Generational Effects of Roundup® and its Components on the Wild Type *C. elegans* and Daf-9 Mutant Strain

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ABSTRACT

When exposed to high stress levels, organisms tend to pass on certain phenotypic traits or tolerance levels to their offspring, sometimes through epigenetics. This experiment explores the epigenetic tolerances that Roundup® and its components create in the model organism, *Caenorhabditis elegans*. If a significant percentage of the nematodes can survive an exposure to a low dilution of Roundup®, their progeny may have a higher tolerance, survival rate, and lessened stress responses in Roundup® due to epigenetic changes caused by the experience of the parent. To expand upon our previous research, we treated both the wild type and a *daf-9* mutant strain, which has been shown to be more stress resistant than wild type. Rather than treating the *C. elegans* with simply Roundup®, separate plates of nematodes were treated with equivalent concentrations of pure glyphosate (Roundup's listed active ingredient), polyethoxylated tallow amine (POEA, the second known ingredient of Roundup®, a surfactant) and a combination of glyphosate and POEA (to detect possible synergistic effects of the two ingredients and the possibility of separate unknown toxic ingredients in Roundup®). These strains were treated with Roundup® concentrations increasing from 2% to 5% in increments of 0.5%, in an attempt to develop a tolerance in the *C. elegans* to the Roundup® over successive generations. In previous research, the final generation in the control plates, which had not been exposed to Roundup in earlier generations, were treated with equal dilutions (2.0% Roundup®) to the test plates, which had been subjected to increasing concentrations of Roundup. In this final generation, the test plates had 100% survival rate while the control plates had 60% and 53% survival. This suggested that the *C. elegans* have the ability to develop a tolerance to Roundup® over multiple generations, but nematodes that are not predisposed to tolerate Roundup® have a lower survival rate when exposed to Roundup®. Contrary to previous results, this year the survival rates for both the generational controls and the experimental groups were all 100% throughout exposure to all of the dilutions of Roundup® and its components. This suggests our previous results are inconclusive as to whether or not the *C. elegans* developed a tolerance against Roundup® and further stresses the importance of repeating experiments. Differences between the experimental methods that were, in part, because of the expanded nature of the study may have created the contrary results. We intend to repeat both of these experiments in an attempt to yield more conclusive findings.

