Efficacy Comparison of Acetic Acid and d-Limonene Combinations for Control of *Amaranthus spinosus* and *Amaranthus albus*

Proposed by Grace Brosofsky, Environmental Engineering

Overview of Proposed Work

According to the Environmental Protection Agency's most recent market estimates, global pesticide usage approximates 5.2 billion pounds with herbicides ranking as the most widely used type of pesticide [1]. This indicates a significant need for innovation as chemical herbicides contaminate the soil, air, and water systems that support human, animal, and plant life, generating numerable consequences. For example, the World Health Organization recently classified glyphosate, a common herbicide currently in use in Georgia Tech's landscape maintenance plan, as "probably carcinogenic to humans" [2]. Research also links glyphosate to autism, Alzheimer's, Parkinson's disease, multiple sclerosis [3], obesity, diabetes, cardiovascular disease, and other health issues [4]. Natural herbicide solutions have generated increasing public interest as they offer a solution to weed control without incurring health and environmental costs and qualify for use in organic agricultural production systems [5]. As these solutions fulfill the same role as glyphosate of providing nonselective postemergent weed control (weed control targeting any already growing plant), the primary obstacle to their widespread adoption for both agricultural and aesthetic use is cost inhibitions. Because of this, research on how to best combine organically registered herbicidal ingredients to provide the greatest control with the least financial input is of critical importance.

The research project outlined in this proposal pursues the optimization of the weed control efficacy of natural herbicide solutions by collecting and analyzing data on the percent control provided by solutions of different ratios of acetic acid, d-Limonene, and adjuvants in a two by three factorial greenhouse study and field study. Acetic acid, found in vinegar, is an aliphatic carboxylic acid, a type of compound that can be generally represented as RCOOH which separates into RCOO anions and H cations during ionization, allowing carboxylic acids to translocate through plant cell walls because they exist in their non-charged forms in the low pH of the cell wall but are ionized once in the high pH of the cytoplasm. D-Limonene is an orange oil derivative, and adjuvants that can augment the efficacy of acetic acid and d-Limonene include citric acid, an aliphatic carboxylic acid in lemon juice. As my previous research featured in *Scientific American* and *The Global Journal* suggested that a "tank mix" of vinegar and d-Limonene provides optimal control out of various mixtures of natural herbicides, refining the composition of this tank mix has potential to yield applicable results. When conducting the aforementioned research, I maintained correspondence with Dr. Charles Webber, Research Agronomist, USDA, who recommended performing such further experimentation with different ratios to enhance the demonstrated efficacy of the tank mix and produce results useful to the scientific community. I am currently leading a research team including environmental engineering undergraduate students Francis Nguyen and Lindsey Tracey, chemical engineering undergraduate student Andrew Vidovich, and materials science and engineering undergraduate student Jamie Curtis.

Materials and Methods

The greenhouse experiment will be established with a two by three factorial design with four replications in each study in the greenhouse on the rooftop of the Cherry L. Emerson Building at Georgia Tech. Main effects will be the two levels of adjuvant use - 5% adjuvant and 0% adjuvant -

and three acetic acid to d-limonene ratios - 1:3, 1:1, and 3:1. A nontreated control will be used as a basis for comparing weed response to herbicide treatment (two nontreated controls per six treatments). Sixty-four total containers containing commercial potting medium (77 to 87 percent aged pine bark, sand, and perlite) will be used in the experiment, and each container represents one experimental unit. Each container has a diameter of 4 cm. Spiny pigweed (*Amaranthus spinosus*) grown from seed will be planted in thirty-two containers, and tumble pigweed (*Amaranthus albus*) grown from seed will be planted in the remaining containers as pigweed is a common invasive broadleaf plant species suited to greenhouse germination. Three seeds have been germinated in each container, and the plants will later be thinned to one per container to provide a uniform stand for experimentation. A parallel study with a randomized complete block design will be performed conditionally using the land outside of the Jesse W. Mason Building and presently growing invasive plant species. In this experiment, environmental variables such as lighting and soil nutrient composition will also be measured and taken into consideration to understand the overall impact of herbicide solutions upon the soil system.

In both studies, treatments will be applied at a rate of 20 L/ha using a CO_2 backpack sprayer contributed by Dr. William Vencill, Research Agronomist. In the greenhouse experiment, this will occur when the pigweed plants reach a height of two to three centimeters with three to four leaves. 25% acetic acid vinegar diluted with deionized water, 100% d-Limonene, and lemon juice concentrate will comprise the herbicidal solutions. Visual observations on a 0 to 100 scale will be recorded at one week intervals up to five weeks after treatment (WAT). Data will be further collected and analyzed using various methods that will be chosen according to financial ability, with options including dot-grid analysis, plot digital photograph classification (PDPC) or other methods of photographic analysis, and statistical analysis using ANOVA.

Objectives and Goals for the Fall 2015 Semester

1. To propose an optimally effective combination of acetic acid, d-Limonene, and adjuvants for broadleaf weed control
2. To gain experience with a broad range of data collection and analysis methods
3. To compile a report meeting the standards of the *Weed Technology* journal
4. To propose a minimum effective volume of application to minimize costs
5. To improve techniques of growing experimental subjects from seed

References

[1] Grube A, Donaldson D, Kiely T, Wu L. Pesticide Industry Sales and Usage: 2006 and 2007 Market Estimates [Internet]. Washington (DC): Environmental Protection Agency (US); 2011 Feb [cited 2015 May 6]. Available from http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf.

[2] Joint FAO/WHO Meeting on Pesticide Residues (JMPR) [Internet]. World Health Organization; 2015 Mar [cited 2015 May 6]. Available from http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/.

[3] Samsel A, Seneff S. Glyphosate, Pathways to Modern Diseases III: Manganese, Neurological Diseases, and Associated Pathologies. Surg Neurol Int. 2015;6:45.

[4] Samsel A, Seneff S. Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases. Entropy. 2013;15:1416-1463.

[5] Abouziena HFH, Omar AAM, Sharma SD, Singh M. Efficacy Comparison of Some New Natural-Product Herbicides for Weed Control at Two Growth Stages. Weed Technology. 2009;23(3):431-437.