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Examination of the Effect of Plant Induced Defenses on Cannibalistic Consumption and Baculovirus Transmission Amongst Spodoptera frugiperda Larvae

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Examination of the Effect of Plant Induced Defenses on Cannibalistic Consumption and
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Abstract

Spodoptera frugiperda (J.E. Smith, 1797) (Lepidoptera: Noctuidae), commonly known as the fall armyworm, is a devastating insect pest with a host plant diet breadth that includes many agriculturally important field crops. Fortunately, fall armyworms are frequently infected with a species-specific lethal virus, *Sf*MNPV (*Spodoptera frugiperda* multiplenucleopolyhedrovirus), which can regulate their population in the environment. Fall armyworms in their intermediate to late larval stages also become cannibalistic. While cannibalism can result in the cannibal becoming infected when consuming an infected conspecific, cannibalism, counterintuitively, is predicted to lower viral spread since transmission between infected individuals and cannibalism is imperfect. The rate of cannibalism is also influenced by food quality, with poorer quality host food plants increasing the rate of cannibalism compared to better quality ones. How cannibalism and host food plant quality influence viral transmission though has not been empirically tested. Thus, to fill this knowledge gap, I examined how cannibalism varies due to differential nutritional food quality and, in turn, affects viral transmission. I first utilized food resources varying in nutritional quality (soybean leaves, artificial diet, and artificial diet supplemented with 2% protease inhibitors, a common plant induced defense) to examine their effect on cannibalism rate. I then explored how these nutritionally different food resources change the rate of cannibalism and *Sf*MNPV viral transmission among fall armyworm larvae. My research ultimately found that a diet composed of non-induced soybean leaves resulted in higher rates of cannibalism when larvae victims were not infected with baculovirus, in comparison to an artificial diet and induced soybean leaves. However, when larval victims are infected, cannibalism rates across all food types remained similar in the rate and total amount of victims consumed. Furthermore, when victims are infected, the number of victims consumed has a significant impact on cannibal mortality due to viral infection. Specifically, an increased

consumption of infected victims significantly effects the cannibal by increasing cannibal infection and mortality. I also determined that consumption of a chemically induced substrate exhibits a strong positive trend of influence on cannibal mortality due to virus.

Introduction

Spodoptera frugiperda, commonly known as the fall armyworm, is a new world species belonging to the order Lepidoptera in the family Noctuidae. *S. frugiperda* primarily exists in the Southern and Midwestern regions of the United States, reaching areas as far west as the Rocky Mountains and as far east as the Atlantic Coast. This insect can also be found in South America, Asia, and Africa (Fan et al 2020). In North America, the insect overwinters in the warm southern states of the U.S., so as to avoid the harsh climate of the north. The pupae, which overwinter in the soil, cannot survive temperatures below freezing. In terms of its ecological significance, the fall armyworm is a known agricultural pest that consumes crops such as cotton, soybeans, corn, and sorghum (Murúa et al 2009). It is found throughout Louisiana and thus is relevant to the health and maintenance of the state's agricultural economy. After the third larval instar, *S. frugiperda* readily become cannibalistic and aggressive towards conspecifics.

Cannibalism is a type of intra-specific predation that occurs frequently in nature, involving the consumption of portions or the entirety of the body of a victim by the cannibal, before the death of the victim occurs (Fox 1975). In relation to the fall armyworm, it is important to note that its cannibalistic behavior is not universal across developmental instars. Cannibalism in the fall armyworm can be stage-structured— both likelihood of cannibalization of conspecifics and likelihood of victimization are reliant on the age of the insect, with younger insects being

more prone to victimization due to their smaller size, and larger insects more likely to be cannibals. As the difference in instar between cannibal and victim widens, the likelihood that a cannibalistic event will occur drastically increases. For example, while larvae of the same instar are faced with a 50% chance of being cannibalized by one another, this likelihood substantially increases as the age gap between cannibal and victim broadens (Van Allen et al 2017).

