Agricultural Vinegar as a growth control agent for both Glyphosate susceptible (GS) *Amaranthus palmeri* and Glyphosate resistant (GR) *Amaranthus palmeri*

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Abstract

Background: Annually farmers in the United States suffer crop losses due to the invasive weed, *Amaranthus palmeri*. The two major reasons for the losses are: the rapid reproduction and adaptability of this aggressive species. The dioecious *Amaranthus* has adapted so rapidly that some populations of *Amaranthus* are resistant to the traditional glyphosate herbicides. Glyphosate was introduced as an herbicide and was considered unlikely to cause resistant populations of plant species. The mechanism of action, lack of metabolic degradation in plants or residual activity in the soil made it a very popular herbicide hoice. Over time glyphosate resistant (GR) *Amaranthus palmeri* species emerged. This new GR *Amaranthus palmeri* calls for a new herbicide, preferably an organic herbicide. Agricultural Vinegar (vinegar with 20% acetic acid) is an effective, non-selective organic herbicide. Over time, a buildup of acetic acid may cause harm to the ecosystem. **Methods:** We are looking at organic herbicide solutions that contain lesser concentrations of acetic acid. For this experiment, we used solutions with 5%, 10%, and 20% acetic acid. These solutions were applied when the plants were young (less than 10 days) and less than 4 cm tall. We are observing the effects of the solution on the epicuticular wax and epidermal layers. We hypothesized that when applied very early, lower concentrations of agricultural vinegar (5%, 10%, or

15%) can slow or stop growth and allow for the desired crop to outcompete the weeds.

Introduction

Amaranthus is a fast-growing C4 (stores carbon as malate) annual weed that can grow up to 10 feet in height.[1] Due to the over-reliance of the chemical glyphosate (Round up™) to combat weeds, many strains of *Amaranthus* have shown signs of glyphosate resistance. Of the glyphosate resistant (GR) plants discovered, *Amaranthus palmeri* commonly known as "Palmer Amaranth" or "careless weed", is the most economically damaging to farmers because of its abilities to produce copious amounts of seeds, adapt quickly, and exhibit allelopathic tendencies towards crops. Further the leaves of *Amaranthus* plants can use diaheliotropism to maximize its photosynthetic capacity and result in extreme heights.[2] Palmer amaranth also exerts allelopathic effects to cause an inhibition of growth in carrots, onions, cabbage, and other plants.[2] These attributes allow the plant to grow quickly and cause billions of dollars to be wasted in yield losses.[3]

This plant possesses the ability to stave off incomplete physical control measures while still growing to produce viable seeds. Prior studies show that *Amaranthus* plants could regenerate after suffering the trauma of physical control measures.[4] Examples of physical control measures are hand weeding and tilling. Tilling the fields affects not only the emerging weeds but also the germination of the dormant seeds and the weed seed bank in the soil.[5] In one experiment, a control plant along with individuals that had their stems cut to heights of 15 cm, 3 cm, and 0 cm above the soil to see their regenerative properties was utilized. At the end of the experiment, the plant that was cut all the way to the soil was still able to produce 23,000 seeds despite a 95% mortality rate, whereby just 5% survived.[4] There is much concern over the ability of many *Amaranthus* plants to bounce back from physical or chemical control measures. One plant can produce anywhere from 200,000-300,000 seeds.[4] If 95% of the seeds die during the winter months, then 5% of the plant remains. If the farmer then kills 99% of the remaining weeds, the farmer will still have more than 3000 pig weed plants to deal with in the future.[6]

Rise of the Resistance

Glyphosate was considered an ideal herbicide for *Amaranthus* plants, because its chemical properties minimized resistance and its mechanism of action meant an absence of metabolic degradation in plants and a lack of residual activity in the soil.[7] *A. palmeri* is a dioecious plant, meaning each individual contains one or the other reproductive organs requiring two separate plants to reproduce.[1] This allows the plant to have the innate ability to increase its genetic diversity giving it an evolutionary advantage in overcoming the stresses of herbicides.[8]

Over time the first GR *Amaranthus* plants emerged in the state of Georgia in 2005.[7] Not all species of *Amaranthus palmeri* were glyphosate resistant. Others were still susceptible to glyphosate herbicides (GS *Amaranthus palmeri*). In the seven subsequent years, GR *Amaranthus palmeri* spread from North Carolina to California. Research showed that *Amaranthus* had built up a resistance to not only glyphosate but also ALS (acetolactate synthase) herbicides, EPSPS (5-enolpyruval-shikmate synthase) herbicides, and other herbicides that operate by affecting the photosystem II in plants.[4] While a strain of *Amaranthus* that is resistant to ALL herbicides has not be found, the fact is it is possible that plants can become resistant after repeated use.[1] Plants can become resistant to glyphosate through target site and non-target site mechanisms.[2] Target site resistance is attributed to altered glyphosate herbicide interaction with the target 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.[4,7] Herbicides select for resistance in the populations by killing off all other genetic competition; farmers unwittingly create an artificial selective pressure increasing the odds that Palmer amaranth will become resistant.[9]

