The Translation of Insecticide Resistance in *Drosophila Hydei* from Organophosphates to Carbamates

Thousand Oaks High School E8 Research Lab

Word Count: 4744

Purpose:

The purpose of this study is to test if conditioned resistance can transfer from two Organophosphates (*Naled/Dibrom* and *Coumaphos*) to a Carbamate (*Bendiocarb*).

Hypothesis:

After ten generations of flies consuming low amounts of the two Organophosphates, there will be a change in susceptibility to *Bendiocarb*, from the control group, which will have no conditione resistance. This susceptibility change will be the most noticeable in the group which received a mutagen in the form of Ultraviolet Radiation, as well as conditioned resistance to the two Organophosphates, *Naled/Dibrom* and *Coumaphos*.

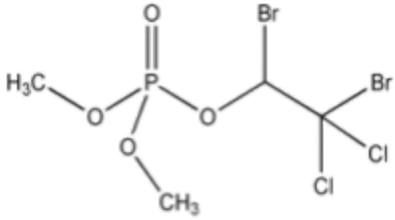
Introduction:

Insecticide resistance has translated from a minor problem in 1990, when resistance was "recorded in at least 504 species of insects and mites" which was "a 13% increase since 1984" (Brogdon, 1998) to a full scale epidemic (Brogdon, 1998). The problem has only grown from there with, over "60 countries have reported resistance to at least one class of insecticide, with a total of 49 of those countries reporting resistance to two or more classes" (Georghiou, 1990). Insecticide resistance is particularly pervasive in highly controlled species like mosquitoes and other carriers of dangerous blood borne diseases (Heckel, 2012). Mosquito populations and Africa and Brazil are reporting record amounts of resistance to multiple types of insecticides, and furthermore, studies of Mosquitos in Ecuador showed that "Lack of insecticide uses does not preclude immigration of resistance genes"(Edi, Koudou, Jones, Weetman, & Ranson, 2012).

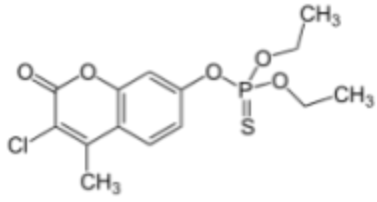
Essentially, we desperately need to know more about insecticide resistance and how it can change and evolve.

Recently the Department of Entomology found that a point mutation in the gene controlling the GABAA neurotransmitter can grant *Drosophila* high levels of resistance to *Picrotoxinin* and *Cyclodiene*, which are "GABAA receptor antagonists which completely displace each other from the binding site." (Ffrench-Constant, 1993)

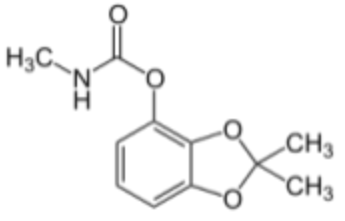
Due to this research one can conclude with a fair degree of certainty that it is possible for a single mutation to grant resistance to multiple insecticides at once. Tests ~~they l~~ater ran found that this single point mutation "may account for over 60% of reported cases of insecticide resistance" (Fillgrove, Pakhomova, Schaab, Newcomer, & Armstrong, 2007). It is highly possible that a similar point mutation on the genes which code for the enzyme Cholinesterase, which is what both Organophosphates and Carbamates target, will grant a widespread resistance to all insecticides acting upon it; in this case *Naled/Dibrom, Bendiocarb* and *Coumaphos.* The Genes which code for Cholinesterase are Ace-1 and Ace-2, and they work in conjunction to form the enzyme, it is currently unknown what mutations on either site could cause insecticide resistance. An extremely similar result was seen in the point mutation on GABAA (Ffrench-Constant, Rocheleau, Steichen, & Chalmers, 1993). Since *Naled/Dibrom* is currently being used in the eradication efforts of Ebola carrying mosquitoes in Central and South America, it would be useful to know the interactions that it, as



Fig, 1: Naled/Dibrom molecule



Fig, 2: Coumaphos molecule



Fig, 3: Bendiocarb molecule

well as the other insecticides have with each other. These interactions can be viewed in the lab by giving populations conditioned resistance to insecticides, which is a resistance acquired over many generations due to prolonged exposure to non lethal doses of the insecticide (Edi, Koudou, Jones, Weetman, & Ranson, 2012).

Procedure:

Basic Procedure:

Drosophila cultures were purchased online, and growth was maintained in an incubator set at 27 degrees celsius over the course of the experiment. The cultures were moved from their original containers into new containers containing the same concentrations of medium, so that the different groups would be as similar as possible.

