The Effects of a Selective and Non-Selective Organic Herbicides on *Amaranthus species*

Chonique S. Long, Nayel N. Novelo, Hanna Derecho and Yolander Youngblood Ph.D.

Department of Biology, Prairie View A & M University

Corresponding Author: Yolander Youngblood, Ph.D.;Department of Biology, Prairie View A & M University, P.O. Box 519, MS 2210, Prairie View, TX 77446; Phone: 936-261-3169; yryoungblood@pvamu.edu

Abstract

The *Amaranthus* species has adaptive abilities that give them competitive advantages and invasive tendencies. Their high seed production, seed viability, quick growth rate, and C4 metabolism have allowed some of the species to become resistant to some types of herbicides, causing soybean, corn, and cotton crop yield losses in North America. For this investigation, different organic herbicide solutions were analyzed to determine their affects on the *Amaranthus species*. Different concentrations of acetic acid, eucalyptus volatile oil, and okanin were combined to test the hypothesis that the unique characteristics of each organic herbicides should safely and effectively deter *Amaranthus* growth, even at low concentrations. The organic herbicide cocktail significantly affected the growth rates and germination percentages of resistant *A. palmeri*, susceptible *A. palmeri*, *A. viridis,* and *A. tricolor*. Spouts died when the solution was applied daily, and seeds did not germinate after application. The solution did not have a large effect on *A. hypochondriacs* and *A. caudatus*, but most of those sprouts' length was diminished, and growth ceased.

Introduction

Amaranthus is a globally diverse species that can be divided into four main classification groups: cultivated, weedy, racial, and landrace (Stallknecht & Schulz-Schaeffer, 1993). Though weedy types are the focus of this research, the cultivated types also have a chance to become an invasive species due to phylogenetic relationships (Stetter & Schmid, 2017). *Amaranthus* are annual herbaceous plants and are a part of the few dicot C4 plant species. Their C4 metabolism allows them to adapt to a diverse range of environmental conditions making them resilient to change and stress (Ward, Webster, & Steckel, 2013). Due to their high seed production, seed viability, quick growth rate, and C4 metabolism, they have become an invasive species in agriculture around the world. In the southern United States, they have caused up to 50% crop yield loss in cotton, soybeans, and corn (Ward et al., 2013). Though farmers have used herbicides to combat their invasion, *Amaranthus* are becoming resistant to the chemical herbicides including: ALS inhibitors, EPSP inhibitors, photosystem II inhibitors, and PPO inhibitors (Dominguez-Valenzuela et al., 2017; Francischini et al., 2014).

Due to chemical overuse, resistant forms of invasive weeds have evolved. Acetic acid, eucalyptus volatile oil, and okanin are alternative herbicides that have been proposed for their unique properties. Acetic acid in a 5-10% concentration strips away the plant's epicuticle protective wax, thereby removing the plant's ability to retain moisture. Small plants are affected by the low acetic acid concentration; however, larger *Amaranthus* plants require higher concentrations (Smith-Fiola & Schulz-Schaeffer, 2017). Even though acetic acid will convert into the water in the soil, the long-term buildup of acetic acid is unknown. Also, due to acetic acid’s conversion into water, the alternative not affect the roots, thereby granting an opportunity for the weeds to recover. Eucalyptus oil’s monoterpenes have been shown to inhibit growth and induce oxidative stress in *Amaranthus*’s roots (Singh, Batish, Kaur, Arora, & Kohli, 2006). Eucalyptus oil’s allelopathic potential, which is naturally occurring, can destroy chlorophyll and reduce seed germination of *Amaranthus viridis* (Kaur, Singh, Batish, & Kohli, 2011). Okanin is a natural pentachalcone in the *Asteraceae* family that selectively inhibits phosphoenolpyruvate carboxylase (PEPC), an essential enzyme in the C4 photosynthetic pathway to allow the plant to store carbon, produce energy, seed germination, CO_2 fixation, cell expansion, and to tolerate abiotic stress (Nguyen et al., 2016; O'Leary, Park, & Plaxton, 2011). When this compound inhibits the C4 plants photosynthetic pathway, the plant's resilience weakens.

