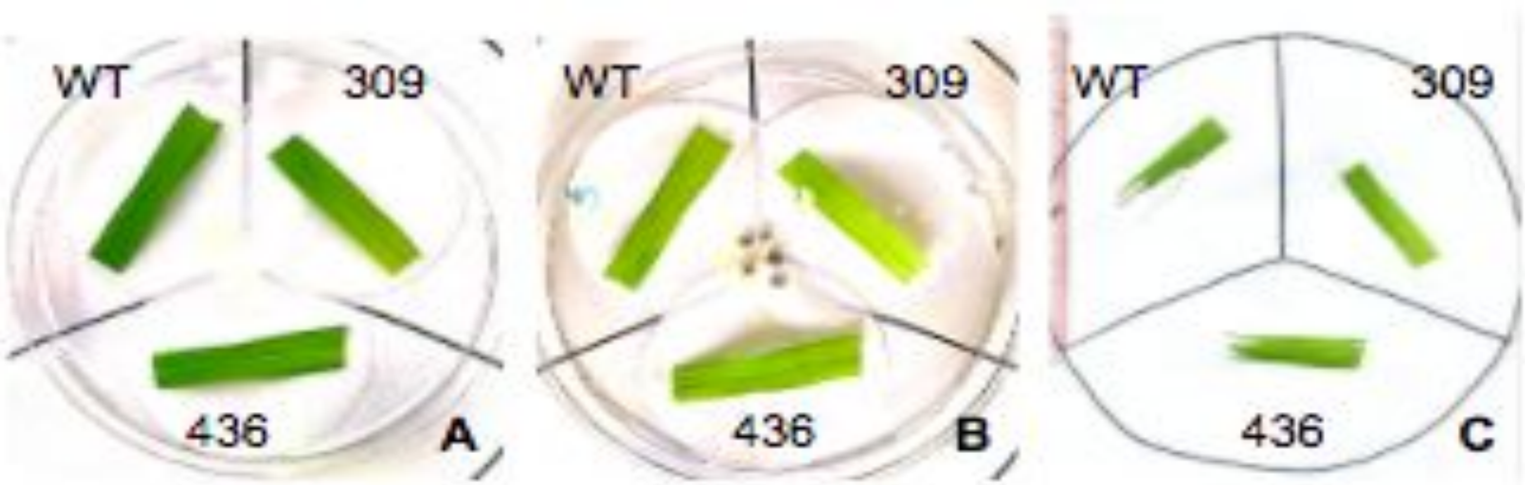


Figure 1. Experimental design of a caterpillar choice experiment. Arena setup of WT, Ds309, Ds436 corn plants that went through drought stress before caterpillar feeding. **a** Five caterpillars placed at the center of the arena setup of WT, Ds309, Ds436 corn plants that went through drought stress. **b** Arena setup of WT, Ds309, Ds436 corn plants that went through drought stress after caterpillar feeding shown in **c**.