The fall armyworm is susceptible to a species-specific baculovirus, the *Spodoptera frugiperda* multiplenucleopolyhedrovirus (*Sf*MNPV). *Sf*MNPV is one of the most common pathogens that circulates within their natural populations (Gardner and Fuxa 1980). *Sf*MNPV infection causes prolific viral reproduction within the host. Eventually, the virus completely liquifies the infected insect causing the insect to rupture on the leaf tissue that it is currently feeding on. Ingestion of leaf tissue that has been contaminated with liquified remains of an infected insect by a new uninfected host can serve as a route of transmission of the virus (Valicente et al 2013). Additionally, viral infection substantially slows/stops insect development, making infected larvae smaller and weaker than healthy conspecifics of their same age. Cannibalism as a potential route of viral transmission has been examined (Van Allen et al. 2017; Chapman et al. 1999)—however, studies on how resource quality influences cannibalism rates have not yet, to my knowledge, been scientifically explored.

Differences in development are not the only driver of cannibalism among fall armyworms. Resource availability and quality is also a factor that impacts the rate of cannibalism (Fox 1975). Specifically for resource quality, experiments examining the relationship between availability of corn and red kidney bean plant foliage showed that percent cannibalism was approximately double for fall armyworm larvae reared on bean foliage than the corn foliage (38.6% vs 17.7%). It was also shown that the percent foliar consumption for the

bean plant was nearly quadruple that of the corn. Furthermore, in a two-choice preference test where insects were individually offered both corn and bean foliage, the majority of larvae exhibited a strong preference for corn foliage. This research demonstrates that this difference in cannibalism rates was likely attributable to plant resource quality (as the chemical composition of the foliage indeed differed) and insect preference (Raffa 1987).

Methyl-jasmonate (MeJA), a phytohormone that is important in signaling wound responses in plants, can be exogenously applied on plants in an effort to chemically increase their induced defenses against herbivory. While defenses as the result of MeJA induction peak at three days post-induction and decline thereafter, defenses are also induced 24-hours post induction, as is done in my study (Underwood 1998). It should be mentioned though that the timeline for induction is species-specific and even genetically-specific (Shikano et al 2017). One benefit of induction— while it is physiologically costly to produce defenses— is that the insect herbivore feeding on the plant declines in development and potentially likelihood of survival, thus resulting in decreased foliar consumption and increasing fitness of the defending plant (Accamando and Cronin 2012). Additionally, plant induced defenses result in decreased plant consumption and increased cannibalism, and individuals are more likely to resort to cannibalism earlier as the plant food source becomes increasingly defended (Orrock et al 2017).

One well-studied portion of plant chemical defenses are protease inhibitors (PIs), which are competitive inhibitors of proteases. Upon damage to a plant such as soybeans, chemical defenses including protease inhibitors greatly increase. As was discussed by Underwood et al (2002), research supports the idea that protease inhibitors are involved in plant defense against Lepidopterans in the environment. Protease inhibitors interfere with the digestive enzymes of the insect, thus impeding the insect's protein digestion and lowering leaf area consumed from the

defending plant. While Underwood et al (2002) states that cysteine protease inhibitors have not been shown to interfere with lepidopteran digestion, soybeans contain a variety of other protease inhibitors, including trypsin and serine protease inhibitors. Some lepidopteran larvae can shift their production of digestive enzymes in response to harmful protease inhibitors circumnavigating this defense, instead producing enzymes that are not affected by the imposed inhibition (Broadway 1995). In addition to protease inhibitors, other defense molecules that result from induction include cyanogenic glycosides, alkaloids, and peroxidases (Mai et al 2016; Peruca et al 2018).

Methyl-jasmonate has been shown to induce high levels of both cysteine and aspartic protease inhibitors, which can affect insect physiology (Bolter and Jongsma 1995). One example of the armyworm's physiological response to induction is reduced mortality by virus, in some cases. For example, research has shown that consumption of jasmonic acid-induced soybean foliage infected with baculovirus results in a decline in mortality, but that this again varied by plant genotype. The same research also showed that for soybean genotypes that are strongly defended upon JA-induction, baculovirus effectiveness was not impacted (Shikano et al 2017). This is further supported by research that shows that while direct benefits of chemical induction do occur (such as a decline in the amount of foliage consumed by the insect herbivore, as was mentioned earlier), one indirect cost of induction is a decrease in insect mortality due to baculovirus when both armyworm and baculovirus are present in high densities (Elderd 2019). In terms of the effect of induction on cannibalistic behavior, these induced defenses result in a decrease in nutritional uptake for the affected insect herbivore, resulting in more food being required to meet the organism's physiological needs. Thus, this makes the insect more prone to searching for nutrition from other, non-toxic food sources. Among insect herbivores, cannibalism

occurs at higher rates when the individuals are nutritionally stressed (Al-Zubaidi and Capinera 1983). Cannibalism as a result of plant induced defenses has also been studied in relation to disease (specifically viral) transmission between cannibal and victims (Elderd 2019).

