Statistically Analyzing the Relationship of the Volume of the Goldenrod Gall with the Organism found in the Gall

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Abstract

When gall flies, *Eurosta solidaginis*, lay eggs in a goldenrod, the larvae tend to form a gall in the stem. Though, these galls can be invaded by three main parasitoids known as *Eurytoma obtusiventris, Eurytoma gigantea,* and *Mordellistena unicolor*. These species kill the gall fly in the gall, which decreases the survival rate of the gall flies. This experiment discusses the effect of the size of the gall on the survival rate of these gall flies. To do this, the measurements of the galls were taken to determine the volume of the galls using vernier calipers. These galls were later dissected using a utility knife and the contents of the galls were recorded. A normal distribution graph was made to compare the mean volumes for the population of gall fly vs. not gall fly, gigantea vs. not gigantes, obtusiventris vs. not obtusiventris, and beetle vs. not beetle in FATHOM. Then, the 95% confidence intervals were calculated to determine the preferred gall volume by each of the species. Lastly, a 2-sample T-test was performed to determine the p-value. If this value was below 5%, it can be concluded that the specific organism prefers a certain type of gall. The p-value for the gall fly vs. not gall fly comparison was 0.00000324, gigantea vs. not gigantea was 0.14, obtusiventris vs. not obtusiventris was 0.42, and beetle vs. not beetle was 0.037. Therefore, a valid conclusion was drawn that gall flies tended to survive more in larger galls, and beetles were more likely to invade smaller galls. The results for the gigantea and obtusiventris populations suggest that these species tended to invade galls of nearly any size.

Introduction

The gall fly, known as *Eurosta solidaginis,* tends to lay eggs in a plant known as the goldenrod, from the *Solidago* plant genus. The eggs hatch and slowly make their way into the stem of the goldenrod while the goldenrod is still growing. Then, the saliva of the larva copies a plant hormone known as the auxin, which causes the goldenrod to develop an increasingly large stem cell growth. This forms a gall around a single egg due to the chewing of the larva and abnormal growing pattern of the host stem. Galls can be any size and shape, but they generally tend to be round or in the shape of an ellipsoid. The larva creates a small chamber in the center of the gall and feeds off of the fibrous material in the gall. The larvae tend to reach their largest size in the fall, where the gall fly larvae tend to be white or a little yellow with an oval shape (Bhansali, 2021).

During this process, however, not all gall flies survive. Sometimes parasitoids and other predators tend to lay their eggs in the galls of goldenrods that have already been formed. These parasitoids tend to find the gall fly in the central chamber of the gall and consume them. It is evident that a parasitoid has affected the Gall fly larva located in the goldenrod when any other larva is found in the gall. The three main predators for the gall fly are: *Eurytoma obtusiventris, Eurytoma gigantea,* and *Mordellistena unicolor* (Abrahamson et al., 1989). *E. obtusiventris* larvae tend to be small and dark brown, *E. gigantea* larvae tend to be white and a little larger but with pointed ends (similar to a football), and *M. unicolor* beetles tend to be white and elongated. When *E. obtusiventris* lays its egg in an already-formed gall, they tend to produce a hormone which causes the gall fly to pupate early. This causes the larva of the Gall fly to enlarge and stops the Gall fly from consuming any more of the fibrous plant material located in the gall. Then, the *E. obtusiventris* larva tends to consume the remaining of the gall and occupy the gall fly's space.

On the other hand, the *E. gigantea* larvae tend to go into the gall and consume the gall fly as a whole. This causes the gall fly to die before it pupates. Lastly, the *M. unicolor* beetle tends to do a similar thing as the *E. gigantea* to occupy the gall and kill the gall fly. Though, *M. unicolor* tends to leave behind a sawdust-like material in the gall it occupies (Bailey et al., 2009). Being known to predatory activity, the abundance of gall flies have slowly been decreasing due to predators and parasitoids occupying galls for their own eggs.

