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Proceeding Paper

Case Study: Characterizing the Response of Young Glyphosate Susceptible and Glyphosate-Resistant *Amaranthus palmeri* (Palmer Amaranth) after Being Sprayed with a Ten Percent Acetic Acid Solution to Control Growth †

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Abstract: In this case study, we investigate the responses of Glyphosate-Susceptible and Glyphosate-Resistant *Amaranthus palmeri* to an organic 10% acetic acid herbicide solution instead of the standard 20% acetic acid solution. We show that although both forms respond differently to glyphosate-based herbicides, both will respond the same way to organic-based herbicides that include acetic acid. After treatment with the 10% acetic acid solution, eighty-five percent (85%) of the Glyphosate Susceptible plants died within 24 h, while 100% of the Glyphosate-Resistant plants died within 24 h. Using a lesser concentration may be better for the environment since there will be less buildup over time and is more cost effective for the farmer.

Keywords: Adaxial surface; abaxial surface; glyphosate susceptible; glyphosate resistant; herbicide

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1. Introduction

Amaranthus palmeri is a dioecious plant and has the innate ability to increase its genetic diversity to allow the species to overcome stresses. Over the years two forms of *Amaranthus palmeri*, Glyphosate Resistant (GR) and Glyphosate susceptible (GS) have evolved. GR *A. palmeri* is resistant to glyphosate-based herbicides, while GS *A. palmeri* is not. Glyphosate is the active chemical found in traditional herbicides. Glyphosate-based herbicides are the most widely used agricultural herbicides around the world. It is used extensively in Roundup Ready corn, cotton, and soybean crops in the Midwestern United States [1]. It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) in plants. This prevents biosynthesis of phenylalanine, tyrosine, and tryptophan, and results in plant death [2,3].

Plants become resistant to glyphosate through two main pathways; target site and no target site mechanisms [1]. Target site resistance is attributed to altered glyphosate herbicide interaction with the target ESPS enzyme. Non-target site resistance is due to altered translocation within the plant and altered translocation is reported to be the most common mechanism of resistance [4,5]. Both GR and GS *A. palmeri* contain the ESPS enzyme, but, GR *A. palmeri*, produce more ESPS enzymes than GS *A. palmeri.* Glyphosate inhibits the ESPS enzyme in both plants, but, because the GR *A. palmeri* has a greater magnitude of the enzyme, it is resistant to the effects of glyphosate [6]. Herbicides select for resistance already present in the populations by killing off all other genetic competition. Farmers unwittingly create an artificial selective pressure increasing the odds that *Amaranthus palmeri* will become resistant [7,8]. The existence of GR A. palmeri was confirmed in the state of Georgia in 2005 [4]. Glyphosate resistance probably evolved once [9]. Over the years, GR A. palmeri spread from North Carolina to California. Because the GR form of A. palmeri has become more prevalent there is a greater need for alternative herbicides [10]. Organic herbicides are a good alternative. One example is Weed Pharm™. It is an agricultural vinegar and thus contains 20% acetic acid [11].

When organic herbicides, such as agricultural vinegar are sprayed directly on weeds, they suppress and kill them [12]. When sprayed early enough (before the desired crop emerges and when *A. palmeri* is less than 8cm tall) it is effective at the 10% level. Because the acetic acid solution is sprayed before the desired crop emerges, it causes no harm to it. When applied to the leaves, the acetic acid eats through the leaf surface, including the epicuticular wax and cuticle. Several studies have shown the effectiveness of strong agricultural vinegar as a weed control product. For instance, agricultural vinegar led to 100% mortality of two-leaf redroot pigweed, *Amaranthus retroflexus*, nine days after treatment [10]. The more acidic the solution, the more effective it will be at controlling weeds. It will also be more dangerous to handle as the concentration increases [13]. As an herbicide, agricultural vinegar has proven to be effective, but produces a build up over time in the soil and may prove harmful to the ecosystem. Therefore, for our project, we applied lesser concentrations of acetic acid to the young leaves of developing *A. palmeri*. The overall goal was to compare the differences in both forms of *A. palmeri* in order to determine how best to control plant growth, prevent loss of desired crops and use as little acetic acid as possible. We do not know the long-term impact of acetic acid deposition to the soil or to the other plants or organisms in the environment. Considering this fact, it is better to lessen the amount of acetic acid used when possible and target it to specific plants. The herbicide will kill all contacted tissue so good spray coverage is essential [14].