Considering that resource quality affects cannibalism rates among insect herbivores, this thus provides support for my examination of manipulation of chemical resource quality via protease inhibitors and induced plant defenses. Additionally, pre-existing research also posits that increases in cannibalism, as typically result from increased resource toxicity or decreased resource quality, might serve as a route of viral transmission. Differing resource quality may play a role in insect viral transmission, although this has not yet been empirically tested. The aim of this study is to elucidate the effects of resource quality on subsequent viral transmission as a result of varying levels of cannibalization using 3rd and 4th instar fall armyworm larvae. I predict that a decrease in resource quality through the addition of protease inhibitors will increase cannibalism rates amongst *S. frugiperda* larvae. I also predict that cannibalism rates will also be higher when the food source is chemically induced with MeJA, and that these increased rates of cannibalism will result in increased death from the baculovirus for the cannibal when the cannibal feeds on infected conspecifics.

Methods and Materials

Experiment 1: Determining the Effective Protease Inhibitor Concentration

S. frugiperda eggs were obtained from Benzon Research Inc. located in Carlisle, Pennsylvania USA. Upon arrival, the eggs were placed in an incubator at 28°C 16 hour day/ 8 hour night until they hatched. Following hatching, the larvae were placed on an artificial diet

made by Southland Products Inc. (Lake Village, AR) following the manufacturer's instructions. The diet was placed in 30mL cups for use. Following setup of the diet, the freshly hatched fall armyworm neonates were placed individually in diet cups and allowed to continue to grow in an incubator at 28°C with a 16 hour day/8 hour night cycle. Growth was permitted until the 4th instar was achieved. Following this, another 49 grams of Southland Products pre-made diet was made— however, 1 gram of protease inhibitor manufactured by Sigma-Aldrich, Inc. (St. Louis, MO) was added to the diet mix once it reached 60°C to prevent potential protein denaturation. Addition of the 1g of protease inhibitor to the artificial diet made a 2% protease inhibitor concentration. 2% protease inhibitor diet was then added in equal amounts to 20 30 mL plastic diet cups. An additional batch of diet lacking protease inhibitor was made and was also added in equal amounts to 20 30 mL plastic diet cups. These two batches were termed the 2% PI and control treatments, respectively.

Once the 2% protease inhibitor and control diet cups were made, the 4th instar larvae were individually labeled in accordance with their treatment and numbered. Next, the caterpillars were individually massed in milligrams using a scale. Their initial masses, termed day 0 masses, were recorded. The larval masses were then recorded over the course of nine days. While the total observational period was nine days, masses were recorded on every third day (days 3, 6, and 9). With each observation, the developmental status— larva or pupa— was also recorded, as well as the state of each larva as alive or dead. At the end of the nine-day period, the weight gain per larva since day 0 was calculated, and the individual insect weight gains was recorded to be used in eventual statistical comparison. When recorded, larval and pupal masses were kept separate, and masses from dead larvae were excluded entirely. This procedure was completed again in another trial of the experiment, except new protease inhibitor concentrations– 0.1% and

1%, in addition to a control group – were used. The same procedure for diet-making, larval massing, and data recording were used. However, due to limitations in the diet-making process, only 17 replicates were produced for the 0.1% and 1% PI treatment groups. The 0.1% PI, 1% PI, and control diets from the same trial were labeled as being from batch A, whereas the 2% PI and its control were labeled as being from batch B. This was done for simplification during data analysis, as well as to avoid potential data skewing due to potential genetic differences between the fall armyworms used in each experiment.