Previously, research has been done on the relationship between the gall diameter compared to the type of organism found in the gall. Scientists have researched the effect of different locations and gall diameters on selection intensities of four different organisms: *E. obtusiventris, E. gigantea, M. unicolor,* and birds such as the *Dendrocopos pubescens*. To do this, they first measured and recorded the diameter of the gall and then they dissected the gall to classify the predatory species located in the gall if not a gall fly larva. An assumption was made where these galls were completely spherical, therefore only one dimension was measured. They concluded that *E. obtusiventris* and *E. gigantea* larvae occupied galls with a larger-than-average diameter. Not only that, these researchers observed that the *M. unicolor* beetle was able to be a predator for all four locations under any circumstances (László et al., 2019). The students of the Academies of Loudoun are planning to take a different approach to investigate the survival of gall flies in correlation to the size of the gall. To do this, the diameters of all three dimensions would be measured instead of one because not all galls are perfectly spherical. This is different from previous research because previously they only measured one diameter to determine the size of the gall. Additionally, the effect of parasitoids that can be found inside the gall would only be observed. For instance, the effect of birds on gall fly survival will not be discussed. Additionally, another approach for the analysis of the data will be taken. Using a normal distribution graph may help us determine the relationship between the mean gall volume for survival of gall flies compared to the mean volume of galls where the gall flies did not survive.

The purpose of this research is to determine the ideal gall size for the survival of the gall fly larvae. Based on previous research, a hypothesis was generated: If the volume of the galls increase, then there is a higher chance for a gall fly larva to be present because larger galls provide more protection, thus making it harder for predatory species to invade the gall. To conduct this experiment, the minimum diameter, maximum diameter, and height of the galls would be measured in centimeters (cm), which is the independent variable. This would help calculate the volume of the gall in centimeters cubed (cm^3). Then, the galls will be cut in half to observe its contents, which is the dependent variable. Throughout this experiment, the time of the year these goldenrod galls were harvested and the materials used were held constant. Additionally, there was no given control group for this experiment. The observations of this experiment were then recorded in a data table, from which valid conclusions were drawn to accept or reject the hypothesis.

Materials and Methods

For this experiment, goldenrod galls were collected from four different locations located in Allenwood, PA; Trout Run, PA; and two different locations in Walworth, NY. They harvested 401 galls and returned them to the Academies of Loudoun. In addition to these galls, vernier calipers, goggles, utility knives, Excel, FATHOM, and INSTAT were used in the process of this experiment. Then, excess stems of the galls were cut so the gall is the only thing that remained. The vernier calipers were then used to take precise measurements of the smallest diameter, largest diameter and height of the galls, which was the independent variable. These measurements were recorded in centimeters (cm) in Table 1 below. Then, the utility knife was used to cut the galls in half, though it was important not to cut through the gall because it would damage the contents inside the central chamber. During this process, adult supervision must be maintained due to the risk associated with sharp objects. Goggles must be worn during this time due to the risk of possible debris getting into the eyes. After this, the contents of the galls were classified using a classification key, which was the dependent variable. If the gall was empty but a sawdust like material was observed, it was classified as a beetle larva. Though, if the gall was empty with no signs of the existence of another organism, then the contents of the gall would be classified as unknown.

After collecting quantitative measurements for all three diameters, the volume formula for an ellipsoid was derived. This formula was then used to calculate the volume of the galls in cubic centimeters (cm^3) and recorded in Table 2. Then the volumes for the galls containing each of the organisms were classified in the following manner: gall fly vs. not gall fly, gigantea vs. not gigantea, obtusiventris vs. not obtusiventris, and then beetle vs. not beetle. For the sorting of these groups, the unknown galls were only located in the not gall fly population because any of the other predatory species could have occupied the gall, but it was certainly not a gall fly. Then, using Excel, the mean, standard deviation, and standard error were calculated for each of these populations and recorded in Table 3. Using the mean and standard error values calculated, normal distribution graphs for each of the populations were generated using FATHOM. Then, the 95% confidence intervals were calculated and plotted onto each of the fathom graphs (Graphs 1, 2, 3, and 4). Lastly, INSTAT was used to conduct a 2-sample T-test for the four comparisons. To do this, the number of galls, mean volumes, and the standard deviation were used. The p-value and degrees of freedom were determined and recorded in Table 3, which later contributed to the results of this experiment.