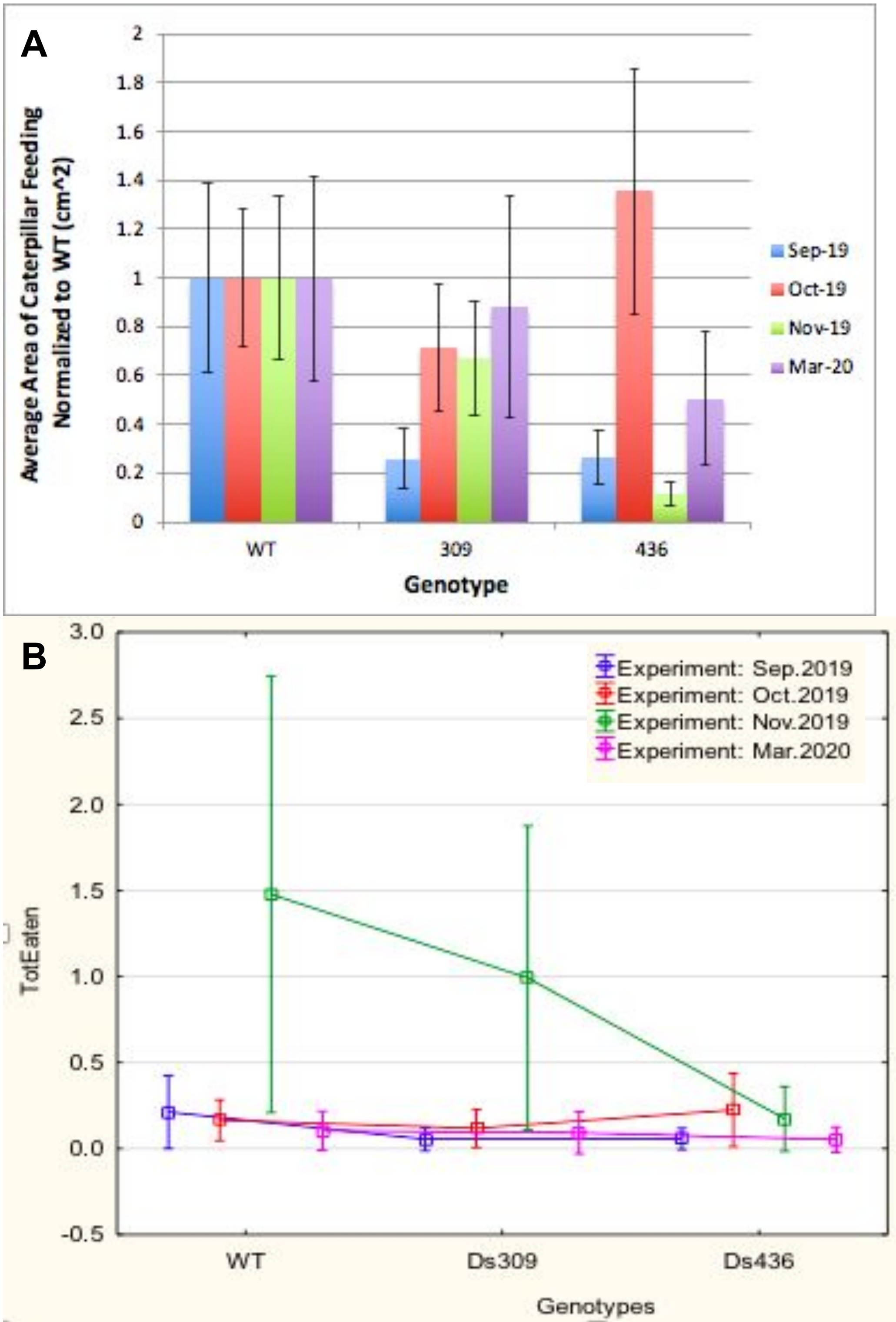


Figure 2. Caterpillar choice experiment for corn that went through salt stress. A Normalized average +/-SE caterpillar feeding in WT, Ds309, and Ds436 corn plants. Averages and SE were normalized to wildtype average values. **B** Mean plot of total area eaten by caterpillars for WT, Ds309, and Ds436 corn plants. Error bars show 95% confidence interval. There is no consistent difference between the wildtype and Ds309 and Ds436 mutants.