After the data from all three treatment groups were collected and finalized, JMP statistical analysis software (JMP® Version 16, 2022) was used to run a one-way ANOVA test for the final larval masses (mg) determined from each treatment group. The data was fit Y by X, with the independent variable being treatment group (0.1% PI, 1% PI, 2% PI, Controls-Batch A, and Controls-Batch B), and the dependent variable being larval masses on day 3 of observation. Given the results of the initial statistical analysis conducted in JMP, post-hoc tests were performed, including a Tukey-Kramer HSD pairwise analysis.

Experiment 2: Examination of Differing Resource Quality on Cannibalistic Consumption

As per the first experiment, *S. frugiperda* eggs were obtained from Benzon Research Inc. The eggs were placed in an incubator at 28°C with a 16 hour day/8 hour night cycle for two days, until hatched. Prior to insects hatching, a batch of Southland Products artificial diet was made. The diet was inserted into 30 mL cups. Following creation of the diet, 660 insects were set up, with one fall armyworm occupying one cup. Larvae were then placed in an incubator at 28°C with a 16 hour day/8 hour night cycle and allowed to grow until they reached the 3rd instar. At

this point, 480 larvae were removed from the 28°C incubator and placed in a 16°C incubator, so as to slow their development while 180 larvae were left in the 28°C incubator. The 480 larvae that were placed into the 16°C incubator were kept as 3rd instars, while the larvae that remained in the 28°C were allowed to grow to 4th instars. Once the fall armyworms in the 28°C reached the 4th instar, both the 3rd and 4th instar groups were removed from their incubators and left on a benchtop for subsequent same-day experimental setup.

Using the same diet-making procedure as was previously described, regular diet was made, as well as a diet that contained 2% protease inhibitor. The diet was added to 30 mL cups and appropriately labeled, according to treatment. Following this, the diet was cut into small slices, and each was massed to weigh approximately 650 mg. These masses were recorded. Next, the diet slices were placed onto 30 small, pre-assembled petri dishes, each containing three small pieces of moistened Whatman filter paper to help with moisture-retention. This process was followed for both the 2% PI diet and the regular (control) diet. For the plant cultivation, Gasoy soybean seeds were placed between two moistened paper towels and kept in the dark at ~23°C for 48 hours until germination was achieved. Germinated Gasoy soybean seeds were then individually planted in cell packs containing a 2:1:1:2:2 soil mixture of Sunshine Mix #3 (Sun Gro Horticulture, Agawam, MA), Perlite, Vermiculite, Osmocote, and water. The plants were allowed to grow in a walk-in incubator at 28°C, 80% humidity, and a 16 hour day/8 hour night cycle for two weeks until they reached the trifoliate stage (Garvey et al 2022). Once the plants were fully developed trifolates, the leaves of approximately 30 Gasoy soybean plants were used in the experiment. Similarly to the diet cubes, the leaves were massed until reaching around 650 mg, and the final masses were recorded. These leaves were also placed into 30 small petri dishes containing moistened Whatman filter paper. In total there were three treatments that varied

resource quality, a control artificial diet treatment, an artificial diet containing 2% PI, and soybean leaves using the Gasoy variety.

To complete the experimental setup, three 3rd instar fall armyworms were placed into each individual petri dish per treatment. After all 3rd instars were added, one 4th instar larva was added to each petri dish and was placed directly on top of the food source. The larvae were then observed at hours 1, 4, 16, 20, 24, and 36 after experiment setup. During each observation, the number of caterpillars remaining was observed and the number of caterpillars cannibalized, labeled as eaten, was noted.

Statistical analysis of the treatment groups was conducted using R and R Studio version Desktop 2022.02.1+461. A statistical model was created using the package glmmTMB, which analyzed the variables in a generalized linear model. Dish individual and plant individual were included in the model as random effects, so as to offset the individual characteristics of each dish/ plant. Using a Poisson error distribution, the model was developed by analyzing the number of 3rd instars eaten over the 36-hour period and was grouped by treatment (food source).