Data

Effect of the Volume of the Goldenrod Gall on the Contents of the Gall

Sample Raw Data				
	Independent Variable			Dependent Variable
Trial (#)	Minimum Diameter "D_{min}" (cm)	Maximum Diameter "D_{max}" (cm)	Height "H" (cm)	Contents
1	2.4	2.48	3.12	Mordellistena Beetle
2	2.53	2.78	2.91	Gall Fly
3	2.45	2.59	2.99	Gall Fly
4	2.09	2.11	2.59	Mordellistena Beetle
5	1	1.21	1.58	Mordellistena Beetle
6	1.51	1.65	2.11	Obtusiventris
7	1.63	1.72	2.73	Gall Fly
8	2.36	2.44	3.53	Gall Fly
9	1.63	1.92	2.15	Gigantea
10	1.45	1.65	2.14	Obtusiventris
11	0.82	0.83	1.07	Obtusiventris
12	1	1.13	2.78	Mordellistena Beetle

Table 1: The data table above represents the minimum diameter, maximum diameter, and height of the 12 sample galls measured in centimeters (cm) for one group. The organism found in the goldenrod gall was also recorded in the last column.

Results

After recording the measurements of the minimum diameter, maximum diameter, and height of the galls in Table 1 above, the volumes were calculated using Equation 1. Then, these values were recorded in Table 2 below. This would determine the size of the galls, which would then help determine the correlation between the size of the gall and gall fly survival.

Volume Equation

$$v = \frac{1}{6} \cdot \pi \cdot D_{\text{min}} \cdot D_{\text{max}} \cdot H$$

Equation 1: The equation above was used to find the volume from the measured minimum diameter, maximum diameter, and height from Table 1.

Volume of the Galls from Table 1

Sample Theoretical Data	
Trial (#)	Volume "v" (cm^3)
1	9.72
2	10.71
3	9.93
4	5.98
5	1.0005
6	2.75
7	4.006
8	10.64
9	3.52
10	2.68
11	0.38
12	1.64

Table 2: The above table represents the volume of each of the 12 sample galls in Table 1, calculated in cm^3.

After calculating the volumes of all 401 galls from the four locations, they were put into Excel. The mean, standard deviation, and standard error were calculated and recorded in Table 3 below. After this, the 95% confidence intervals were calculated using Equation 2. Out of the 2 values calculated, the lower value would be the lower 95% confidence interval and the larger value would be the higher 95% confidence interval. Both of these values are recorded in Table 3 below. The significance of the 95% confidence interval is to determine the range in which the true mean gall volumes are located for the true gall population while being 95% sure.

Confidence Interval Equation

$$CI = \overline{x} \pm 1.96\,(SE)$$

Equation 2: The equation above was used to determine the Confidence Interval for each of the populations using the mean and Standard Error. The Confidence Intervals were calculated and recorded in Table 3 and Graph 1-4.

After calculating the confidence intervals, a 2-sample T-test was performed in INSTAT using the number of galls, mean, and standard deviation of the following populations: gall fly vs. not gall fly, gigantea vs. not gigantes, obtusiventris vs. not obtusiventris, and beetle vs. not beetle. This gave us the degrees of freedom and p-value. The degrees of freedom is significant because it helps graph a curve for the normal distribution. Additionally, the p-value was calculated to check if the true mean volumes for each of the compared populations were truly equivalent. If the true means were significantly different, the p-value would be less than 5%, which is the universal significance level for the T-test. The difference of means would determine if gall flies prefer larger gall volumes compared to smaller ones. These values are located in Table 3 below:

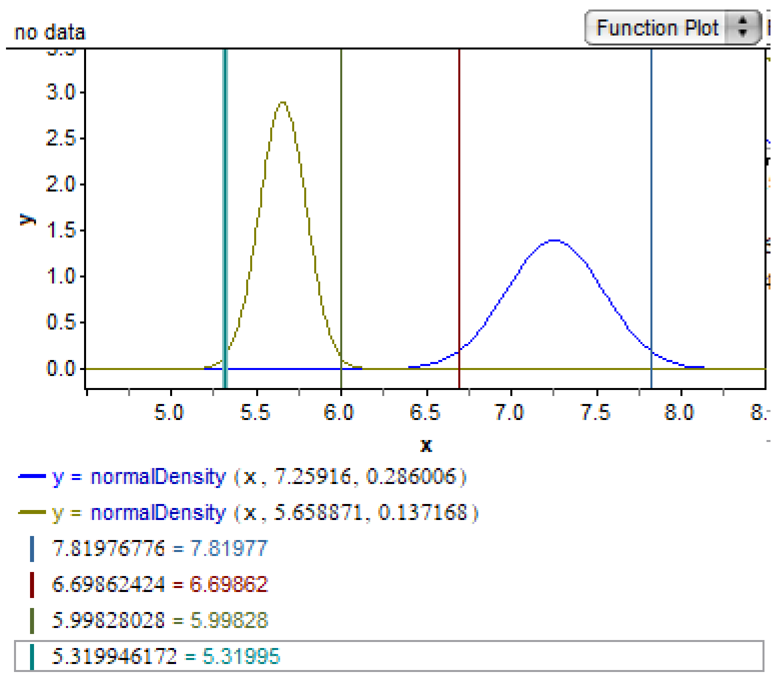
Statistical Analysis of the Different Sample Populations

Statistical Theoretical Data								
Contents	Number of Galls "n"	Mean "m" (cm)	Standard Deviation "SD" (cm)	Standard Error "SE" (cm)	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Degrees of Freedom	P-value
Gall Fly	117	7.26	3.09	0.29	6.699	7.82		
Not Gall Fly	284	5.66	2.92	0.17	5.32	5.998	205.33	3.24E-06
Gigantea	34	5.67	3.02	0.52	4.65	6.68		
Not Gigantea	306	6.49	3.09	0.18	6.14	6.83	41.03	0.14
Obtusiventris	76	6.18	2.58	0.296	5.603	6.76		
Not Obstusiventris	264	6.47	3.22	0.198	6.08	6.86	148.64	0.42
Beetle	113	5.89	3.25	0.31	5.29	6.49		
Not Beetle	227	6.66	2.98	0.198	6.27	7.05	207.47	0.037

Table 3: The above data table represents data for all 401 galls collected for the students of the Academies of Science. The mean, standard deviation, and standard error for all of the populations are calculated using Excel, and were measured in centimeters (cm). Then, a two-sample T-test was performed using INSTAT for each of the compared populations to determine the degrees of freedom, confidence intervals, and p-value. The established significance level for these T-tests was 0.05, therefore if the p-value is below that, then there is a significant difference in the true mean volumes for the two compared sample populations.

