A person smiling for the camera
Description automatically generated with low confidence

Student Picture

An Analysis of the Effects of Nicotine on Characteristics of *Drosophila melanogaster*

Madison Echols

Abstract

Currently, the world suffers from an epidemic: the epidemic of nicotine products. Using the *Drosophila melanogaster* as a model organism, a correlation between nicotine exposure and decreased longevity, impaired locomotive activity, phenotypic changes, and reduced mass was tested. Two Drosophila diets were used: a control diet using a standard medium and a nicotine diet. Three trials were tested, with four groups, one for each sex and type combination. This experiment ran for a week and a half. The data was analysed using a one-way ANOVA test. Significant data showed that the survival rate of nicotine-treated flies decreased at a higher rate than that of the control flies. In addition, the mass of the nicotine flies decreased compared to that of the control flies, and locomotive activity in female flies was impaired. Despite the female significance, the male negative geotaxis assay results were not significant, and correlation could not be determined. This study further demonstrates the correlation between behavior, longevity, locomotive ability, and nicotine exposure that is also shown in humans. Further experiments sequencing the flies' DNA and testing varying nicotine concentrations will lend to more clarity regarding the ramifications of nicotine products.

Introduction

Outside of the current COVID-19 pandemic, the world suffers from an epidemic of a different cause: tobacco products. According to the World Health Organization, the tobacco epidemic is one of the biggest public health threats the world has ever faced, killing more than eight million people a year around the world (WHO 2021). Over 80% of the 1.3 billion tobacco users worldwide live in low and middle-income countries; the burden of tobacco-related illness and death is the heaviest there (WHO 2021). Furthermore, the constant tobacco usage in these countries contributes to poverty by redirecting funds for survival and quality of life to tobacco. This tragedy has been exacerbated by tobacco companies, like British American Tobacco, pushing claims that nicotine offers substantial benefits and even protection against COVID-19. Additional research surrounding the health ramifications caused by nicotine exposure can be used to debunk claims driven by the tobacco industry, promote the need for more awareness in less developed countries, and ultimately mitigate the tobacco pandemic. This project aims to provide that research by analysing the effects of nicotine on the longevity, locomotive behavior, phenotype, and mass of *Drosophila melanogaster*. Fruit flies have high similarity to the human genome, with 60% of their genome corresponding (Mirzoyan, 2019). This, compounded with their short generational period and life cycle, low cost to maintain, and availability, make them effective models for this study.

Hypotheses

H_0: There is no correlation between nicotine exposure and decreased longevity, impaired locomotive activity, phenotypic changes, and decreased mass.

H_a: There is a correlation between nicotine exposure and decreased longevity, impaired locomotive activity, phenotypic changes, and decreased mass.

Methodology

Wild-Type *Drosophila melanogaster* were reared in a light-dark controlled Climatarium under standard conditions. The control flies were raised on a rehydrated potato flakes medium, and the nicotine flies were reared on the same ready-made medium. However, during the rehydration process, 300 µL of .1 M nicotine solution was mixed in with the 12 mL water and added to 4.4 g of dry medium.

Using Triethylamine to anesthetize the flies, they were first separated into males and females within one day of eclosion to ensure age synchronicity. Twenty-five flies were placed in each vial, with each trial having a male nicotine vial, male control vial, female nicotine vial, and female control vial. This was repeated three times, making 150 male and 150 female flies in total. Each fly sex remained separate throughout the experiment. Their phenotypes were observed before the experiment to ensure no perceivable mutations.

Figure 1: Negative Geotaxis Test tube made with 2 stacked culture vials.

The negative-geotaxis climbing assay was used to study the difference in locomotive behavior in nicotine-treated flies and control flies. The flies from the different food and sex vials were introduced into their respective negative geotaxis tubes one by one. The flies were observed climbing up the tube, and the number of flies reached the marked orange ring at 10 cm height within 10 sec. The percent climbing activity was calculated using the formula:

Text
Description automatically generated

Where E= Number of flies reaching 10 cm mark in 10 sec, and T= Total number of live flies in the culture tube.

Control and nicotine flies were collected separately, with 20 flies in each group, and weighed on an analytical balance at the end of the experiment to determine changes in adult weight. The value on the balance was then divided by 20 to find the average weight.

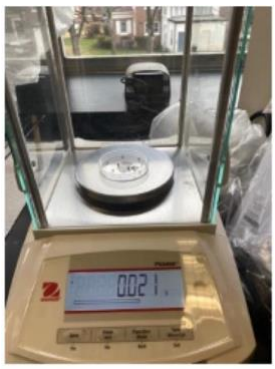


Figure 2: Analytical balance used to find the average weight of each of the fly types.

The number of dead flies was counted every day (not including skipping days from weekends) and used to calculate the percent survival of the flies.

The data was statistically tested using a one-way ANOVA test.