Experiment 3: Comparing Differing Rates of Cannibalistic Consumption Derived from Diet vs Plant Food Source on Baculovirus Transmission Amongst Spodoptera frugiperda larvae

As per the resource quality experiment, *S. frugiperda* eggs were ordered from Benzon Inc. and, upon hatching, placed in 30 mL cups with artificial diet and reared at 28°C on a 16 hour day/8 hour night cycle. Once the larvae reached the 3rd instar, 540 of the insects were then moved to a 16°C incubator with a 16 hour day/8 hour night cycle so as to slow food consumption and development for two days. During this time, the remaining 180 insects were left in the 28°C

incubator and allowed to grow until they reached the 4th instar. One day prior to experiment

setup, 270 of the 3rd instar larvae were removed from their traditional diet cups and fed a small

diet cube containing an LD95 dose of the *Sf*MNPV baculovirus (isolate Slavicek)— 3 µL of

solution per diet cube with 100,000 viral occlusion bodies per microliter. The LD95 dosage was

determined by infecting a separate 30 insects with the dose and tallying the death due to viral

infection (28/30, rounded to an LD95). The soybean leaves that were to be used in the

experiment were taken from Gasoy variety of soybean plants that had been grown in a walk-in

incubator at 28°C, 80% humidity, and a 16 hour day/8 hour night cycle. The plant propagation

methods were the same as from experiment 2. Additionally, control soybean plants were sprayed

heavily with a 1% EtOH solution, whereas the induced soybean plants were sprayed with a 2

mM MeJA solution (made with EtOH) one day prior to setup. On the day of initial observation,

the same procedure as the resource quality experiment was followed, this time using artificial

diet, soybean leaves, and soybean leaves sprayed with MeJA as treatments. Each petri dish

contained one 4th instar cannibal and three 3rd instar potential victims that varied in terms of

whether or not they were infected with baculovirus. That is, two treatments were made for each

resource— one containing 3rd instar victims infected with an LD95 dose of *Sf*MNPV, the other

containing healthy, non-infected 3rd instars. Each treatment consisted of 30 petri dishes.

The individual dishes were observed at hours 1, 4, 16, 20, 24, 28, 36, and 42. At each

observation, the number of cannibalized victims was recorded. Additionally, the 4th instar

caterpillars were observed for any occurrence of death. After the 42-hour period ended, the 4th

instars were kept separately on regular diet and stored at 28°C with a 16 hour day/8 hour night

cycle. The 4ths were then observed for death over the course of a week. If the cannibal appeared

deceased, a simple "splat" test was performed to check for presence of virus. The "splat" test,

which consists of knocking the diet cup against a hard surface, can be used as a diagnostic due to the intense liquefaction of the insect that occurs with baculovirus infection. If the caterpillar liquefied upon the splat test, it was recorded as positive for virus. For those cannibals that were deceased but did not liquefy, an autopsy under a compound microscope was conducted to detect the presence of virus or lack thereof, using 1M KOH-induced lysing of the virus occlusion bodies as an indicator of viral infection. KOH ruptures the occlusion bodies of the virus, a process that is visible under the microscope, thus making this an appropriate diagnostic technique.

Finally, after the observation period ended and all data was recorded, statistical analysis using R Studio version Desktop 2022.02.1+461 was performed to analyze any statistically significant differences between treatment groups. Again, a statistical model was created using the package glmmTMB, which analyzed the variables in a generalized linear model. Dish individual and plant individual were again included as random effects. Using a Poisson error distribution, the cannibalism-consumption model was developed by analyzing the number of 3rd instars eaten over the 42-hour period and was grouped by treatment (food source). This same process was used for the creation of the cannibal mortality model, which used a binomial error distribution to analyze cannibal mortality in relation to cannibalistic consumption per treatment group.

Results

Experiment 1: Determining the Effective Protease Inhibitor Concentration

A boxplot containing the day 3 larval masses (mg) for each treatment group is shown below (Figure 1). The 0.1%, 1%, and its respective control group all shared similar median final