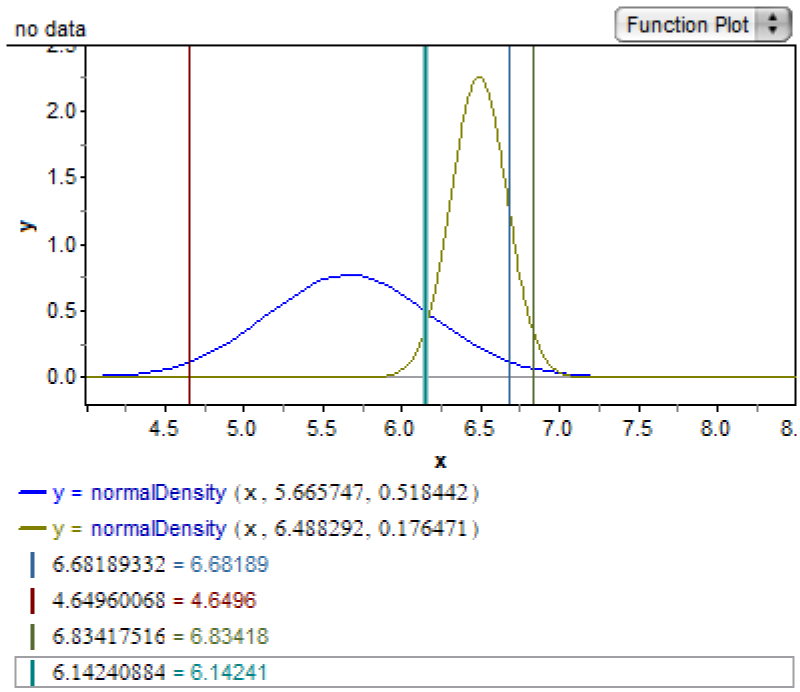
Using FATHOM, the normal distribution curves for the four compared populations. To graph this, the equation normalDenisty(x, mean, standard error) was used. Then, the 95% confidence intervals recorded in Table 3 were graphed, as represented in the Graphs below. The gall fly vs. not gall fly population normal distribution is represented in Graph 1, gigantea vs. not gigantea in Graph 2, obtusiventris vs. not obtusiventris in Graph 3, and beetle vs. not beetle in Graph 4. In each of the graphs below, the x-axis represents the volume of the galls in cm^3, and the y-axis represents the population density.

Normal Distribution Graph for Gall Fly vs. Not Gall Fly



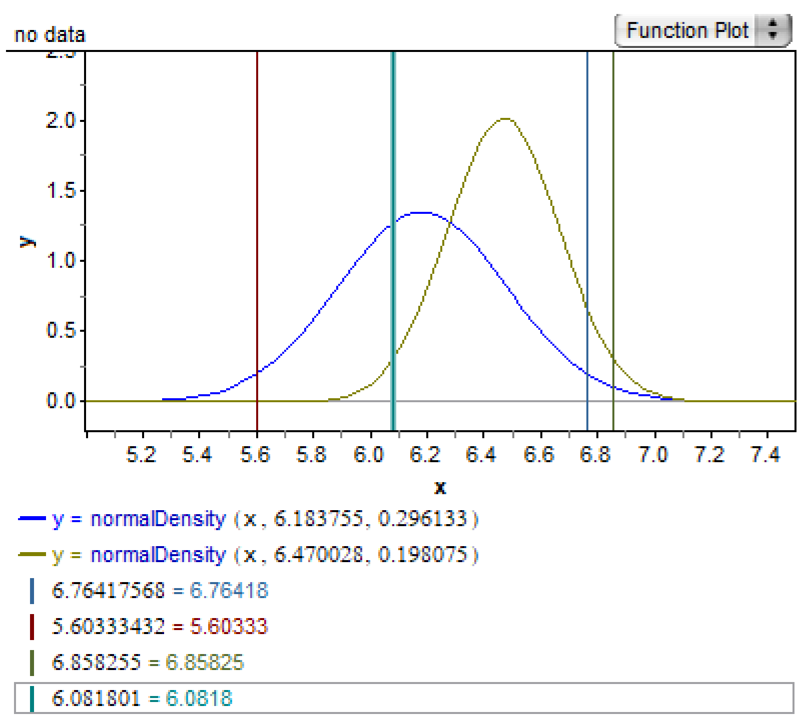
Graph 1: In the above graph, the blue curve represents the normal distribution for the Gall Fly population and the yellow curve represents the normal distribution for the Not Gall Fly population. The vertical lines represent the upper and lower 95% confidence intervals for both populations from Table 3.