2. Materials and Methods

In the greenhouse *Amaranthus palmeri* was grown in 24 pots using Carolina® Seed Starter mix. We measured cardboard pieces to create a four section divider for each pot. The seeds were loosely placed in the soil about an inch from the top. The lighting was placed on a timer and set for 12 h of light and 12 h of dark.Once 2 to 8 leaves per plant were apparent, the young Glyphosate-Susceptible and Glyphosate-Resistant *Amaranthus palmeri* leaves were sprayed with a 10% acetic acid solution. We then watched our plants and watered when necessary and waited until enough growth was present in both GS and GR *Amaranthus palmeri*. We noticed that our GS plants germinated before GR plants. Once plants germinated and their leaves were apparent, they were then treated at the 0 h and 2 h stages with 10% acetic solution. After treatment we viewed the upper surface of the leaves using the Scanning Electron Microscop to see if stomata were open. These plants are C4 plants and thus have stomata on their adaxial and abaxial leaf surfaces. Most leaves have stomata only on their abaxial surfaces.

3. Results

Results shows that *Amaranthus palmeri* stop growing when sprayed with a 10% solution of Acetic Acid (see Figure 1). All seedlings died after being sprayed. They were all young and under 10 cm tall. Their decline was visible within 4 h of being sprayed. They started to wilt. There was a slight difference in the death rate in that 100% of the glyphosate Resistant seedlings died within 24 h of treatment. 85% of the Glyphosate Susceptible seedlings died within 24 h of treatment. 100% died within 72 h of treatment.

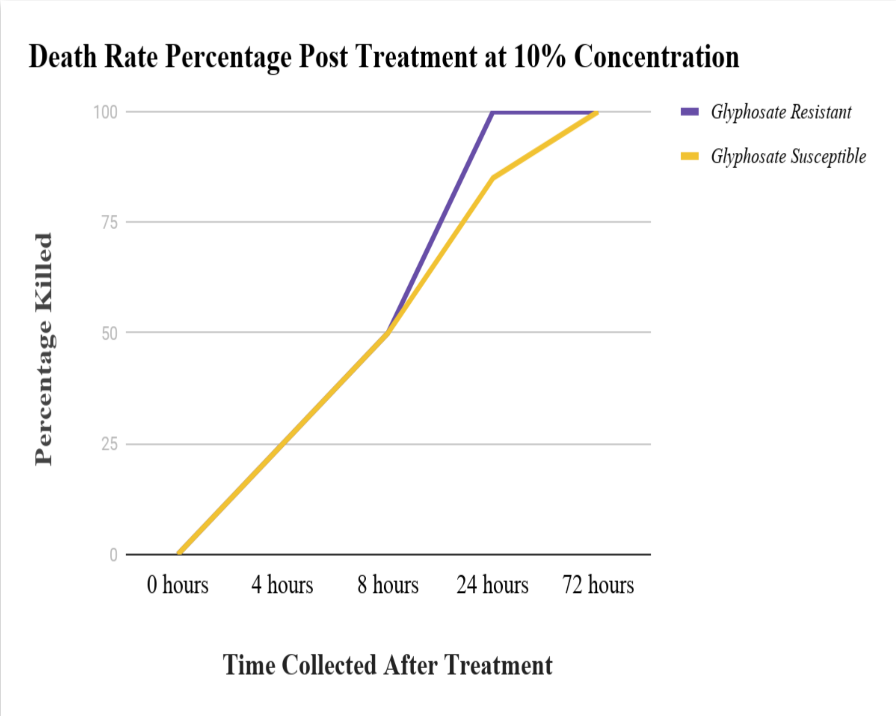

Figure 1. The 10% acetic acid treatment kills 100%of the Glyphosate Resistant (GR) *Amaranthus palmeri* seedlings and 85% of Glyphosate Susceptible (GS) *Amaranthus palmeri* seedlings within 24 h. All other seedlings die within 72 h. *t*-test for significance: 0.4575. There is no statistical difference in death rate.

Figures 2 and 3 show that the stomata start to close. soon after treatment. These stomata are on the upper surface and are in direct contact with any herbicide that is sprayed. Figures 4 and 5 represent stomata as they appear normally, before treatment.