Establishing a Baseline for *Naled/Dibrom*:

Four new cultures were created. These four cultures were a 100 ppb *Naled/Dibrom* medium solution, 1 ppm *Naled/Dibrom* medium solution, 10 ppm *Naled/Dibrom* medium solution, and a 100 ppm *Naled/Dibrom* medium solution.

The 100 ppb medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppb *Naled/Dibrom* solution. The *Naled/Dibrom* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppb *Naled/Dibrom* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila*

container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 1 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 1 ppm *Naled/Dibrom* solution. The *Naled/Dibrom* solution was created through serial dilution. A 1% stock solution was diluted down to a .1% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 1 ppm *Naled/Dibrom* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 10 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 10 ppm *Naled/Dibrom* solution. The *Naled/Dibrom* solution was created through serial dilution. 5 µl of a 1% *Naled/Dibrom* stock solution was added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 10 ppm *Naled/Dibrom* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were

introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 100 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppm *Naled/Dibrom* solution. The *Naled/Dibrom* solution was created through serial dilution. 50 µl of a 1% *Naled/Dibrom* stock solution was added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppm *Naled/Dibrom* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approqaximating the survival rate.

Establishing a Baseline for *Coumaphos*:

Four new cultures were created. These four cultures were a 100 ppb *Coumaphos* medium solution, 1 ppm *Coumaphos* medium solution, 10 ppm Coumaphos medium solution, and a 100 ppm *Coumaphos* medium solution.

The 100 ppb medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppb *Coumaphos* solution. The *Coumaphos* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppb *Coumaphos* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then

mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 1 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 1 ppm *Coumaphos* solution. The *Coumaphos* solution was created through serial dilution. A 1% stock solution was diluted down to a .1% solution, 5 µl was then added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 1 ppm *Coumaphos* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 10 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 10 ppm *Coumaphos* solution. The *Coumaphos* solution was created through serial dilution. 50 µl of a .1% *Coumaphos* stock solution was added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 10 ppm *Coumaphos* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were

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The 100 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppm *Coumaphos* solution. The *Coumaphos* solution was created through serial dilution. 500 µl of a .1% *Coumaphos* stock solution was added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppm Coumaphos solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

Establishing a Baseline for *Bendiocarb*:

Four new cultures were created. These four cultures were a 100 ppb *Bendiocarb* medium solution, 1 ppm *Bendiocarb* medium solution, 10 ppm *Bendiocarb* medium solution, and a 100 ppm *Bendiocarb* medium solution.

The 100 ppb medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppb *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppb *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then

mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 1 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 1 ppm *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. A 1% stock solution was diluted down to a .1% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 1 ppm *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 10 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 10 ppm *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. 50 µl of a 1% *Bendiocarb* stock solution was added to a 50ml volumetric flask using a P200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 10 ppm *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the

container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

The 100 ppm medium solution was created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppm *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. 500 µl of a .1% *Bendiocarb* stock solution was added to a 50ml volumetric flask using a P1000 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppm *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. Approximately 200 *Drosophila* were introduced into the container, and their survival rate was observed over the next seven days. This was done through measuring their movement every day, and approximating the survival rate.

Control without UV Radiation for Generation 1:

The culture for control without UV Radiation for generation one was created by adding 50 ml of *Drosophila* Instant Medium to 50 ml of DI water. The culture was left to sit for 5 min before 200 *Drosophila* were introduced. The culture was stored in the half of the incubator which did not have UV radiation for 7 days, and the numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

Experimental without UV Radiation for Generation 1:

The culture for the experimental group for generation one was created by adding a .5 ppm *Coumaphos*, 50 ppb *Naled/Dibrom* solution to 50 ml of *Drosophila* Instant Medium. This solution was created by adding 1 ml of the .01% *Naled/Dibrom* solution to 1 ml of the .1% *Coumaphos* solution. 5 µl of this solution was then then added to a 50 ml volumetric flask using a p200 micropipette, which was then filled the rest of the way with DI water. The *Naled/Dibrom* and *Coumaphos* solution was then mixed using a vortexer, then stirred into the *Drosophila* Instant Medium. The culture was left to sit for 5 min before 200 *Drosophila* were introduced. The culture was stored in the half of the incubator which did not have UV radiation for 7 days, and the numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

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Testing for Generation 1:

Two cultures of 100 ppb Bendiocarb medium solution were created. These cultures were created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppb *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppb *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. 50 *Drosophila* from each the experimental and the control were added to the

containers and left in the incubator for 7 days. The numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

Control without UV Radiation for Generation 2:

The culture for control without UV Radiation for generation two was created by adding 50 ml of *Drosophila* Instant Medium to 50 ml of DI water. The culture was left to sit for 5 min before all remaining *Drosophila* from the control of generation one were introduced. The culture was stored in the half of the incubator which did not have UV radiation for 7 days, and the numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

Experimental without UV Radiation for Generation 2:

The culture for the experimental group for generation two was created by adding a .5 ppm *Coumaphos*, 50 ppb *Naled/Dibrom* solution to 50 ml of *Drosophila* Instant Medium. This solution was created by adding 1 ml of the .01% *Naled/Dibrom* solution to 1 ml of the .1% *Coumaphos* solution. 5 µl of this solution was then then added to a 50 ml volumetric flask using a p200 micropipette, which was then filled the rest of the way with DI water. The *Naled/Dibrom* and *Coumaphos* solution was then mixed using a vortexer, then stirred into the *Drosophila*

Instant Medium. The culture was left to sit for 5 min before all remaining *Drosophila* from the experimental of generation one were introduced. The culture was stored in the half of the incubator which did not have UV radiation for 7 days, and the numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

Testing for Generation 1:

Two cultures of 100 ppb *Bendiocarb* medium solution were created and two cultures of 1 ppm *Bendiocarb* media solution were created.

The 1 ppb *Bendiocarb* cultures were created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 100 ppb *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 5 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 100 ppb *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. 50 *Drosophila* from each the experimental and the control were added to the containers and left in the incubator for 7 days. The numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

The 1 ppm *Bendiocarb* cultures were created by combining 50 ml of *Drosophila* instant medium, and 50 ml of a 1 ppm *Bendiocarb* solution. The *Bendiocarb* solution was created through serial dilution. A 1% stock solution was diluted down to a .01% solution, 50 µl was then added to a 50ml volumetric flask using a p200 micropipette. The remainder of the volumetric flask was then filled with DI Water. The 1 ppm *Bendiocarb* solution was mixed thoroughly on a vortex mixer, and added to the *Drosophila* Instant Medium in the *Drosophila* container, then mixed thoroughly. The medium was left to sit for 5 minutes. 50 *Drosophila* from each the experimental and the control were added to the containers and left in the incubator for 7 days. The numbers of alive and dead *Drosophila* were counted each day. This was done by sectioning off the container into four quadrants using a X shaped piece of paper and counting the individual quadrants survival rates. The survival rates in each quadrant were then added together as to have maximum accuracy.

Results:

It should be noted that the UV radiation, which was being used in low levels to increase mutation rates in the *Drosophila*, resulted in a hundred percent mortality rate for all *Drosophila.* Because of this, all results from UV are excluded from the graphs in order to minimize confusion, as it did not play any part in the conclusions drawn from the results.

The exact data is also provided directly under the figures where it is hard to determine the survival rates from the graph due to the extreme similarities between the control and experimental E.g. Table 5 and Table 7