Normal Distribution Graph for Gigantea vs. Not Gigantea



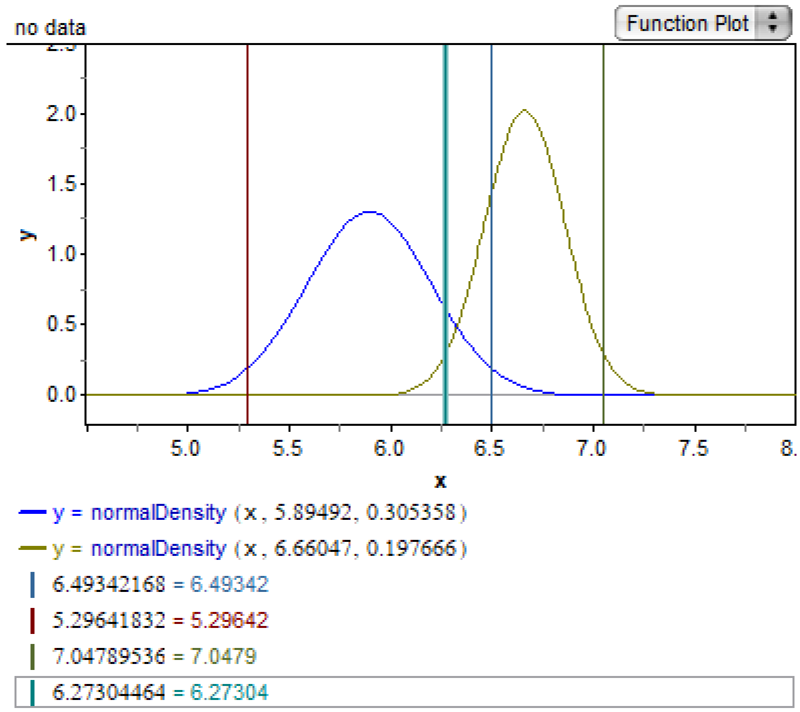
Graph 2: In the above graph, the blue curve represents the normal distribution for the Gigantea population and the yellow curve represents the normal distribution for the Not Gigantea population. The vertical lines represent the upper and lower 95% confidence intervals for both populations from Table 3.

Normal Distribution Graph for Obtusiventris vs. Not Obtusiventris



Graph 3: In the above graph, the blue curve represents the normal distribution for the Obtusiventris population and the yellow curve represents the normal distribution for the Not Obtusiventris population. The vertical lines represent the upper and lower 95% confidence intervals for both populations from Table 3.

Normal Distribution Graph for Mordellistena Beetle vs. Not Mordellistena Beetle



Graph 4: In the above graph, the blue curve represents the normal distribution for the Beetle population and the yellow curve represents the normal distribution for the Not Beetle population. The vertical lines represent the upper and lower 95% confidence intervals for both populations from Table 3.

Discussion

Based on Table 3, there is enough evidence to support a difference in the mean gall volumes for the gall fly and not gall fly populations since the p-value is less than the established 0.05 significance level. If there were no difference in the true population means for the gall Fly and not gall fly population, there would be only a 0.000324% probability of getting a difference of mean gall volumes by sampling variability alone. Therefore, sufficient evidence from the sample data that was collected to conclude that there is a statistically significant difference in the true mean gall volumes of the populations, where the mean volumes of galls for the survival of gall flies are greater than the mean volumes of survival for any other species. According to

Graph 1, there is 95% confidence that the true mean gall volume for gall fly survival is between 6.699 cm^3 and 7.82 cm^3. There is also 95% confidence that the true mean gall volumes of the survival of organisms that are not gall flies is between 5.32 cm^3 and 5.998 cm^3. Since the confidence intervals do not overlap, enough evidence was collected from the sample data to conclude that the mean gall volumes in which gall flies survive are significantly higher than the mean gall volumes in which they do not survive.

Additionally, based on Table 3 there is insufficient evidence to support a difference in the mean gall volumes for the gigantea and not gigantea populations since the p-value is greater than the established 0.05 significance level. If there were no difference in the true population means for the gigantea and not gigantea population, there would be a 14% probability of getting a difference of mean gall volumes by sampling variability alone. Therefore, sufficient evidence from the sample data that was collected to conclude that there is not a statistically significant difference in the true mean gall volumes of the populations for the survival of gigantea. According to Graph 2, there is 95% confidence that the true mean gall volume for gigantea survival is between 4.65 cm^3 and 6.68 cm^3. There is also 95% confidence that the true mean gall volumes of the survival of organisms that are not gigantea is between 6.14 cm^3 and 6.83 cm^3. Since the confidence intervals overlap, there is not enough evidence to conclude a significant difference in the gall volumes.