The invention and propagation of Round up™ (glyphosate)-ready technology allowed the overuse of glyphosate herbicides. The technology also initially increased production in the agricultural sector, as it was used in 85% or more of the soybeans and 95% or more of the cotton crops.[9] However, this means these same fields are now prone to resistant weeds, the rising of which could dramatically affect production and cost to the public.

Organic Growth Control Agents

Recently there has been increased consumer interest in organic vegetables. In meeting this demand, many organic herbicides have been developed to assist the organic farmer in his ever-evolving struggle against undesirable weeds. These herbicides use many different pathways to kill their selected target. Corn gluten meal, for example, is a byproduct of corn processing whose composition of 60% protein and 10% nitrogen, coupled with its herbicidal properties, make this organic herbicide a good weed and feed.[10] Microbial herbicides like Phoma Macrostoma Stain, are weak plant pathogens. Macrostoma works by colonizing the leaves and secreting compounds called macrocidins that bleach the leaves, causing chlorosis. Streptomyces is another microbial utilized for weed management. This microbe is produced in a facility where the organism's secretions are harvested after it produces them. The secretions are then used for its herbicidal properties.[10] However, in recent years, fear over possible mutations has slowed the growth in microbial herbicides.

Agricultural vinegar is a relatively recent addition to the organic herbicide market. A popular brand Weed Pharm™ is 20% acetic acid. The acetic acid is applied to the weeds that are targeted for termination. The herbicide has shown great promise killing *Amaranthus retroflexus*.[10] In multiple studies, 20% agricultural vinegar concentrations were able to completely kill weeds after nine days of treatment.[10] When applied to the leaves, the acetic acid eats through the leaf surface, including the epicuticular wax and cuticle. The long-term effects of acetic acid deposition to the soil or to the other organisms in the environment are unknown. Considering this fact, the minimal concentration of acetic acid should be used when or if possible. The earlier a herbicide is applied, the more effective it will be. The *Amaranthus* plants are often already 12 inches high before farmers start weeding, so another step would be to be able to intervene earlier in the growth cycle.[11]

Hypothesis –Agricultural vinegar concentrations of less than 20% acetic acid can serve as effective growth control agents for both GS *Amaranthus palmeri* and GR *Amaranthus palmeri*.

Materials and Methods

Varying concentrations of acetic acid (5%, 10%, and 20%) were used as a means of controlling the growth of both GS *A. palmeri* and GR *A. palmeri*. Since 20% seems to be an effective industry standard, that concentration was used as a standard of practice control, and two lower concentrations were compared. As this was a preliminary study, a non-treatment control group was not used (plants treated with only water).

All seeds were planted in 905 cm^3 pots with Miracle Gro© Potting Soil, that contains pre-mixed fertilizer.

A new batch of plants were seeded every week for seven weeks. Cycle 1 consisted of planting 10 pots of glyphosate susceptible (GS) *A. palmeri* and 10 pots of GR *A. palmeri*. Cycle 1 was watered 2 times per week.

Cycle 2 consisted of 10 pots of GS *A. palmeri* only, and another 10 pots with GS *A. palmeri* coupled with beans. Beans were seeded to see how they would respond to the 5% acetic acid along with being placed with the GS plants. Cycles 3 and 4 each contained 10 pots of GR *A. palmeri* and 10 pots of the GS strains. Cycle 2 and all subsequent weeks were watered only once a week.

All the vinegar was prepared from mixing 100% acetic acid with deionized water except for the 5% table vinegar. To procure the 5% acetic acid, Heinz distilled white vinegar was purchased. For the stronger concentrations, 20 mL 100% acetic acid was mixed with 180 mL deionized water to achieve 200 mL of a 10% acetic acid

solution. To make the 20% acetic acid solution, 40 mL of 100% acetic acid was mixed with 160 mL deionized water.
The application of acetic acid took approximately ten (10) Days, where Day 0 was the day each cycle was planted. Cycles 1 and 2 received 5% acetic acid by volume, which was applied to leaves via a spray bottle. Cycle 3 received 10% acetic acid by volume applied directly to the leaves *and hypocotyl* of the plants. The application of acetic acid on the hypocotyl was do the lack of growth uptained in the greenhouse from the Amaranthus species. The majority of the plants did not develop true leaves, so the acetic acid was applied to the cotyledons and the hypocotyl. The 4^{th} cycle received the 20% acetic acid solution applied in the same manner as the 10% solution.