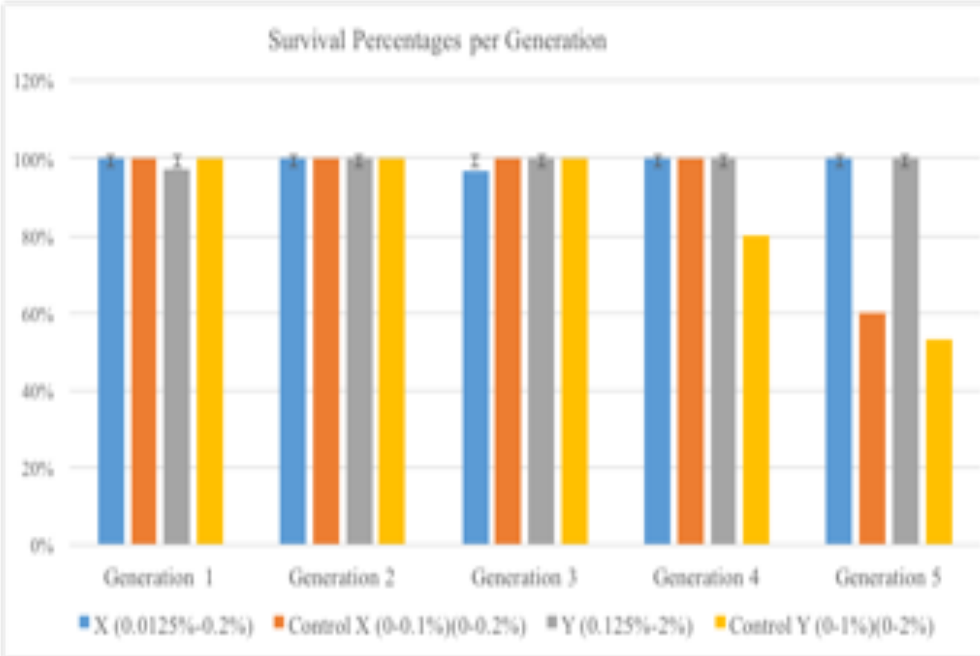
INTRODUCTION

The herbicide Roundup® has been at the center of many studies throughout the years. Its active ingredient, glyphosate, targets the shikimic acid pathway found in plants, some bacteria, and fungi. This pathway is critical for making aromatic amino acids essential for protein production. Since the shikimic acid pathway is not present in animals, companies such as Monsanto market Roundup® as a safe herbicide with no negative effects on the environment. Polyethoxylated tallow amine, or POEA, is the only other known component of Roundup®. In Roundup® it acts as a detergent to stabilize the mixture. POEA itself is animal fat that is often bought very cheap to make detergents and is thus used in Roundup® since it is such a mass produced product. The *C. elegans* is an ideal model organism to test this claim due to its similarities to other organisms found in crop fields, many of which are sprayed with Roundup® products.

When stressed, organisms tend to produce more progeny to ensure survival of the species. This creates an ideal scenario for epigenetics research, the study of how an organism's experiences affect the gene expression of its progeny. During our initial experiment, as seen in Figure 1, we observed the nematodes across five generations. The test plates appeared to have grown a tolerance to Roundup® with 100% survival rates when compared to the control plates which had lower survival rates of 60% and 53% when exposed to the same level dosage of Roundup®. From this we inferred that when not exposed to Roundup® gradually over generations, the *C. elegans* respond negatively, usually resulting in their death.

GOAL

The goal of this experiment is to test if two different strains of *C. elegans*, the wild type and Daf-9 strains, are capable of building a tolerance across multiple generations to a stress-inducing chemical such as Roundup®. Daf-9 is part of the IGF-1 signal pathway, an evolutionarily conserved pathway which responds to insulin-like factors in the *C. elegans*. Daf-9 is disabled in the Daf-9 mutant strains, increasing the lifespan and stress tolerance of the *C. elegans*. Testing the Daf-9 would highlight how Roundup® affects the Daf-9 strain compared to wild type *C. elegans*.



X plates	X control	Y plates	Y control
0.0125%	0%	0.125%	0%
0.025%	0%	0.25%	0%
0.05%	0%	0.5%	0%
0.1%	0%	1%	0%
0.2%	0.2%	2%	2%

Figure 1: Previous results showed that there was correlation between exposure and survival rates.

METHODS AND MATERIALS

- Wild Type *C. elegans*
- Daf-9 Mutant *C. elegans*
- Plates
- Platinum wire pick
- Roundup Super Concentrate
- Glyphosate

Dilutions:

Roundup®, glyphosate, POEA, and a mixture of POEA and glyphosate were respectively diluted into 1 mL of water each to create dilutions of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% of each chemical.

Transferring Nematodes:

Each of the six plates in generation one contained 15 adult *C. elegans* worms. Following the first generation, nematodes were picked at the L1 larvae stage, which is the first larval stage after hatching. After taking two days to mature and produce progeny in their separate dilutions, 15 L1 larvae from each plate were transferred to new plates with higher concentration (see Table 1). The nematodes were transferred using a platinum wire pick under a microscope. 1 mL of each respective dilution was pipetted onto each new plate following the transfer of the larvae.

Classification:

To monitor the success of the progeny, death counts were conducted to calculate the percentage of the population that survived. If the nematodes ceased to move and became stick-like, they were most likely dead. To verify their state, the platinum pick was used to stimulate the worms in an attempt to provoke a response. If the nematodes did not respond, they were classified as dead.

Stress Responses:

When under stressful circumstances the nematodes exhibit four main signs of stress: high motility, egg expulsion, low motility, and cuddling. To further see the effects of Roundup® on the nematodes, when death counts were administered, these signs of stress were noted along with their survival rates.