Table One

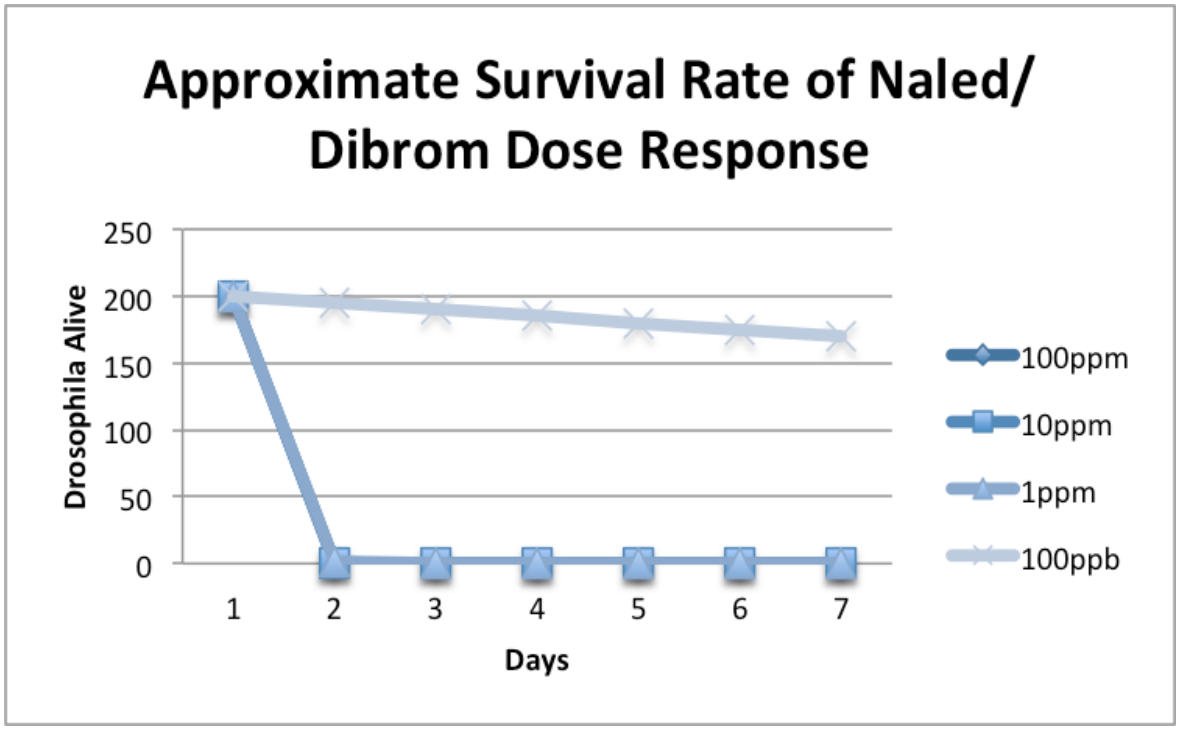
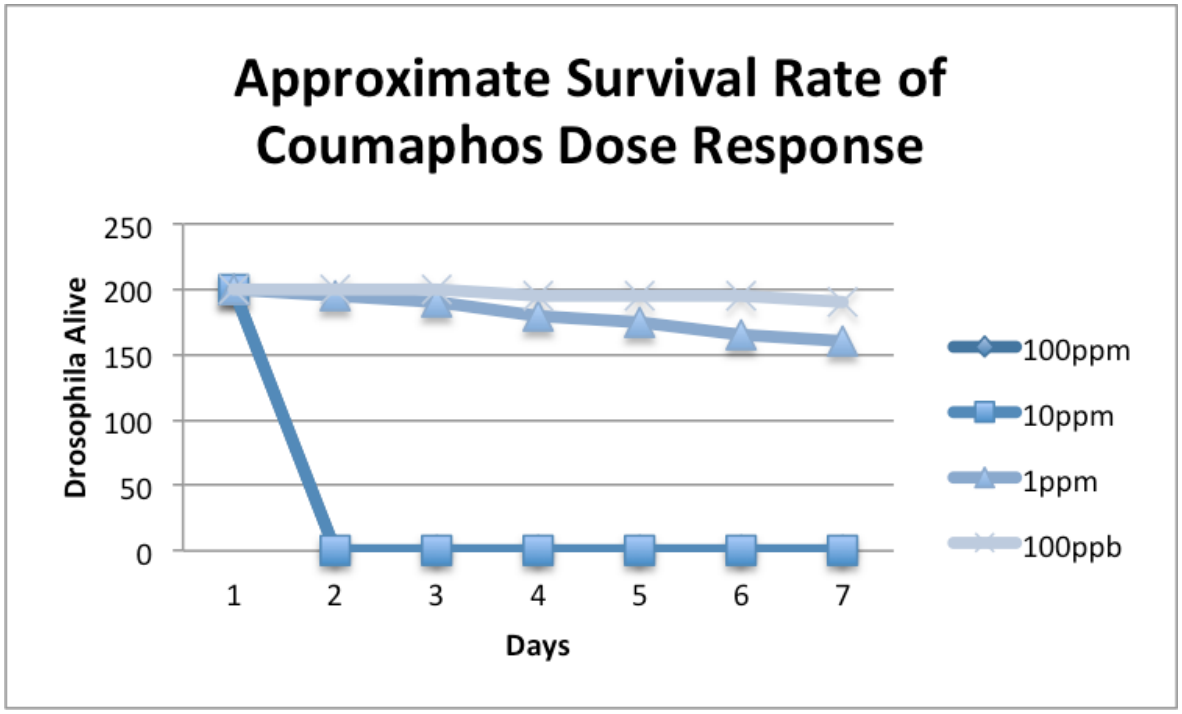