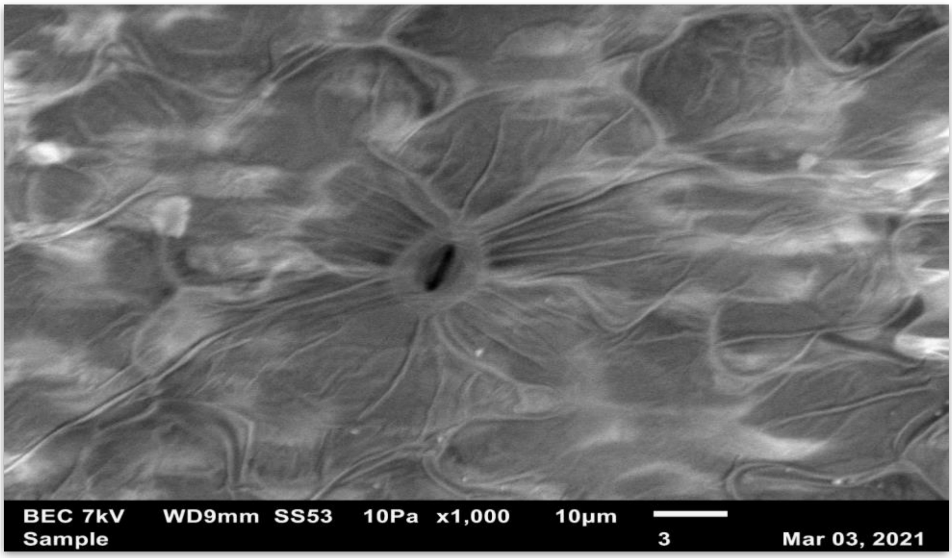

Figure 2. This is the adaxial surface of a Glyphosate Susceptible *Amaranthus palmeri***.** leaf. This micrograph was taken with the JEOL Scanning Electron Microscope immediately after the leaves of *Amaranthus aplmeri* were sprayed and treated with a 10% acetic acid solution. Stomata are showing stress.

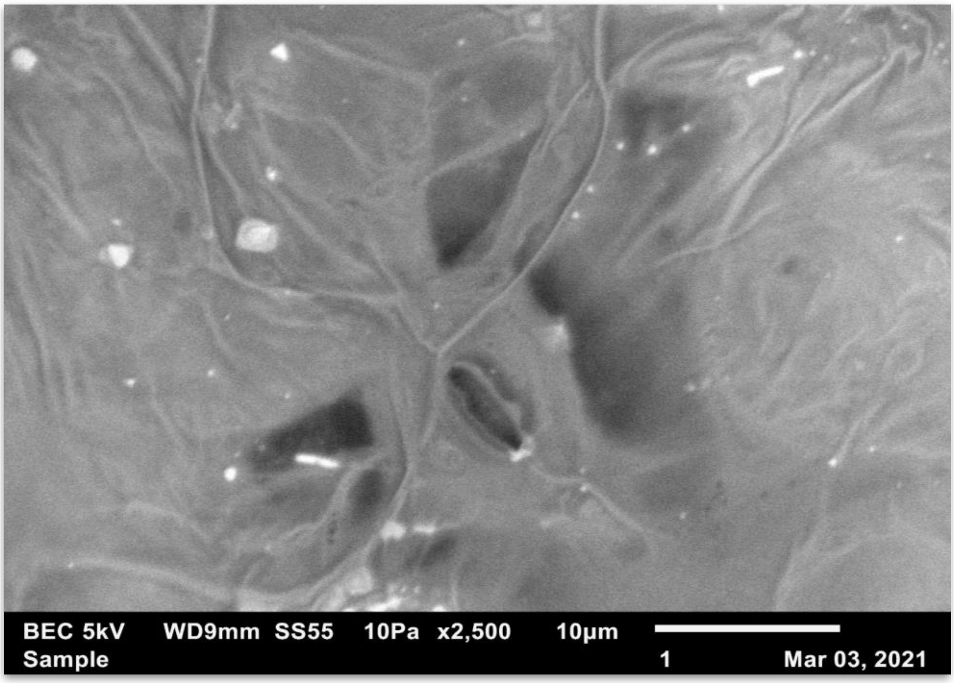

Figure 3. This is the adaxial surface of a Glyphosate Susceptible *Amaranthus palmeri* leaf. This micrograph was taken with the JEOL Scanning Electron Microscope immediately after the leaves of *Amaranthus palmeri* were sprayed and treated with a 10% acetic acid solution. Stomata are showing stress. They start to close soon after treatment.

