Do Non-Lethal Doses of Dicamba Induce Epigenetic Effects on Palmer Amaranth?

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Introduction

- Palmer amaranth (*Amaranthus palmeri*) is a competitive weed species
  - Threat to cotton and soybean crops
    - Can grow up to 8 feet tall
    - Spreads lots of seed
    - Decreases crop yield
  - Resistant to glyphosate, 2,4-D, atrazine and other herbicides
  - In 2019, a the first dicamba-resistant Palmer amaranth was found in Kansas
- Dicamba is a volatile herbicide that easily vaporizes
- Repeated drift of low, non-lethal levels of dicamba has been a rising concern
  - Drift could lead to rapid evolution of resistance to dicamba
  - Resistance influences the weed management practices needed to combat weeds
- Can this drift affect the "memory" of the plant?
  - Stress-induced epigenetic signal
    - Plant responding to environment without changes in the genome
  - Will drift impact the resistance of the current generation and/or the progeny?

Objectives

1. To determine if Palmer amaranth has a phenotypic response to low, non-lethal doses of dicamba
2. To determine if dicamba drift can affect the genetics and/or epigenetics of current and future populations of Palmer amaranth

Methods

Applying the Stress

- Palmer amaranth seed was sprinkled across small pots and thinned to one per pot after germination
- Plants were repotted at symptoms of being root-bound
  - Root system confined in pot
- Once plants were 6-8inches in height, they were sprayed with varying dilutions of XtendiMax® (Bayer) a.i. dicamba
  - See Table 1 for dilutions
- Sprayed in DeVries Manufacturing Generation 4 Research Track Sprayer at 15 gal/acre at 4 mph.

Analyzing Stress Response

- Height of plants measured every week, starting day before first spray
- Youngest fully developed leaves were sampled from every plant two weeks after spraying
- Sampled leaves stored in -80ºC CryoCube F570h (Eppendorf)
- DNA extracted using FastDNA™ Spin Kit for Plant and Animal (MP Biomedicals)
  - For future use (See Future Work)
- Plants sprayed a second time (3 weeks later)
  - To see if there is an epigenetic difference in response between naïve and treated population
  - Half of previously sprayed plants were sprayed again
    - Treated-treated and treated- naïve population
  - Half of control plants sprayed with 1/10x
    - Naive-treated and naïve-naïve population

Treatment #	Dilution
1	0x (DI Water)
2	1/10x
3	1/50x
4	1/100x
5	1/500x
6	1/1000x
7	1/5000x
8	1/10000x

Table 1: Dilutions of XtendiMax® herbicide sprayed on Palmer amaranth. The 1x rate is 22 fl oz. of product per acre. There were 10 plants per treatment, 8 treatments, for a total of 80 plants. 1/10x concentration made with 1.14mL of XtendiMax® and 1L of DI Water. The 1/10x was then utilized as stock solution for all other concentrations.

Preliminary Results

Objective 1: Did the plants have a phenotypic response to the herbicide application?

- No differences between initial and final plant height were detected, Figure 1; (p=0.2332)
- No phenotypic response to varying treatment concentrations

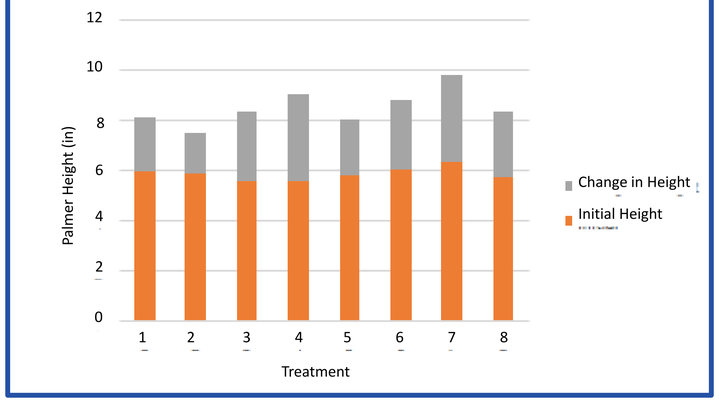
Objective 2: Did the treatments induce a genetic or epigenetic response?

- To be determined
- DNA has been extracted, will run MS-AFLP test
  - See Future Work

Challenges Encountered

- Damping off pathogen, *Pythium* spp., caused seedling mortality
- First set of plants became root bound
  - Need to be transplanted sooner
- Developmental stages varied at time of spraying
  - Presence of inflorescence
- Lack of randomization following spraying
- All of the above contributed to too much genetic variability between plants
  - Will likely not be able to conclude if differences in methylation are due to treatment or other variability

Figure 1: Average initial height and average final height of 8 plants in a treatment. Determining if there is a phenotypic difference between the treatments resulting from the initial spray.



Future Work

- Methylation Sensitive - Amplified Fragment Length Polymorphism test (MS-AFLP)
  - Completed utilizing DNA extracted from leaves
  - Identifying methylated sites on the Palmer genome; This is part of the epigenetic signal that could be a response to herbicide treatment
- Exploring effect of dilute herbicides applied to Arabidopsis
  - Arabidopsis is a model plant; the genome has been more intensively studied
  - To confirm the treatment is what is contributing to changes in methylation
    - Three lines grown to confirm a change in methylation (an epigenetic signal) is occurring
      - 1. Wild-type, 2. Methylation Sensitive, 3. Demethylation Sensitive
  - Germinated and grown in Environmental Growth Room
  - Selfed to get population of seeds to be grown and sprayed
- Planting more Palmer amaranth
  - More controlled setting
  - Spraying the plant earlier, before too much variability
  - Randomizing plants in trays after spraying
  - Spraying higher concentrations of XtendiMax to see a phenotypic affect (See Figure 5)

Figure 2: Palmer amaranth showing signs of being root bound

Figure 3: Plant #4-8. No DNA was extracted from this plant as there are no viable leaves

Figure 4: Phenotypic variability within a treatment of plants before initial spray

Figure 5: XtendiMax® dicamba damage on Palmer amaranth at a higher concentration (1/2x). Picture taken one day after spraying. This is the phenotypic effect we are expecting with higher concentrations.

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