Investigators hypothesize that combining the non-selective acetic acid with the selective eucalyptus oil and okanin will deter *Amaranthus* growth, even at lower concentrations due to a synergistic combination effect.

Methods

Potting

Field soil obtained from Prairie View A&M's agriculture land was collected in a basin and brought into the Botany greenhouse for use. The soil was placed in 12 plastic pots with dimensions of 11cm X 15cm. The first six pots contained the control set for comparison and the next six planted later contained the sprayed set. The control and sprayed sets both contained around 100 seeds of *Amaranthus* species, two of which (*A. palmeri* susceptible and *A. palmeri* resistant) were obtained from Dr. Youngblood. *A.tricolor, A.hypochondriacus,* and *A. caudatus* were obtained online from David's Garden (El Segundo, CA), and *A. viridis* was obtained online from Frozen Seed Capsules (Palm beach, Florida).

Pots were monitored during weekdays and were watered daily with double ionized water with a spray bottle daily to keep the soil moist. Pots were placed under Industrial Glowlight WS, UV light (manufactured in Mexico), 24/7.

An additional pot of *A. Palmeri* was planted with the intent to allow growth to the adult height.

Materials

The material used for the solution was Carolina Methanol (Carolina, Burlington, NC), DMSO 99.7% (Fisher BioReagents, Pittsburgh, PA), BDH Tween 20 (Millipore Sigma, Darmstadt, Germany), Glacial acetic acid (Carolina Biological Supply, Burlington, NC), Eucalyptus Globulus Oil (DeLaCruz, Los Angelos, CA) The okanin extract was obtained from the plant *B. pilosa*, received from the Mercer Botanic Gardens (22306 Aldine Westfield Rd, Humble, TX).

Extraction

The adult plants of *B. pilosa* were washed then dried in an air dryer for 24 hours and manually ground with a marble pestle and mortar. Once ground into a powder, it was mixed with 70% methanol for ultrasonic extraction with a Q500 Sonicator system with standard ½ diameter probe. It was extracted for 30 minutes at 40hz and 500W with 10 seconds rest intervals. The solution was then vacuum filtered to obtain the solvent that would then be placed in a rotary evaporator, Rotavapor R-100 for 2 hours. The remaining solute and oil was vacuumed filtered again to remove remaining residue. The resulting solution was placed in a transparent glass vial.

Solution

In a 500mL beaker, a 200mL solution was made with 5% acetic acid, 0.2% eucalyptus oil, 17.65% of the *B. pilosa* extract, 0.1% of Tween 20, and 2.4% DMSO in DI water. The solution was then placed in an 800mL plastic spray bottle.

Application

The application of the herbicide solutions was started on day 2 of measurements, when growth was seen, with a two-day intermission due to the weekend. Control groups were sprayed daily with deionized water. The experimental groups of the *Amaranthus spp.* were daily sprayed with the solution described above (two full pumps each). The plants were sprayed on Days 0, 1, 2, 4, 5, 6, 7, and 8. Results were measured at the time of application.

Another *A. palmeri* plant that was potted for the intent of growing to adult height, received only deionized water applications until they reached 20cm to test the herbicides application on larger plants. At that time, it was sprayed from top to base of the stem until thoroughly coated with the solution described above.

Analysis

The measurements and daily log were recorded in a lab notebook. Line graphs were generated via Microsoft Excel® and included the daily height data and the daily average growth data (calculated by averaging the daily formula: $\frac{y_2-y_1}{T_2-T_1}$). Germination rates were calculated using formula: $(Number\ of\ germinated\ seed \div Total\ number\ of\ seeds) \times 100$.