masses—204 for 0.1% PI, 188 for 1% PI, and 190 for the control. Furthermore, the 2% PI treatment possessed a median final mass of 150 mg, whereas its control counterpart possessed a median value of 154 mg. The various treatment groups are comparable in their maximum and minimum values, however the control from trial 2 exhibits a maximum value that is fairly far from the 3rd quartile (75th percentile). Additionally, the 0.1% PI treatment possesses an interesting outlier at 373 mg. Again, for simplicity, the diet that was used in the trial testing protease inhibitor concentrations of 0.1% and 1% and its respective control will be referred to as from batch A, whereas the control diet and 2% PI treatment will be referred to as being from batch B.
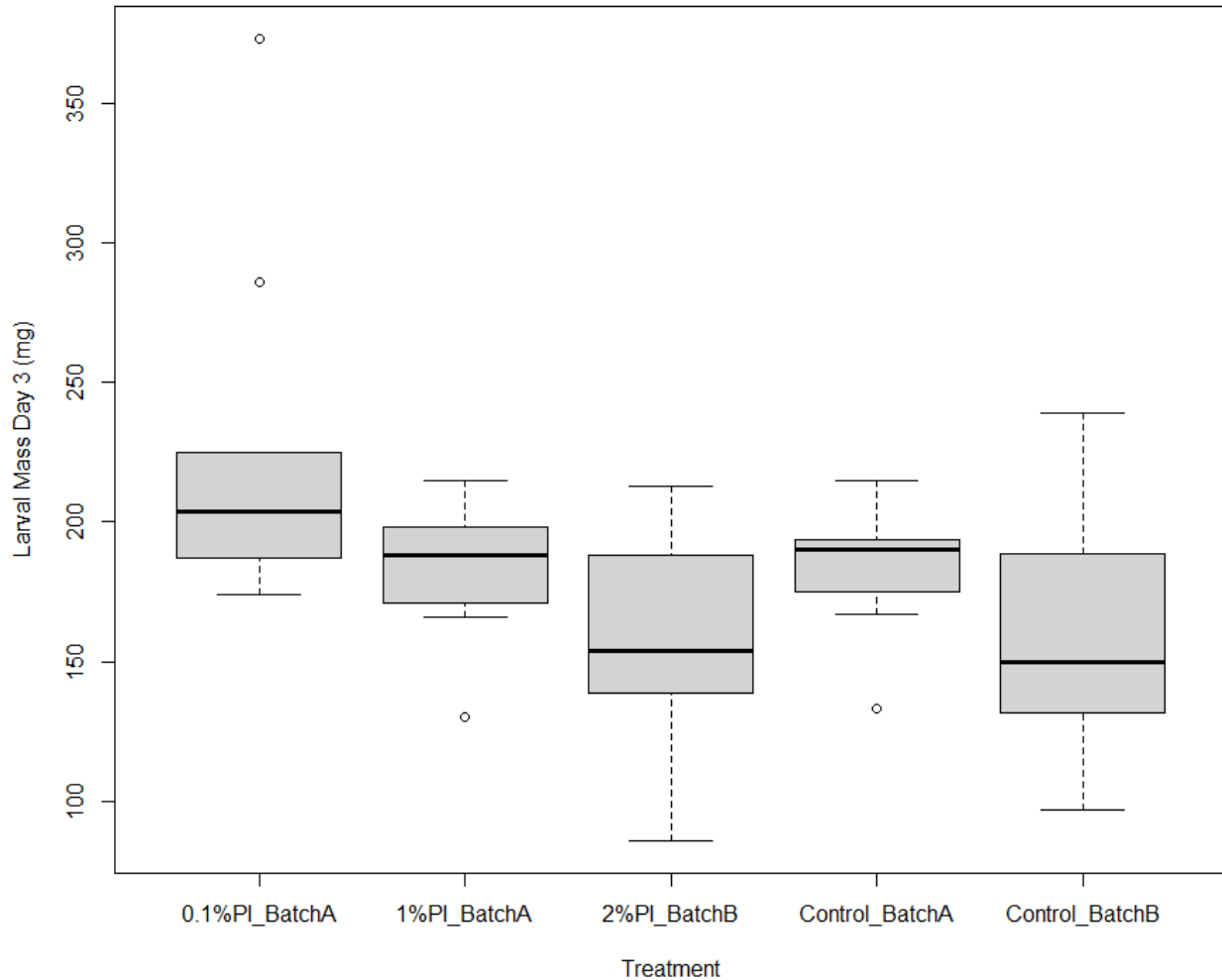
Figure 1: A box plot of day 3 larval mass (mg) vs treatment group. Lines located in the box interiors indicate the median larval weights (mg) for each treatment group, and the box limits represent the 25th and 75th percentiles. The whiskers extend beyond the box limits by 1.5 times the interquartile range. Points outside of the whiskers are considered outliers.