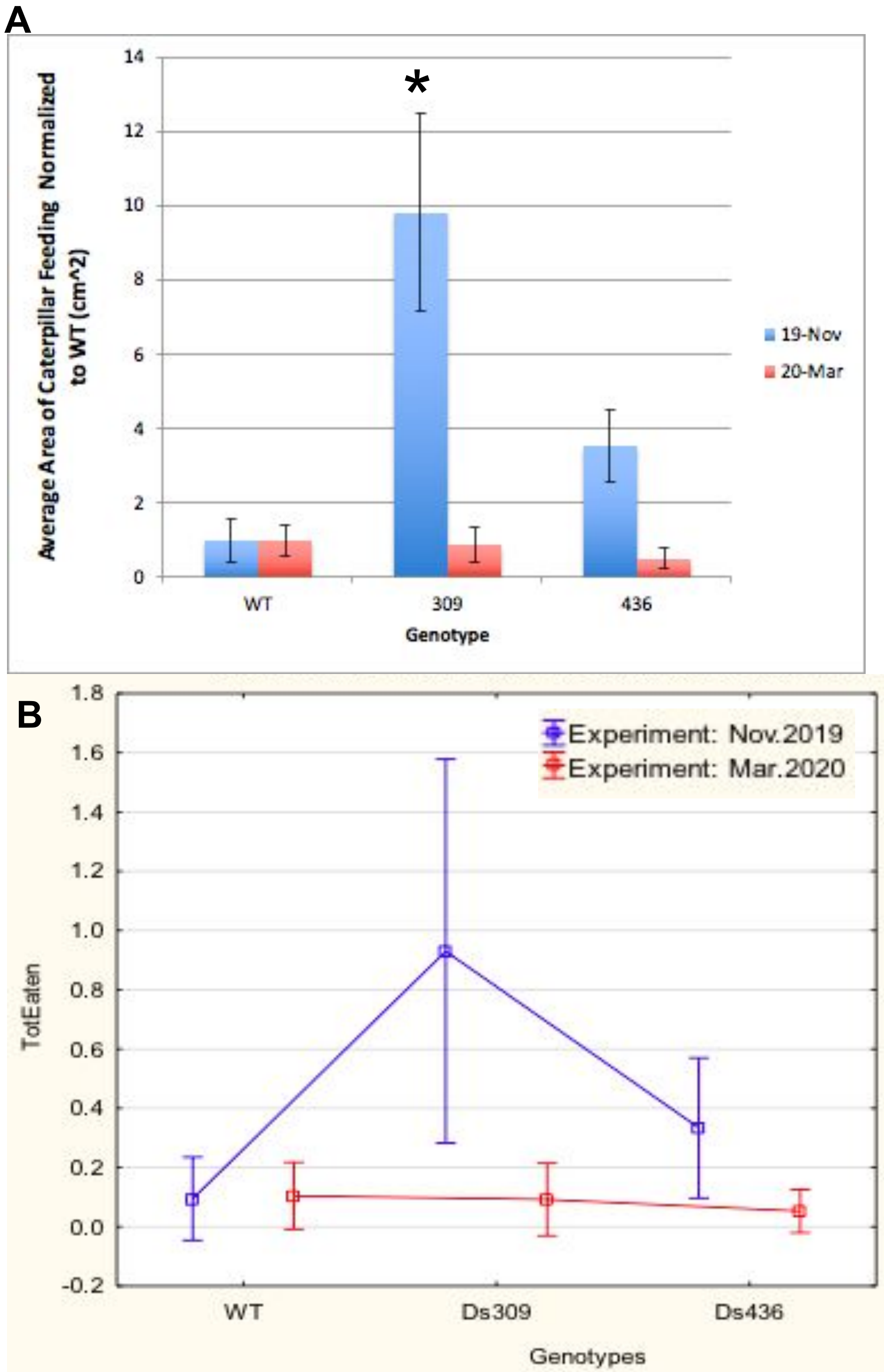


Figure 3. Caterpillar choice experiment for corn that went through salt and drought stress. A Normalized average +/-SE caterpillar feeding in WT, Ds309, and Ds436 corn plants, after stress and caterpillar feeding in November 2019, there was significantly more feeding on the Ds309 plants than wildtype and Ds436 plants (* $P < 0.05$) as determined by ANOVA with the Tukey HSD test. N = 6. **B** Mean plot of total area eaten by caterpillars for WT, Ds309, and Ds436 corn plants. Error bars show 95% confidence interval.