Results and Discussion

The average height for each plant sprayed was about 4cm tall (Table 1). The research suggested that 20% concentration of acetic acid was already established as an excellent means of control for *Amaranthus* plants, so this investigation aimed to elucidate the lowest effective concentration. Through the course of the experiments, three different concentrations of acetic acid were applied to the susceptible and resistant *Amaranthus* plants: 5%, 10%, & 20%. The 20% and the 10% concentrations of acetic acid were most effective at killing the young amaranth, less than 21 days old. The 20% acetic acid solution took less than 24 hours to kill the entire sample on which it was sprayed. While the 10% acetic acid solution killed a majority of the plants on which it was sprayed, it was not 100% effective. In Cycle 3, the 10% solution reduced the total overall GS weed number by 85% and killed all GR plants. The 5% solution was not very effective. Plants who received 5%, did have a significant amount of mycelium growth in the pots. The specific effect of the mycelium growth is unknown.

Table 1 Amaranthus Growth Chart. Plants grown in June and July 2016

Day	Type	# of Plants	Height	# of Leaves
Day 3	Cycle 1 R	< 10	1 cm	2
Day 3	Cycle 1 S	> 30	1 cm	2
Day 7	Cycle 1 R	< 10	2.5 cm	2
Day 7	Cycle 1 S	< 15 in all but 2 water damage	3.5 cm	2
Day 9	Cycle 1 R	< 10	4 cm	2
Day 9	Cycle 1 S	< 10	3.5 cm	2
Day 9	Cycle 2 S	No New Growth	No Growth	0
Day 9	Cycle 2 S & Beans	No New Growth	No Growth	0
Day 15	Cycle 1 R	2 pots > 10 8 pots < 5	2.5 cm	2
Day 15	Cycle 1 S	< 10 in all pots water really hurt them	3.5 cm	2
Day 15	Cycle 2 S	5 pots > 20 1 pot 15 4 pots < 10	1/2 3.5 cm 1/2 4 cm	2
Day 15	Cycle 2 S & Beans	4 pots > 30 2 pots about 15 4 pots < 10	3.5 cm Avg. bean 23 cm	2
Day 16	Cycle 1 S	< 5 in all pots	3.5 cm	2
Day 16	Cycle 2 S	All sprayed 5% acetic acid (2 sprays)	4 cm	2
Day 28	Cycle 1 S	< 5	4 cm	2
Day 28	Cycle 2 S & Beans	< 20	4 cm	2
Day 28	Cycle 3 S	10 pots > 30	3.75 cm	2
Day 28	Cycle 3 R	5 pots about 15 & 5 pots about 10	3.5 cm	2
Day 29	Cycle 3 R&S	6 of Cycle 3 sprayed 10% acetic acid	3.5 cm	2
Day 31	Cycle 1 R&S	Same	4 cm	2
Day 31	Cycle 2 S	Same	4 cm	2
Day 31	Cycle 3 R	Same	4 cm	2
Day 31	Cycle 3 S	10 pots > 25	3.75 cm	2

Table 1 Continued

Day	Type	# of Plants	Height	# of Leaves
Day 35	Cycle 1 R&S	Same	4 cm	2
Day 35	Cycle 2 S	Same	4 cm	2
Day 35	Cycle 3 R	Same	4 cm	2
Day 35	Cycle 3 R sprayed	0 (Dead)	0 cm	2
Day 35	Cycle 3 S	Same	4 cm	
Day 35	Cycle 3 S sprayed	< 5	4 cm	2
Day 35	Cycle 4	> 30	4 cm	2
Day 35	Cycle 4	< 15	4 cm	2
Day 36	Cycle 4	Half of each sprayed w/20% acetic acid	4 cm	2
Day 37	Cycle 1 R & S	Same	4 cm	2
Day 37	Cycle 2 S & Beans	Same	4 cm	2
Day 37	Cycle 3 R & S	Same	4 cm	2
Day 37	Cycle 4 S not sprayed	> 20	4 cm	2
Day 37	Cycle 4 R & S sprayed	0 (Dead)	0 cm	2

R = Resistant S= Susceptible

The results indicate that 10% concentrations could be used, and further research could scale studies on concentrations of 18%, 16%, 14%, and 12% to further refine the impact of the solutions on weed growth.