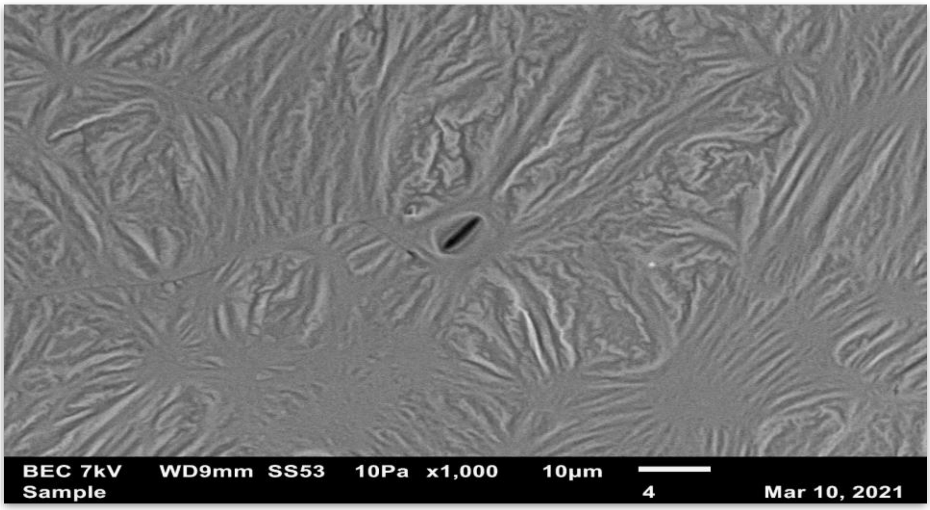

Figure 4. This is a micrograph of the adaxial leaf surface of Glyphosate Resistant *Amaranthus palmeri* taken with JEOL Scanning Electron Microscope. It has not been treated with acetic acid. It represents the control. The above stomata appears normal and open. It is not under stress.

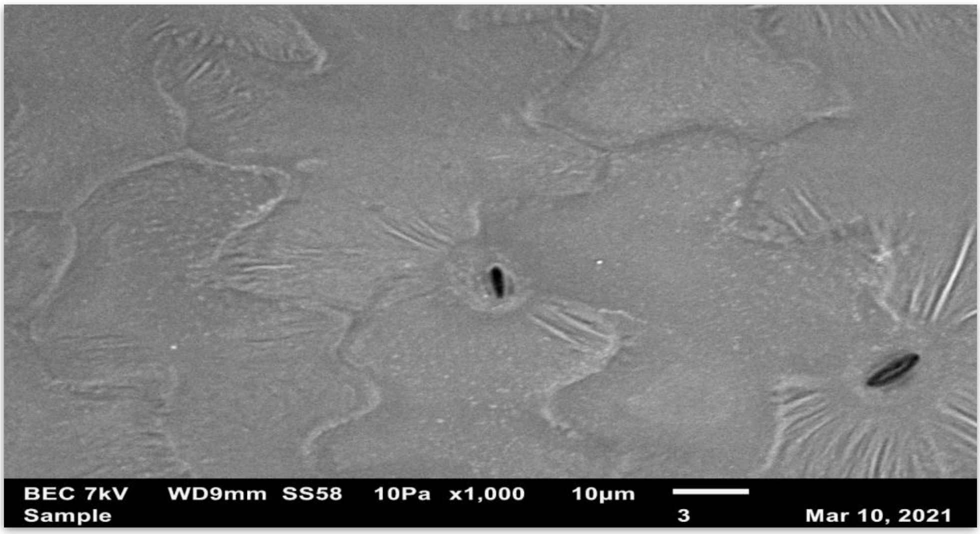

Figure 5. This is a micrograph of the adaxial leaf surface of Glyphosate Susceptible *Amaranthus palmeri* taken with JEOL Scanning Electron Microscope. It has **not** been treated with acetic acid. It represents the control. The above stomata appears normal and open. It is not under stress.

4. Conclusions

In the field, *Amaranthus palmeri* grow rapidly, up to 2–3 inches a day. The growing season typically occurs between March and September and temperatures may be between 5 °C and 35 °C. Under similar conditions in our laboratory, the Susceptible and Resistant strains were able to grow up to 14 inches in height. Our results show that within 72 h of treatment, all treated *Amaranthus* seedlings die. Using a 10% solution of acetic acid as a growth control agent is successful and impacts the environment less. **Future studies** will assess genomic data at different spraying times.

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