Furthermore, based on Table 3, there is not enough evidence to support a difference in the mean gall volumes for the obtusiventris and not obtusiventris populations since the p-value is greater than the established 0.05 significance level. If there were no difference in the true population means for the obtusiventris and not obtusiventris population, there would be a 42% probability of getting a difference of mean gall volumes by sampling variability alone. Therefore, sufficient evidence from the sample data that was collected to conclude that there is not a statistically significant difference in the true mean gall volumes of the populations for the survival of obtusiventris. According to Graph 3, there is 95% confidence that the true mean gall volume for obtusiventris survival is between 5.603 cm^3 and 6.76 cm^3. There is also 95% confidence that the true mean gall volumes of the survival of organisms that are not obtusiventris is between 6.08 cm^3 and 6.86 cm^3. Since the confidence intervals overlap, there is not enough evidence to conclude a significant difference in the gall volumes.

Lastly, based on Table 3, there is enough evidence to support a difference in the mean gall volumes for the beetle and not beetle populations since the p-value is less than the established 0.05 significance level. If there were no difference in the true population means for the beetle and not beetle population, there would be only a 3.7% probability of getting a difference of mean gall volumes by sampling variability alone. Therefore, sufficient evidence from the sample data that was collected to conclude that there is a statistically significant difference in the true mean gall volumes of the populations, where the mean volumes of galls for the survival of beetles is less than the mean volumes of survival for any other species. According to Graph 4, there is 95% confidence that the true mean gall volume for beetle survival is between 5.29 cm^3 and 6.49 cm^3. There is also 95% confidence that the true mean gall volume of the survival of organisms that are not beetles is between 6.27 cm^3 and 7.05 cm^3. Since the confidence intervals only overlap slightly, there is evidence from the sample data to conclude that the mean gall volumes in which beetles survive are significantly lower than the mean gall volumes in which they do not survive.

Though, assumptions and errors must be accounted for. To perform a 2-sample t-test, four main assumptions were made: the sample galls taken were random, independent, normally distributed, and less than 10% of the whole population. Additional error includes instrumental error because some students may have not taken measurements from the vernier calipers properly. Also, students may have rounded their values to different decimal places, causing inaccurate and inconsistent results. Another source of error is the location these galls were harvested from. Four main samples were taken in the collection of all 401 galls, and the predatory species in all four locations may have been different. This may cause certain locations to have a greater gall fly survival rate than other locations, providing inaccurate results. As observed in Graph 4, the confidence intervals for the beetle and not beetle populations overlapped slightly, even though the p-value is less than 0.05. This may have occurred because of these errors. To further improve this experiment, all samples should be taken from the same location. Additionally, more precise instruments could be used to measure the diameter of the galls because the vernier calipers used a separate scale to find the decimal place the diameter rounds to. Additionally, all groups can round their values to three significant figures to maintain consistency with the measurements.

In conclusion, gall flies tend to prefer larger galls compared to smaller ones, therefore the hypothesis formulated is correct. Additionally, *E. gigantea* and *E. obtusiventris* did not prefer a specific gall size for laying eggs. However, *M. unicolor* beetles tend to prefer smaller galls, which supports the hypothesis. Since they choose smaller galls, gall flies are more likely to survive in larger galls due to less predatory species invading them. Therefore, larger galls tend to be the ideal gall size for gall fly survival. This is supported by previous research because previously, gall flies tended to occupy galls with a larger diameter (Abranhamson, 1989). Since a larger diameter generally correlates with a larger volume, this experiment supports

Abrahamson's findings. In the future, the conclusions from this experiment may be used to find further relationships between preferred gall size and the survival rate of these gall flies.

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