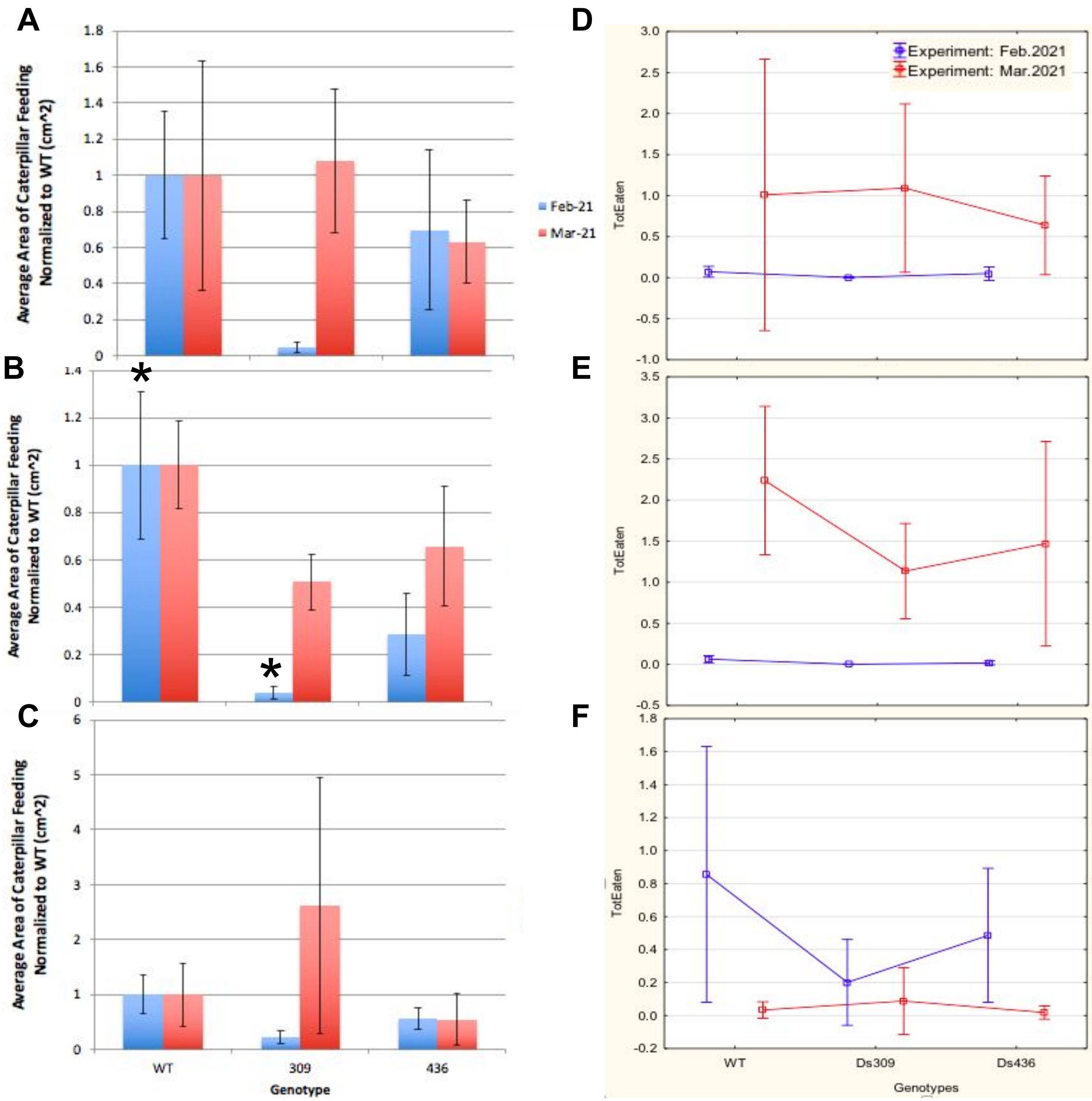


Figure 4. Caterpillar choice experiment for corn in salt, drought and combination salt and drought stress. Normalized average +/-SE caterpillar feeding in WT, Ds309, and Ds436 corn plants that went through salt stress **A** drought stress **B** salt and drought stress **C.** Averages and SE were normalized to wildtype average values. Mean plot of total area eaten by caterpillars for WT, Ds309, and Ds436 corn plants that went through salt stress **D** drought stress **E** salt and drought stress **F.** Mean plot error bars show 95% confidence interval.