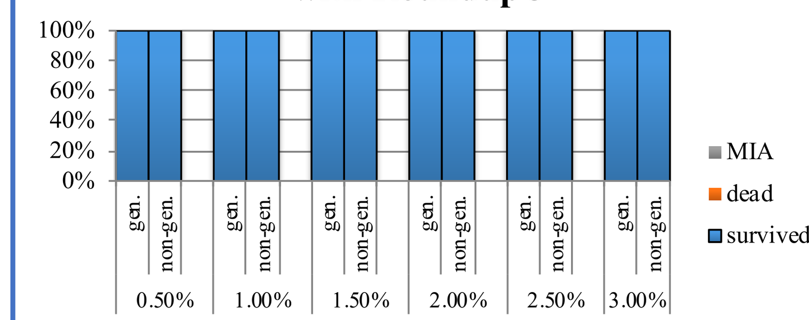
RESULTS AND DISCUSSION

This year's experiment yielded results contradictory to last year's. This year, no development of tolerance was shown in any of the strains of worms, even in the wild type, which had shown some form of tolerance development in last year's experiment. This means that all of the groups sustained 100% survival rates throughout all of the generations and doses, the same as both the non-generationally tested (previously untreated *C. elegans* treated at increments corresponding to the test groups, but whose progeny was not transferred into the proceeding concentration level) and the baseline control (untreated *C. elegans*) as seen in Figures 2 through 17.

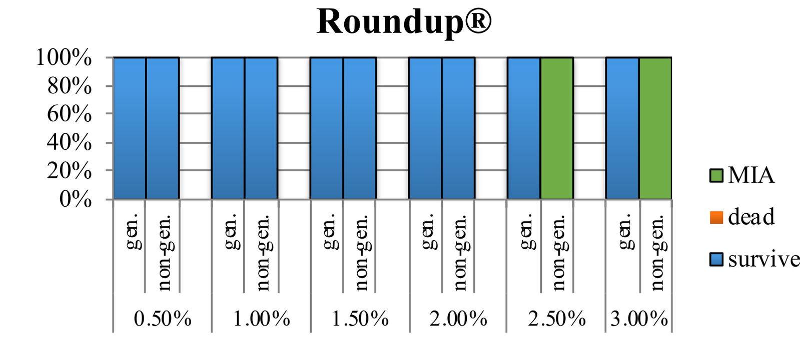
We repeated our experiment twice, each time seeing the same results. During one of our repeated experiments, seeing no deaths up to the generation 5, we decided to break the sequence and expose the progeny of generation 5 to the dilution intended for generation 8. This jump in dosage provoked no difference in the worms' behaviors, making our results, once again, inconsistent with last year's and therefore inconclusive overall.

In the future, this experiment would be repeated using the methods from last year in terms of only using wild type for a dose response to determine if our results from last year are replicable. From there, those results could be used to repeat this year's experiment in terms of using both wild type and Daf-9 strains and collecting more conclusive results on the effects of the known components of Roundup®.

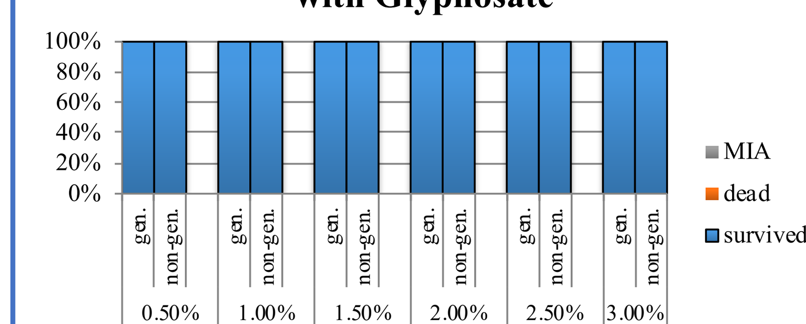
Survival Rates of Wild-Type treated with Roundup®



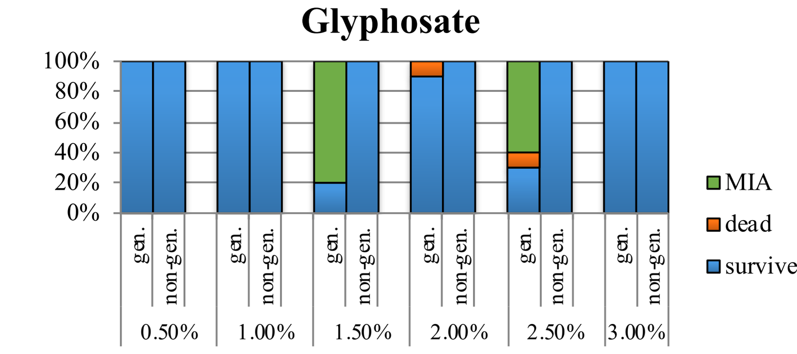
Survival Rates of Daf-9 treated with Roundup®