Table Two



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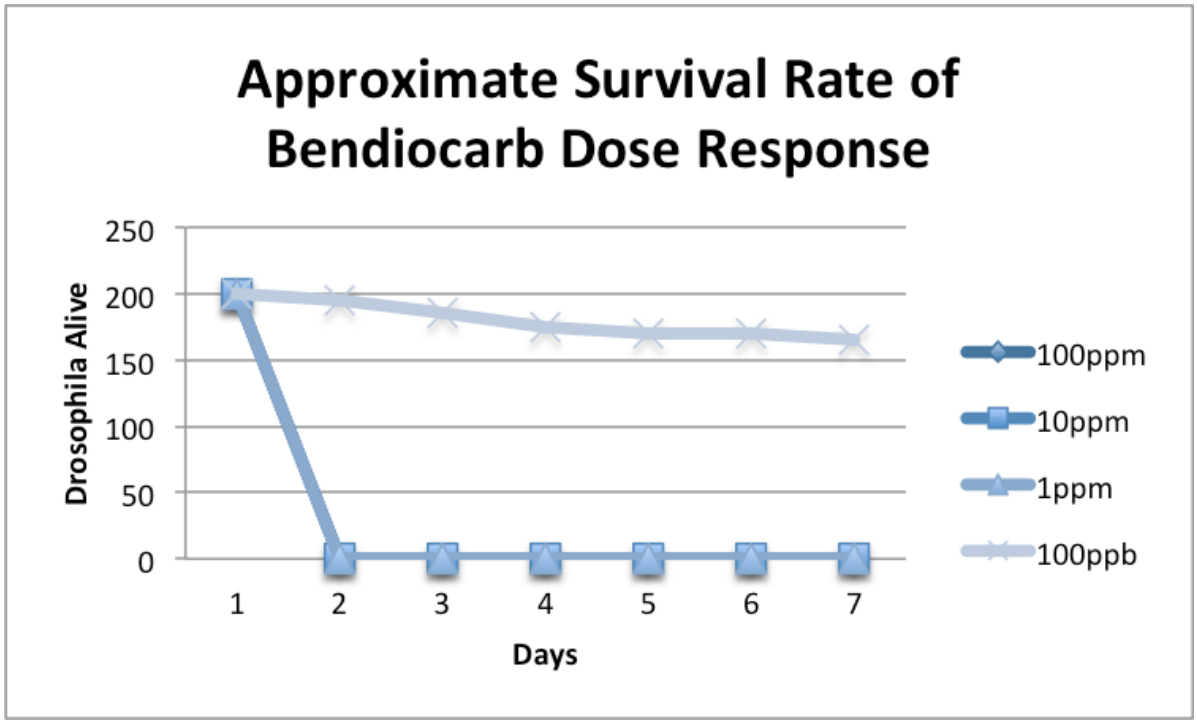

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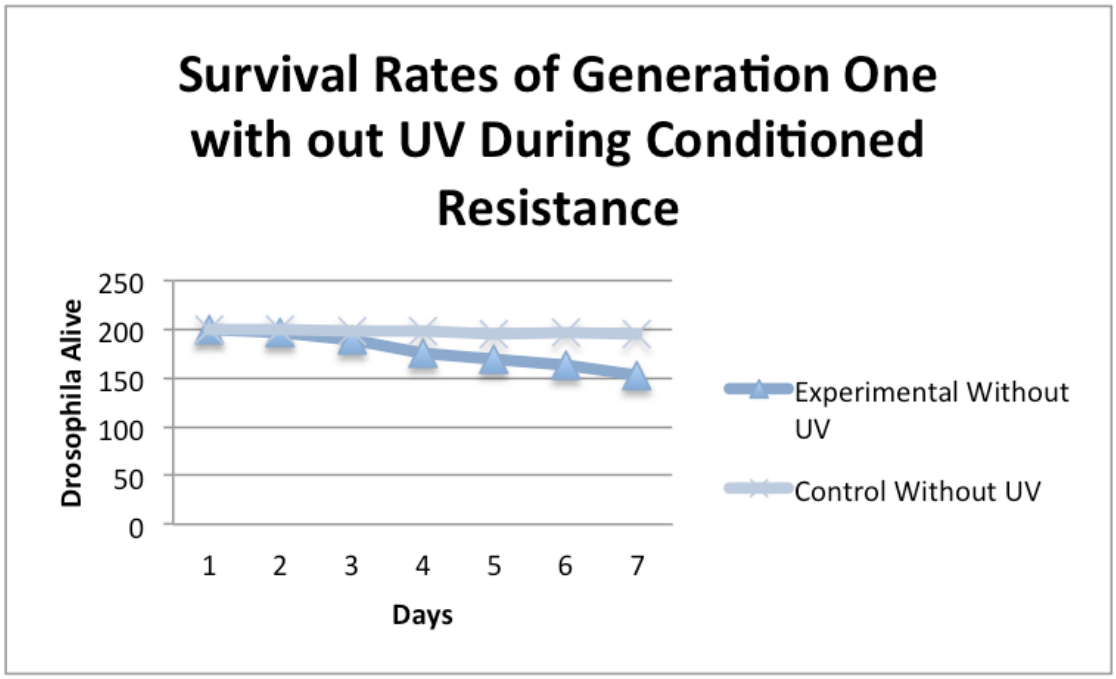

