Epigenetic effect of non-lethal doses of dicamba on Palmer amaranth

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Introduction

Palmer amaranth (*Amaranthus palmeri*) is a competitive weed species, and is particularly a threat to cotton and soybean crops. Palmer amaranth can grow up to eight feet tall, spreads lots of seed, and decreases crop yield. Palmer amaranth is also resistant to many herbicides such as glyphosate, 2,4-D, atrazine, making the weed harder to kill. In March of 2019, the first dicamba resistant Palmer amaranth was discovered in Kansas. Dicamba is a volatile herbicide that easily vaporizes, causing drift to occur. The repeated drift of low, non-lethal levels of dicamba could lead to rapid evolution of resistance to dicamba. Resistance influences the weed management practices needed to combat weeds. This project aims to determine whether this drift can affect the "memory" of the plant. Whether the plant's "memory" has been affected is assesed through a stress-induced epigenetic signal. This signal is the plant responding to the environment, without changing the genome of the plant. The epigenetic signal, identified through methylation or demethylation, could impact the resistance of both the current generation and progeny of Palmer amaranth.

There are two objectives in this experiment:

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

To confirm that the treatment is what is contributing to changes in methylation (the epigenetic signal) in the Palmer amaranth genome, Arabidopsis will undergo the same treatment as the Palmer amaranth. Arabidopsis is a model plant, and the genome has been more intensively studied than Palmer amaranth. Three lines of Arabidopsis will be utilized: Wild-type, methylation sensitive, and demethylation sensitive.

Methods

This experiment was separated into three sections: Palmer Trial 1, Palmer Trial 2, and Arabidopsis.

Palmer Trial 1

Growth:
Palmer amaranth seeds were sprinkled across small pots and placed in greenhouse in Lexington, KY. The small pots contained a soil mixture of 2 ProMix : 2 Sand : 1 Field soil. The seedlings were thinned to one plant per pot after seeds had germinated. The plants exhibited signs of the damping off pathogen and were treated accordingly. Plants eventually had signs of being root

bound to the pot, so the plants were repotted into bigger, four inch pots. After plants reached six to eight inches in height, the 80 viable plants remaining were sorted into eight treatments of ten plants. Each treatment was sprayed with a varying dilution of XtendiMax® (Bayer) a.i. dicamba (See Table 1 for dilutions).

Dilutions and Spraying:
The 1x rate of XtendiMax® herbicide (active ingredient dicamba) is 22 fl oz of product per acre. The stock solution utilized for the dilutions was 1/10x, made with 1.14mL of XtendiMax® and one liter of DI water. To make the 1/100x dilution, 10mL of the stock solution was mixed with 90mL DI water. Further dilutions were mixed using the same volumes: 10mL of 1/100x to create 1/1000x, 10mL of 1/1000x to create 1/10000x. To make the 1/50x dilution, 20mL of the stock solution was mixed with 80mL DI water.

Table 1: Dilutions of Xtendimax® sprayed on Palmer amaranth for Palmer Trial 1

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

The dilutions were sprayed in DeVries Manufacturing Generation 4 Research Track Sprayer at 15 gal/acre at 4 mph. The dilutions were sprayed in order of lowest concentration to highest concentration to reduce risk of contamination. In between treatments, the sprayer was rinsed with ammonia, followed by DI water. The plants were left outside, spaced apart by treatment for 24 hours following the spray, then moved back into the greenhouse.

Analyzing the Stress Response:
The height of every plant was measured weekly starting the day before the first spray, and every week following. The youngest fully developed leaves were sampled from the plants two weeks after spraying. The sampled leaves were placed in liquid nitrogen immediately following sampling, and stored in the -80° CryoCube F570h (Eppendorf) upon return to the lab. The DNA was extracted using FastDNA™ Spin Kit for Plant and Animal (MP Biomedicals) following the associated protocol.

Palmer Trial 2

The same protocol used in Palmer Trial 1 was utilized for Palmer Trial 2, with a few changes. The plants were repotted into larger pots before there were signs of being root bound. There were changes in the dilutions (See Table 2), now including two higher concentrations. There were 200

plants total split evenly across two repotting dates. Each treatment had ten plants from each repotting date, totaling 20 plants per treatment.

Table 2: Dilutions of XtendiMax® sprayed on Palmer amaranth for Palmer Trial 2

Treatment #	Dilution
1	1/2x
2	1/5x
3	1/10x
4	1/50x
5	1/100x
6	1/500x
7	1/1000x
8	1/5000x
9	1/10000x
10	0x (DI Water)

For the above dilutions, 1/2x was utilized as the stock solution, made with 5.71mL of XtendiMax® and 1L of DI water. To create the 1/5x dilution, 40mL of stock was mixed with 60mL of DI water. To create the 1/10x dilution, 20mL of stock was mixed with 80mL of DI water. The remaining dilutions were made the same way as Palmer Trial 1.

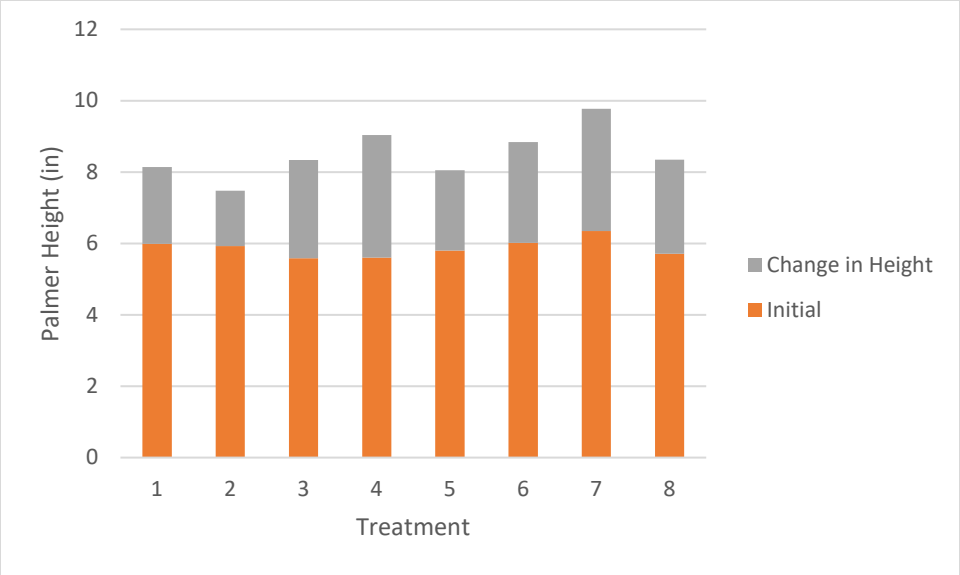
Arabidopsis

The Arabidopsis was grown in the Environmental Growth Room in the Plant Science Building in Lexington, KY. This Arabidopsis was grown and selfed for seed. The seed was then to be used to grow plants for the treatments. Four inch pots were filled with moist soil and sprinkled with seed from one of the three lines: wild-type, methylation sensitive, and demethylation sensitive. There were four pots per line. Each pot was covered with a petri dish lid to aid in germination. Due to the presence of gnats in the growth room, Nematodes were mixed with the water when the plants were first watered.

Preliminary Results

Palmer Trial 1

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.



Palmer Trial 2

Signs signaling dicamba damage have been identified in plants from the higher concentrations (T1, T2, and T3). Two weeks after spraying, some of the plants damaged by higher concentrations of dicamba have stockier stems than those without visible damage. Analysis on plant height has not been completed

Arabidopsis

No results yet. There has been difficulty growing the Arabidopsis for seed in the Environmental Growth Room.

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