Results

Growth Comparison

A. palmeri resistant control's average growth rate in an 8-day period is 0.33cm/d. *A. palmeri* resistant sprayed average growth rate in an 8-day period is a negative -0.20, due to there being a two-day gap (Days 2 & 3) in sprays, thereby giving an opportunity for sprouts to grow. Once spray was applied, the sprouts wilted and died, but no other seeds would sprout after.

As seen in *Figure 1*, the growth of control and sprayed growth was similar at the beginning of measurements, but once the solution was applied again, no more growth was seen on Days 5-8. The initial application of the solution did not seem to affect the growth rate of sprayed pot.

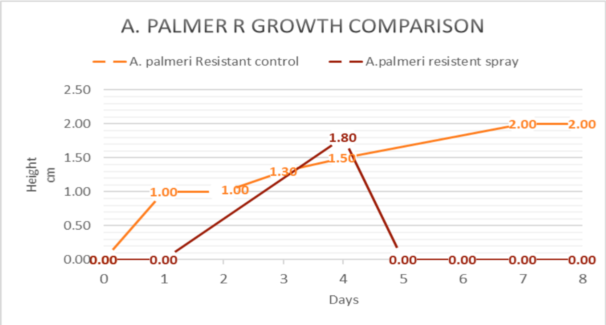

Figure 1: The daily height of control and sprayed set of A. palmeri resistant over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate of *A. palmeri* susceptible control is 0.36cm/d, and *Figure 2* shows the gradual development of the control. *A. palmeri* susceptible spray's average growth rate over 8 days was in the negative at -0.13cm/day due to the application of the solution causing the plant's length to decrease and growth to stop. In *Figure 2*, the initial treatment of solution decreased the growth rate when compared to control. The sprayed sprouts length greatly decreased after Day 4 with the continuous daily application.

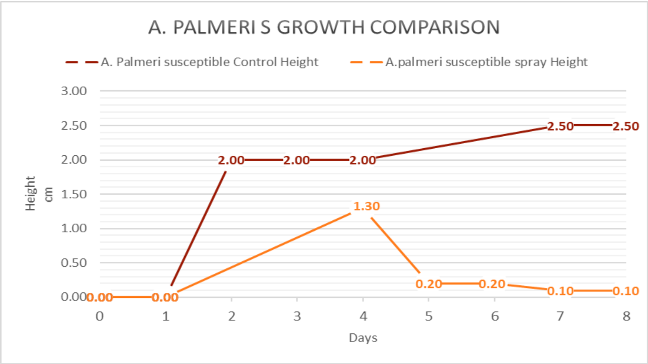

Figure 2: The daily height of control and sprayed A. palmeri susceptible over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate for *A. viridis* control is 0.56 cm/day over the 8-day period, while the average growth rate of *A. viridis* sprayed is negative -0.13 cm/day due to sprouts decreasing in length after application of solution on Day four as seen in *Figure 3*. The initial application of the solution on Day 1 did affect the growth rate as compared to control.

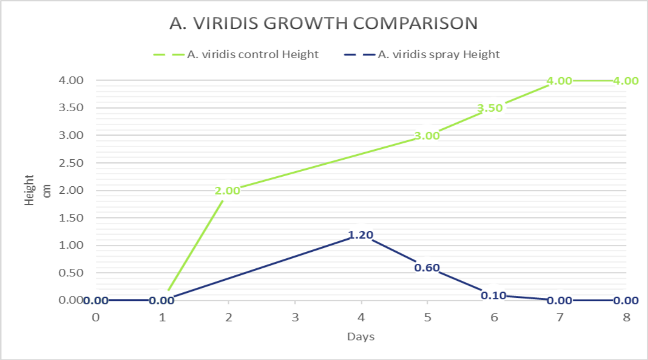

Figure 3: The daily height of control and sprayed A. viridis over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate of *A. hypochondriacus* control in the 8-day period is 0.38 cm/day, while the average growth rate of *A. hypochondriacus* sprayed is -0.07 cm/day due to sprouts decreasing in length, as seen in *Figure 4*, after Day 4. The initial application of solution did affect growth rate once compared to control.