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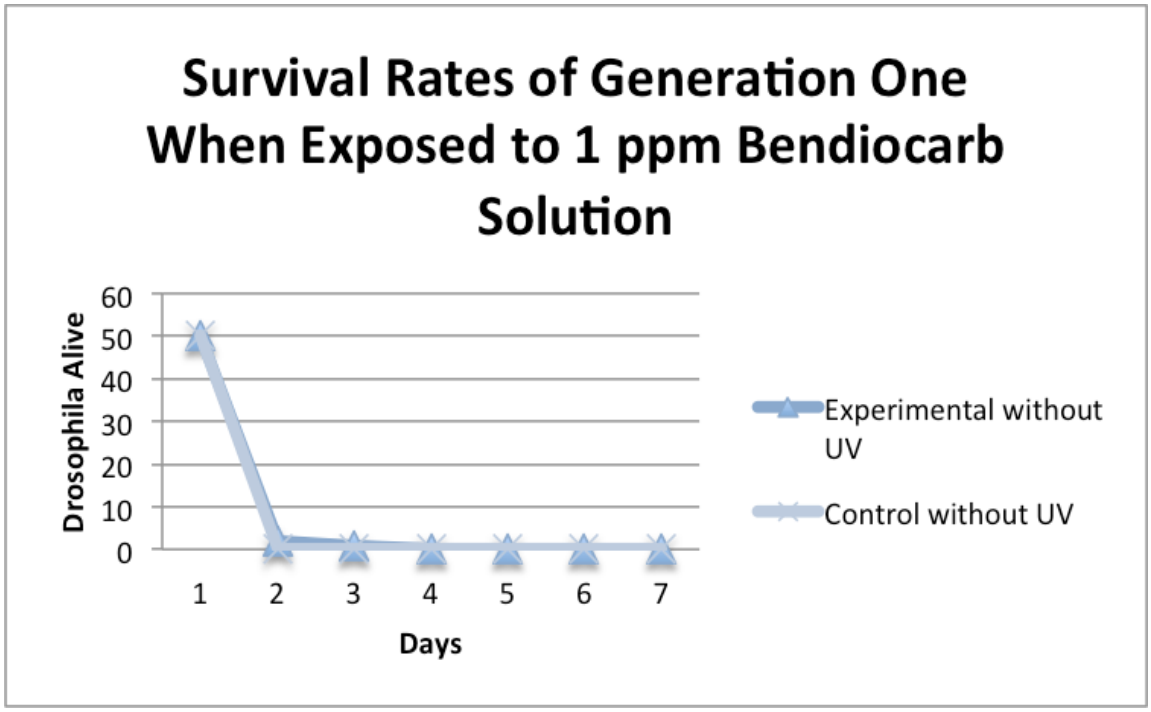


Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Survival Rate for Experimental without UV	**50**	**2**	**1**	**0**	**0**	**0**	**0**
Survival Rate for Control without UV	**50**	**0**	**0**	**0**	**0**	**0**	**0**