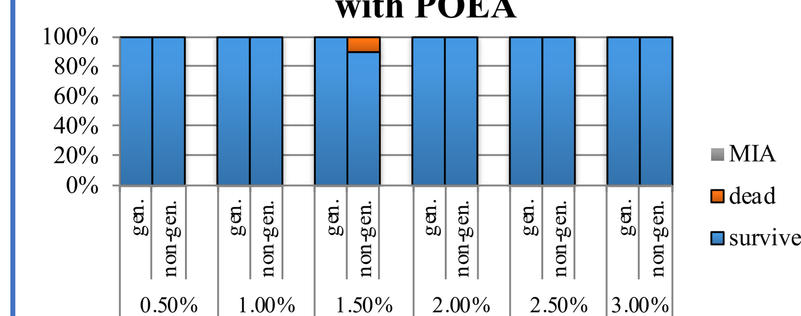
Survival Rates of Wild-Type treated with Glyphosate



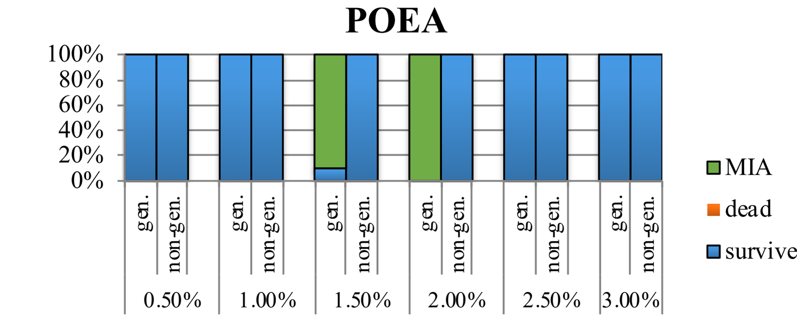
Survival Rates of Daf-9 treated with Glyphosate



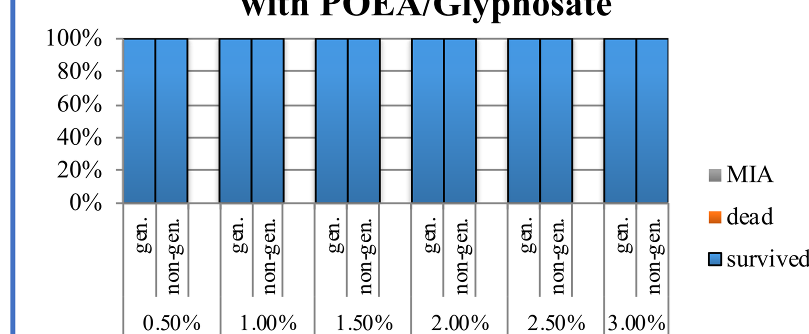
Survival Rates of Wild-Type treated with POEA



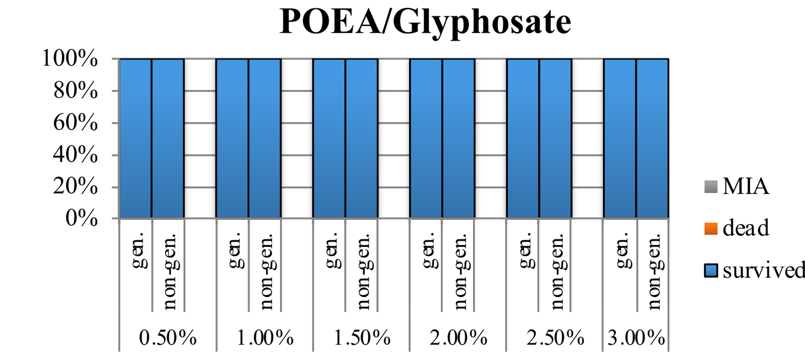
Survival Rates of Daf-9 treated with POEA



Survival Rates of Wild-Type treated with POEA/Glyphosate



Survival Rates of Daf-9 treated with POEA/Glyphosate



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