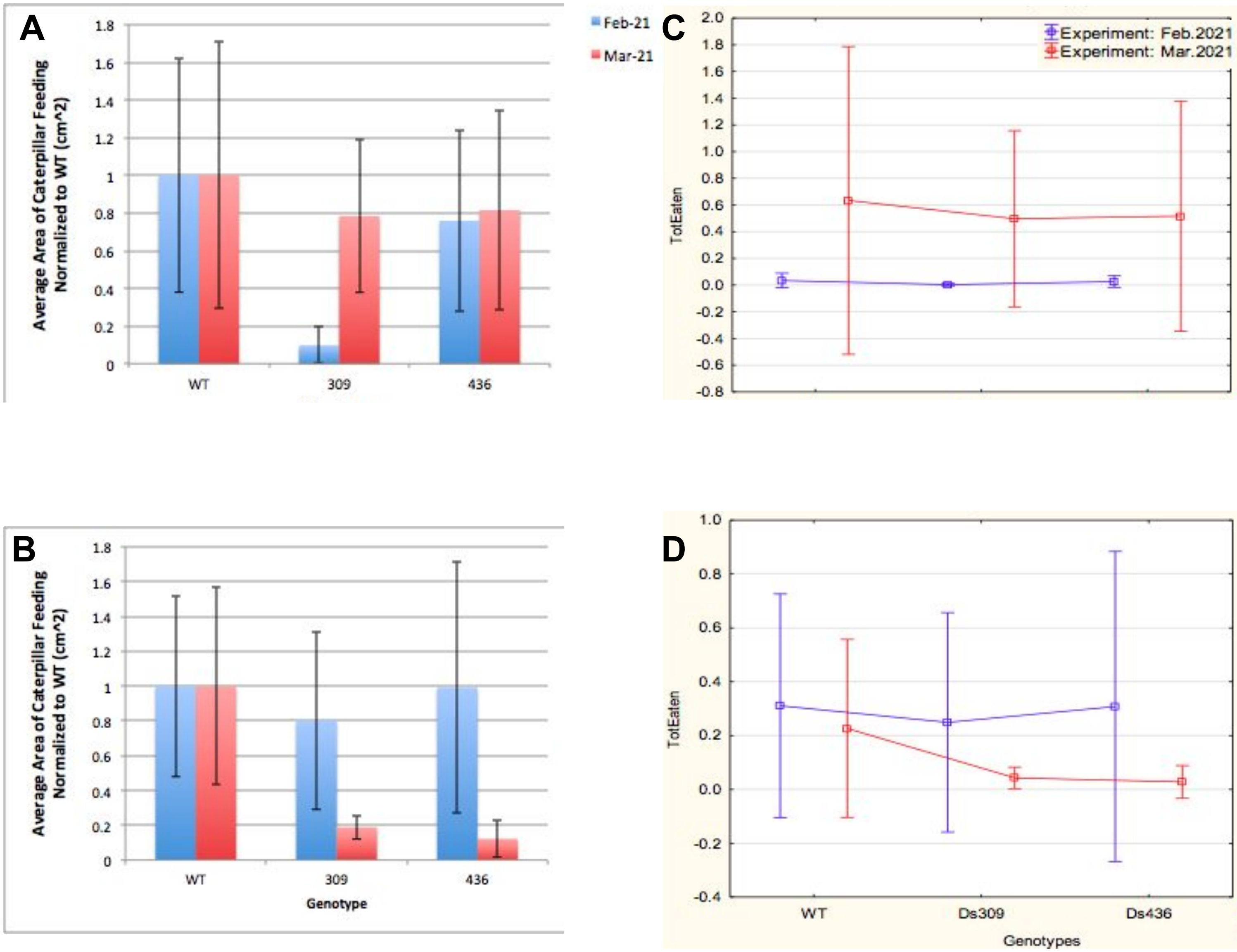


Figure 5. Caterpillar choice experiment for corn in heat, drought and combination heat and drought stress. Normalized average +/-SE caterpillar feeding in WT, Ds309, and Ds436 corn plants that went through heat stress **A** heat and drought stress **B.** Averages and SE were normalized to wildtype average values. Mean plot of total area eaten by caterpillars for WT, Ds309, and Ds436 corn plants that went through heat stress **C** heat and drought stress **D.** Mean plot error bars show 95% confidence interval.

Results

Sept. 2019: Treated corn with salt stress and performed a caterpillar choice experiment

Oct. 2019: Repeated caterpillar choice experiment with corn treated with salt stress.

Nov. 2019: Repeated caterpillar choice experiment with corn treated with salt stress. Accidentally applied drought stress in addition to salt stress to corn and performed another caterpillar choice experiment.

Mar. 2020: Repeated caterpillar choice experiment with salt stress and another caterpillar choice experiment with a combination of salt and drought stress.

Mar. 2020-Feb. 2021: Redesigned the experiment. Used growth chambers instead of greenhouse (smaller plants).

Feb. 2021: Applied the following treatments to corn: salt, heat, drought, combination of salt and drought, and combination of heat and drought. Then, performed caterpillar choice experiments.

Mar. 2021: Repetition of the experiments done in Feb. 2021.

- Sept. 2019- Mar. 2020: with salt stress, wildtype was being eaten more than the mutants.
- Nov. 2019: with salt and drought stress, mutants, specially Ds 309, was preferred by the caterpillars.
- Mar. 2020: Did not see a significant difference between genotypes with combination of salt and drought stress.
- Feb. 2021: Overall, small amount of feeding was difficult to analyze and therefore could not conclude if there was any significant difference between the genotypes.
- Mar. 2021: No consistent difference between the wildtype and Ds309 and Ds436 mutants.

Conclusion

- We were trying to see if there was any significant difference between the mutants and the wild type corn right after stress.
    - A chemical difference should have impacted the caterpillar feeding behavior.
    - We did not observe a genotype effect.
- The single mutants Ds309 and Ds436 do not seem to taste different to caterpillars since the caterpillars eat all the genotypes.
- Further direction to probe the function of these CYP72A enzymes:
    - Allow longer feeding: This may allow caterpillars to eat on the corn plant which might help see some trends and significant differences between the genotypes.
    - Obtain double mutant: This might show a greater impact on corn’s ability to defend itself against the biotic stress of caterpillar feeding after being treated by abiotic stress(es) such as salt, heat, and drought.
    - Study the assimilation of plants over time as they induce defenses against caterpillars

Acknowledgements

Prall, W., Hendy, O., and Thornton L. E. (2016). Utility of a Phylogenetic Perspective in Structural Analysis of CYP72A Enzymes from Flowering Plants. *PLoS One* **11(9)**: e0163024.

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