Table Six

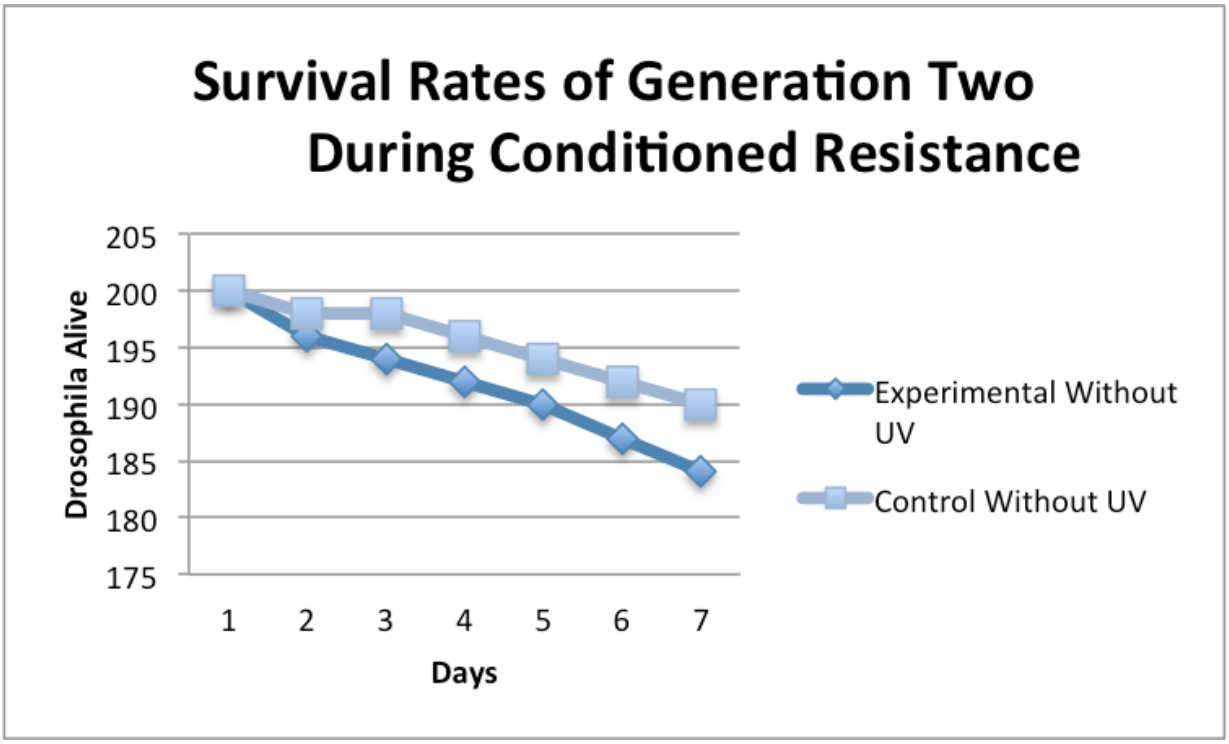
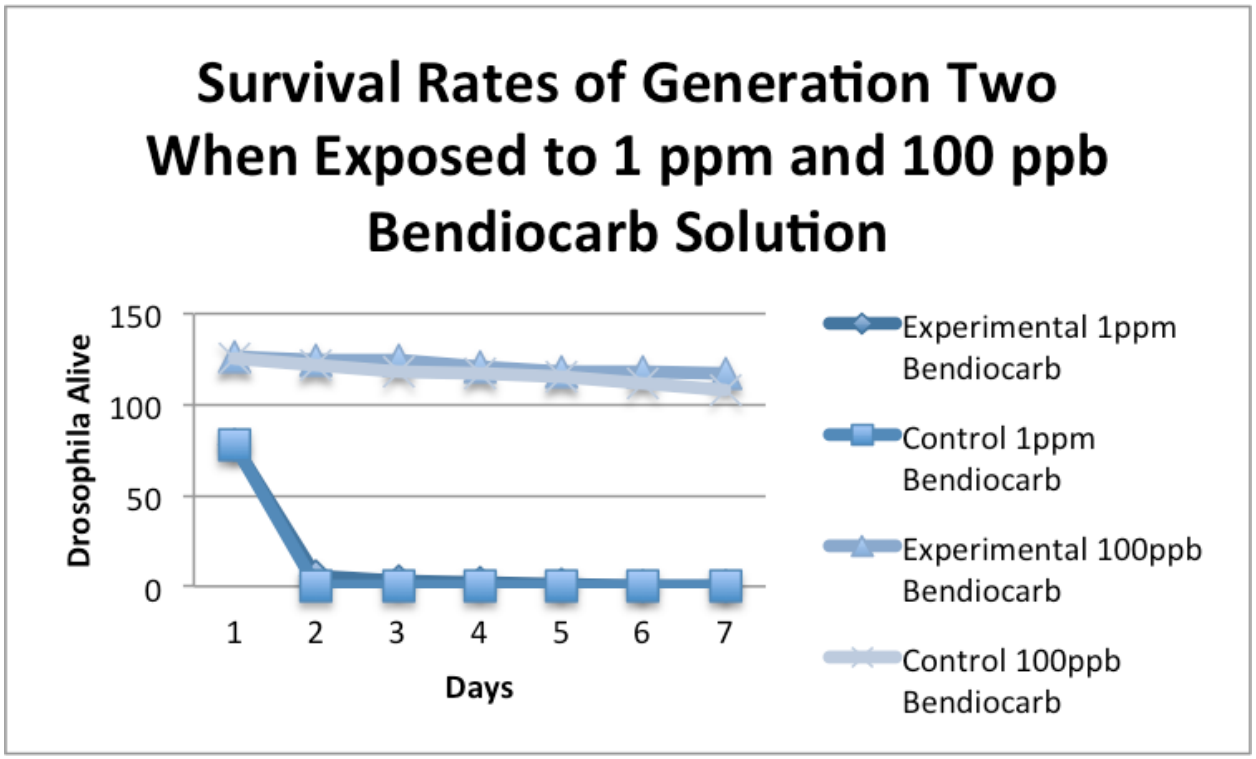