Results

Negative Geotaxis:

The f-ratio value of the percent of female flies that climbed past 10 cm is 6.232. The p-value is .03165. The results show significance at $p < .05$, showing a correlation between exposure to nicotine and an impaired locomotor ability. Regarding the male's Negative Geotaxis Test, though biological significance between impaired climbing ability and nicotine exposure was shown, the f-ratio value is 1.674. The p-value is 0.2100. The results are not significant at $p > .05$, meaning a conclusion of locomotor impairment could not be correlated with nicotine exposure.

Survival Rates:

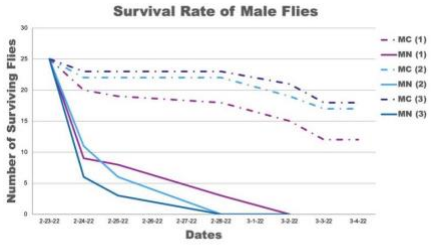


Figure 3: Effect of nicotine on the survival rate of male flies.

The survival rates of male nicotine treated flies were significantly less than that of the control flies. The f-ratio value is 45.25. The p-value is < .00001. The result is significant at $p < .05$. The same can be said about the female flies with the survival rates of nicotine treated flies were significantly less than that of the control flies.

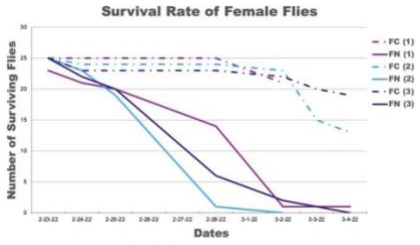
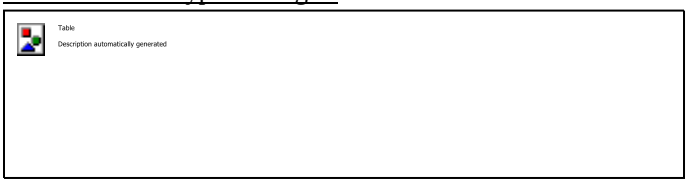


Figure 4: Effect of nicotine on the survival rate of female flies.

Mass and Phenotypic Changes:



The weighing of each fly group showed a significant difference between the control flies and the nicotine flies, with the weights decreasing in the nicotine flies.

The mass changes are further backed up by the phenotypic changes seen in the flies themselves. In both males and females, the nicotine-treated flies shown are smaller in mass than the control fly. The abdomens appear thinner as well as the heads.

Figure 6: Effect of nicotine on the phenotype of male flies.

Figure 7: Effect of nicotine on the phenotype of female flies.

Discussion

Significant data showed that the survival rate of nicotine-treated flies decreased at a higher rate than that of the control flies, the mass of the nicotine flies decreased compared to that of the control flies, and locomotor activity in female flies was impaired. Given the significance of the results, the nulls and the alternates were accepted for all but the locomotor activity. However, despite the female significance, the results of the male negative geotaxis assay were only biologically significant but not statistically significant, and therefore the null altogether was accepted.

This study further demonstrates the correlation between behavior, longevity, locomotor ability, and nicotine exposure that is also shown in humans. Further experiments sequencing the flies' DNA and testing varying nicotine concentrations will lend to more clarity regarding the ramifications of nicotine products.

Sustainable Goal Number Three

The Sustainable Goal of this project is Good Health and Well-Being, which promotes healthy living and well-being for all. By gaining further information on the potential effects of nicotine, this project could turn people away from using tobacco products, mitigating poverty through the reduced usage of nicotine, leading to a healthier world overall.

Future Research and Implications

There are future plans for research regarding the sequencing of DNA of the control flies and nicotine flies to understand better the genetic mutations of using nicotine products such as E-Cigarettes.

Reliable Equipment / More Experience

Due to inexperience, the flies were not accurately sorted, giving rise to pupae before the experiment was over, which led to limitations regarding the longevity assay. In addition, the use of non-standard medium and Triethylamine, which are not as reliable in anesthetization compared to CO_2, could have varied the results in the experiment. Going forward, the usage of materials standard to *D. melanogaster* experimentation will provide more accurate results.

References

Chandran, G. Negative geotaxis or climbing assay for assessment of locomotor function in adult *Drosophila* (2021). Experiments with *Drosophila* for Biology Courses. (Eds: S.C. Lakhotia & H.A. Ranganath). (pp. 471-475).

Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (2019). Drosophila melanogaster: A Model Organism to Study Cancer. Frontiers in genetics, 10, 51. https://doi.org/10.3389/fgene.2019.00051

Velazquez-Ulloa NA. (2017) A *Drosophila* model for developmental nicotine exposure. PLoS ONE 12(5): e0177710. https://doi.org/10.1371/ journal.pone.0177710

World Heath Organization (2021). Tobacco. Tobacco. (2021). Retrieved 12 February 2022, from https://www.who.int/news-room/fact-sheets/detail/tobacco.