The JCM 5000 NeoScope™ tabletop scanning electron microscope (SEM) was used to analyze images of each experimental group to document the physiological changes, if any, to the epicuticular waxes located on the leaves. Micrographs of the adaxial leaf surface were taken before and after the application of the varying concentrations of acetic acid to document the change in the sample. This gave a viable overall picture of the effectiveness of each varying concentration of acetic acid.

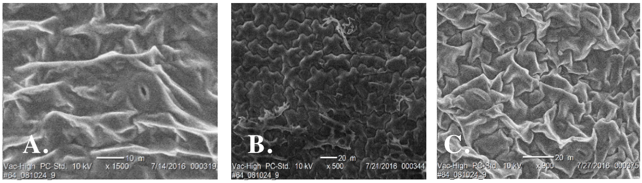

Figure 1. Scanning Electron Micrographs of *Amaranthus palmeri* leaves. **A.** 5% Acetic Acid Applied (Day 14). **B.** No vinegar (Day 14). **C.** 20% Acetic Acid (Day 10). Note changes in epidermal cells. Samples A) and C) are comparable in that there are changes. Sample B) represents the typical leaf surface with no damage.

The SEMs taken corresponded with the data collected (See the SEM's in Figures 1). All of the samples that were sprayed with acetic acid showed signs of stress regardless of the concentration. Figure 1A shows deformation in the dermal layer cells that occurred in the leaves when sprayed with 5% acetic acid. It took 14 days to show this change. While the 5% did cause some changes to the cuticle layer, the effects of the 20% acetic acid solution were seen earlier and were more significant (Figure 1C). The high dosage of acetic acid caused massive plasmolysis (loss of structure due to dehydration) in the upper layers of cells resulting in a loss in turgor pressure causing wilting as seen in Figure 1C; this process culminated in plant death. In Figure 1B you can see normal cells that look taut and rigid in comparison to its counterparts. This was to be expected in the sample with no exposure to acetic acid.

Conclusions

Overall, the experiments show that if caught early enough, with in the first 30 days, the young *A. palmeri* may be treated with applications of 10% acetic acid or 20% acetic acid regardless of its glyphosate resistance. This is beneficial for the environment because it will decrease the buildup of acetic acid in the soil. This minimizes the chance for making the soil and surrounding environment more acidic. It also brings down the price per acre of producing produce. This in turn brings down the price at the supermarket. Future work can refine what concentration between 10 and 20% remains effective, and further analysis of how the herbicide affects the soil could also lead to more discovery, optimization and further savings.

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References

1 Morichehette, S., Ferrell, J. & Leon, R. *University of Florida: IFAS Extension*, <http://edis.ifas.ufl.edu/> (2012).
2 Chahal, P., Aulakh, J., Jufulam, M. & Jhala, A. in *Herbicides, Agronomic Crops and Weed Biology* (eds A Price, J Kelton, & L Sarunaite) (2015).
3 Jabran, K., Mahajan, G., Sardana, V. & Chauhan, B. Review: Allelopathy for weed control in agricultural systems. *Crop Protection* **72**, 57-65 (2015).
4 Sosnoskie, L., Culpepper, A., Grey, T. & Webster, T. Severed stems of Amaranthus palmeri are capable of regrowth and seed production in Gossypium hirsutum. *Annals of Applied Biology* **165**, 147-154 (2014).
5 Morrison, L. Herbicide-resistant weeds: the tillage dilemma. *Corn & Soybean Digest* **74**, 24-30 (2014).
6 Roberson, R. Pigweed control is a numbers game. *Southeast Farm Press* **39**, 1 (2012).
7 Whitaker, J., Burton, J., York, A., Jordan, D. & Chandi, A. Physiology of Glyphosate-Resistant and Glyphosate-Susceptible Palmer Amaranth (Amaranthus palmeri) Biotypes Collected from North Carolina. *International Journal of Agronomy*, 1-6 (2013).
8 Legleiter, T. & Johnson, B. (ed Purdue Extension) (https://www.extension.purdue.edu/extmedia/ws/ws-51-w.pdf, 2013).
9 Yancy, J. Weed scientists develop plan to combat glyphosate resistance. *Southeast Farm Press* **32**, 1 (2005).

10 Quarles, W. *The IPM Practitioner: Monitoring the Field of Pest Mangement*, <http://www.birc.org/MayJune2010.pdf> (2010).
11 Smith, R. Herbicide resistance changing Sunbelt production options. *Southeast Farm Press* **40**, 1 (2013).

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