Table Seven



Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Survival rate for Experimental 1ppm Bendiocarb	**78**	**6**	**3**	**2**	**1**	**0**	**0**
Survival rate for Control 1 ppm Bendiocarb	**78**	**0**	**0**	**0**	**0**	**0**	**0**
Survival rate for Experimental 100 ppb	**126**	**124**	**124**	**121**	**118**	**118**	**117**

Bendiocarb							
Survival rate for Control 100 ppb Bendiocarb	**125**	**122**	**118**	**117**	**115**	**112**	**108**

There was a small increase in resistance levels from generation one, to generation two. This resistance increase was most apparent in the final test of 100 ppb as shown in figure 7.

The P values, found using a standard T-test, are as follows,

Generation One survival rates when exposed to 1 ppm *Bendiocarb* without UV: P=.97

Generation Two survival rates when exposed to 100 ppb *Bendiocarb*: P= .91

Generation Two survival rates when exposed to 1 ppm *Bendiocarb*: P= .11

Discussion:

Over the course of the study there was a small increase in insecticide resistance. The survival rate increased from a 2% survival rate when exposed to 1 ppm *Bendiocarb* in generation one, to a 3% survival rate when exposed to 1 ppm *Bendiocarb* in generation two. The most significant increase in resistance levels was present in generation two when exposed to 100 ppb *Bendiocarb*. What these results, as well as the wider range of studies show, is the nature of insecticide resistance. There are basically two types of resistance, a specific resistance to a single insecticide caused by a mutation in the activation site for the insecticide, and a generalized resistance due to increased levels of metabolic detoxification in the insects. While this type of resistance can normally be combated by increasing the dosage of the insecticide and

overwhelming the enzymes which break down the different toxins, or by switching insecticide class, as they usually have different enzymes responsible for detoxification. However, this is still only a temporary solution, and a substandard one at that. In both cases, more harmful chemicals are introduced to the environment, and the insects in question only gain more types of resistances to increasingly toxic chemicals. When it comes down to it we only really have one insecticide, a general pest killer which, if we continue to use to excess like we do now, will cease to work.

Conclusion:

Due to the high P values, the Null Hypothesis, which states that there is no statistical difference between the experimental and control groups, must be accepted. However, there is still a correlation between the translation of resistance between *Naled/Dibrom* and *Coumaphos* to *Bendiocarb* which should not be ignored due to the lack of statistical significance. As seen by Figure 7, there is a positive correlation which is most noticeable, and most significant with low levels of *Bendiocarb*. This correlation still, as shown by the P values, could be due sources of error. While this study does show promise, it is inconclusive to the extreme with almost contradictory results depending on generation and concentration.

Further Work:

The most important thing that can be done to extend the relevance, and confirm of deny the tentative results seen, is to extend the study length to the proposed 10 generations instead the two generations that are seen here. This would allow for a much greater level of resistance

which, when compared to the control in a T test could yield a higher lower P value. In addition, this research could also be applied to other fields of insecticide resistance and could possibly be tested in mosquitos, which was the overall purpose of the experiment in the first place.

Work could also be done in eliminating the sources of error, due to the unknown heredity of the *Drosophila* that were used, it was impossible to determine whether or not they were truely wild type. Error also was incurred while counting the *Drosophila*, as they were not knocked out with Carbon Dioxide gas.

After determining whether or not there is a transference in resistance, it would also be beneficial to look into specifically what mutation on either Ace-1 or Ace-2 gene allows for the widespread insecticide resistance to insecticides which act upon cholinesterase.

Acknowledgements:

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