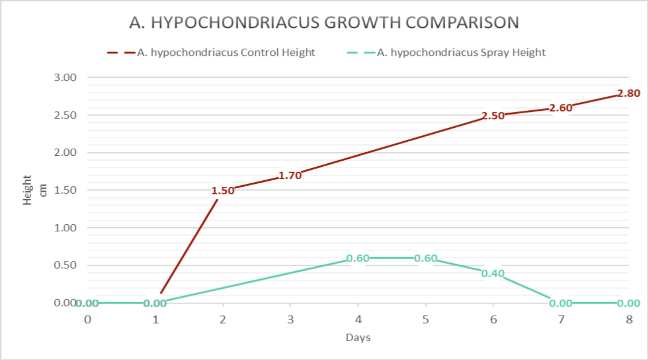

Figure 4: The daily height of control and sprayed pots of A. hypochondriacus. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

A. caudatus control's average growth rate is 0.34 cm/day, while *A. caudatus* sprayed average growth rate is -0.08 cm/day in the 8-day span due to sprouts length decreasing once the solution was applied.

As seen in *Figure 5*, the growth of sprayed and control have similar increases in growth until Day 4, when the solution was applied to the sprayed pots that cause sprouts to wither, stop seed germination, and cease radicle growth.

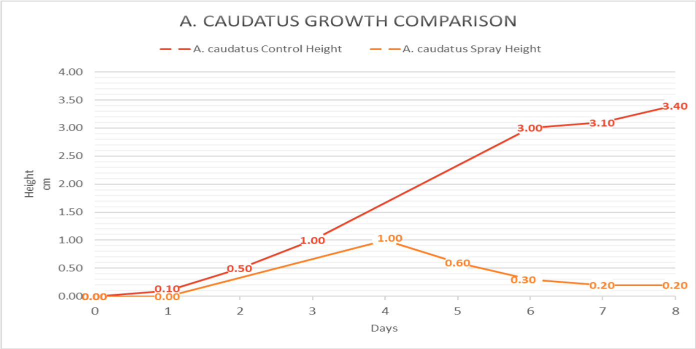

Figure 5: The control and sprayed daily height of A. caudatus in an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

A. tricolor control's average growth rate over the eight-day period is 0.46 cm/day, while the average growth rate of sprayed *A. tricolor* is -0.10 cm/day due to sprouts length decreasing after continuous daily application of the solution (*Figure 6*). The initial treatment reduced the growth rate when compared to control. After Day 4, sprayed pot length decreased, and no other seeds germinated with continuous daily spray.

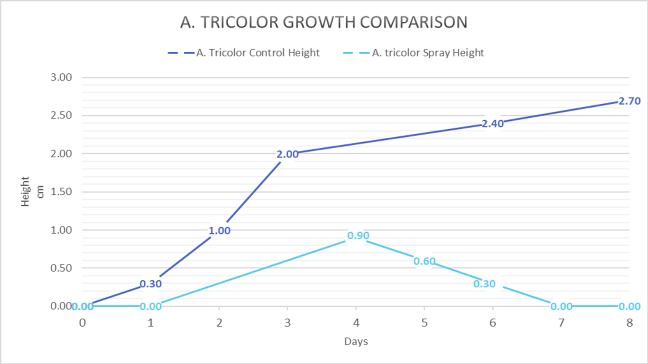

Figure 6: Daily height of control and sprayed A. tricolor in the 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

Germination

The germination percent of the control *Amaranthus spp.,* as seen in *Figure 7*, shows *A. viridis* with a germination percentage of 45%--the highest of the control species. Susceptible *A. palmeri* germination percentage at 19%, is on par with *A. caudatus* and *A. hypochondriacus*. The lowest percentages of control were resistant *A. palmeri* at 7% and *A. tricolor* at 10%.

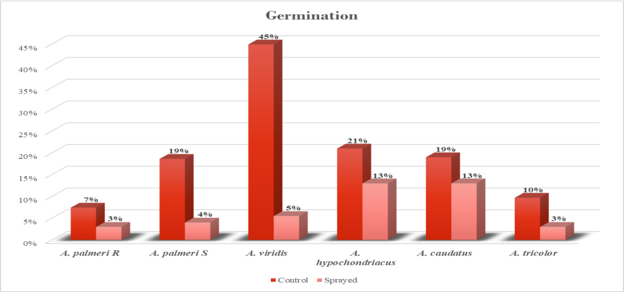

Figure 7: The germination percentage of Control and Sprayed sets of Amaranthus spp. in an 8-day period.

For the sprayed pots, the solution affected all species germinations percentages. The largest effects were on *A. viridis* and susceptible *A. palmeri* of 40% and 15% different respectively when compared to control. *A. hypochondriacus* and *A. caudatus* germination percentage is 13%, only a slight decrease from control percentage.

Adult Amaranthus palmeri *application*

For the second experiment, the adult plant was fully erect before the solution was applied, but three hours after application the plant shows visible drooping, wilting, and spots of lighter discoloration appear on leaves. Three days after application, most leaves wilted along with parts of the stem.

Discussion/Conclusion

The average growth rate and high germination percentage of the control susceptible *A. palmeri* plants confirms the invasive characteristics previously reported. Resistant *A. palmeri*, on the other hand, has a lower growth rate and a low germination rate; thus, it poses less of a threat to an invasive species as they are less likely to survive, compared to the susceptible species. *A. viridis* high growth rate and high germination rate, which means it is more invasive than *A. palmeri* and should be monitored in agriculture to prevent infestations. *A. hypochondiacus,* mainly used for grain, has a slightly higher growth rate and germination percentage than susceptible *A. palmeri*; therefore, it could also become an invasive species. *A. caudatus*' growth rate and germination percentage are on par with susceptible *A. palmeri*. Though *A. caudatus* are used for their grains, they have a possibility to become just as invasive as susceptible *A. palmeri* in unwanted areas. *A. tricolor's* germination percentage is low, and their growth rate is high; therefore, they have a medium chance to become an invasive species.

The organic herbicide cocktail significantly affected the growth rates and germination percentages of resistant *A. palmeri*, susceptible *A. palmeri*, *A. viridis,* and *A. tricolor*. Spouts died when the solution was applied daily, and seeds did not germinate after application. The solution did not have a large effect on *A. hypochondriacs* and *A.*

caudatus, but most of those sprouts' length was diminished, and growth ceased. Comparing the difference in growth and germination rates of control and sprays, the organic herbicide solution affected *A.viridis* and *A. palmeri* the most, but the less invasive species were not affected to the same extent. A plausible reason for less invasive species being less affected would be that the eucalyptus volatile oil does not have allelopathic potential against *A. hypochondriacus,* and *A. caudatus*.

The organic herbicide solution had a noticeable effect on the larger plant when applied thoroughly. The organic herbicide solution also significantly affected the adult *A. palmeri*, causing it to wilt within 3 hours. After three days, parts of the adult are unaffected, probably due to the solution not being applied evenly. Because okanin is known to cause a lighter discoloration in leaves of *A. retroflexus* (Nguyen et al., 2016), we believe the same effect three hours occurred in this experiment and that the application of the okanin on *A. palmeri* affected the PEPC pathway and caused the plant to be more susceptible to the acetic acid and eucalyptus volatile oil.

While further research is needed, the organic herbicide containing acetic acid, volatile eucalyptus oil, and okanin shows promise. Future experiments should investigate application timing procedures and also should explore starting the application at different stages of growth. Further, experiments should be run on the key crops (corn, soybean, etc) that are harmed by this weed, to see how the herbicide will affect their growth.

Due to the low solute concentration, this solution should not cause a meaningful change or build up in the soil and environment; however, future experiments should analyze the soil.

In conclusion, due to the components availability and low cost, the proposed organic herbicide cocktail could be a cheaper and effective alternative to traditional herbicides.

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