A one-way ANOVA statistical analysis of the data illustrated a P-value of <0.0001 for the treatments, indicating that one or more treatments was significantly different from another. Furthermore, the ANOVA produced an R-square value of 0.3068 for the relationship between

the treatment groups vs final weight in mg. This R-square value indicates that approximately 31% of the variation in the data can be explained by treatment. The results of the one-way ANOVA are recorded in Table 1 below.

Table 1: A summary of the fit derived from the conducted one-way ANOVA statistical analysis. It contains the data source, degrees of freedom, mean square, and F-ratio, as well as the P-value. A P-value of <0.05 is significant and is indicated in bold.

Source	Degrees of Freedom	Mean Square	F Ratio	P-value
Treatment	4	23483.6	8.853	**<0.0001**
Error	80	2652.6		
C. Total	84			

A Tukey-Kramer HSD pairwise analysis of the various treatment groups was conducted to examine differences in the pairwise treatments (Figure 1). The results showed that the 0.1% PI and 1% PI treatments, as well as their respective trial control, were statistically similar, indicated with the letter "A" to denote the similar grouping. The control batch A and 0.1% PI groups were statistically different from the 2% PI treatment and control batch B group, which both were assigned the letter "C." 1% PI (batch A) and control diet batch B both received the letter B, indicating that these two treatments were statistically similar. The report summarizing the results of the Tukey-Kramer analysis is provided in Table 2.

Table 2: JMP-generated Tukey-Kramer HSD pairwise analysis of the three varying treatment groups and the two control groups. Groups marked "A" are statistically similar to one another, as are those in group "B" and group "C." Different letter assignments indicate statistically different results.

Level	Letter Assignment	Mean Larval Weight ± Standard Deviation (mg)
0.1% Protease Inhibitor (Batch A)	A	237.0 (± 61.63)
Control Diet (Batch A)	A	221.1 (± 46.65)
1% PI (Batch A)	AB	206.1 (± 48.78)
Control Diet (Batch B)	BC	169.7 (± 55.09)
2% Protease Inhibitor (Batch B)	C	146.8 (± 46.65)

A more in-depth, pairwise analysis of the Tukey-Kramer results was also generated. While no concentration of protease inhibitor was determined to be statistically different from its respective control diet treatment, the strongest differing trend was visible between the batch B control diet and 2% protease inhibitor concentration (batch B), with a P-value of 0.6928, although this P-value is fairly non-significant.

Experiment 2: Examination of Differing Resource Quality on Cannibalistic Consumption

GLM analysis on treatment groups in relation to cannibalism rate revealed significant differences between cannibalism and time, indicating that cannibalism notably increased over time. Leaf and protease inhibitor treatments also significantly influenced cannibalism rate, with P-values of 0.0003 and 0.0164, respectively. These results are found in Table 3.

Table 3: A GLM analysis of variables for the varying treatment groups using a Poisson error distribution. Treatment individual was included as a random effect in the model. Significant P-values indicated in bold, and interactions between variables are indicated by the presence of a colon (:). Interactions were not statistically significant between time and the leaf treatment nor time and the PI treatment.

Variable	Estimate	Standard Error	Z-Value	P-Value
Intercept	-1.305	0.3319	-3.932	**8.42e-5**
Time	0.0285	0.0070	4.086	**4.38e-5**
Leaf	-2.105	0.5818	-3.618	**0.0003**
Protease Inhibitor	-1.239	0.5164	-2.400	**0.0164**
Time: Leaf	0.0230	0.0149	1.543	0.1229
Time: Protease Inhibitor	0.0039	0.0039	0.324	0.7461

Comparison of the cannibalism rates between each treatment group revealed a significant difference between the rates of the control diet vs leaf (P-value 0.0003) and control diet vs 2% protease inhibitor (P-value 0.0164). When subjected to a poisson fit, this difference is clearly visible. While cannibalism rates did indeed increase over time across all treatment groups, the initial cannibalism rate for the control diet group was markedly higher than that of its two opposing treatments. Similarly, the control diet group reached a higher overall number of average caterpillars eaten per dish, approximately 1.25 caterpillars. It should also be noted that the leaf and PI treatments were not significantly different from each other. These metrics are visible in Figure 2.
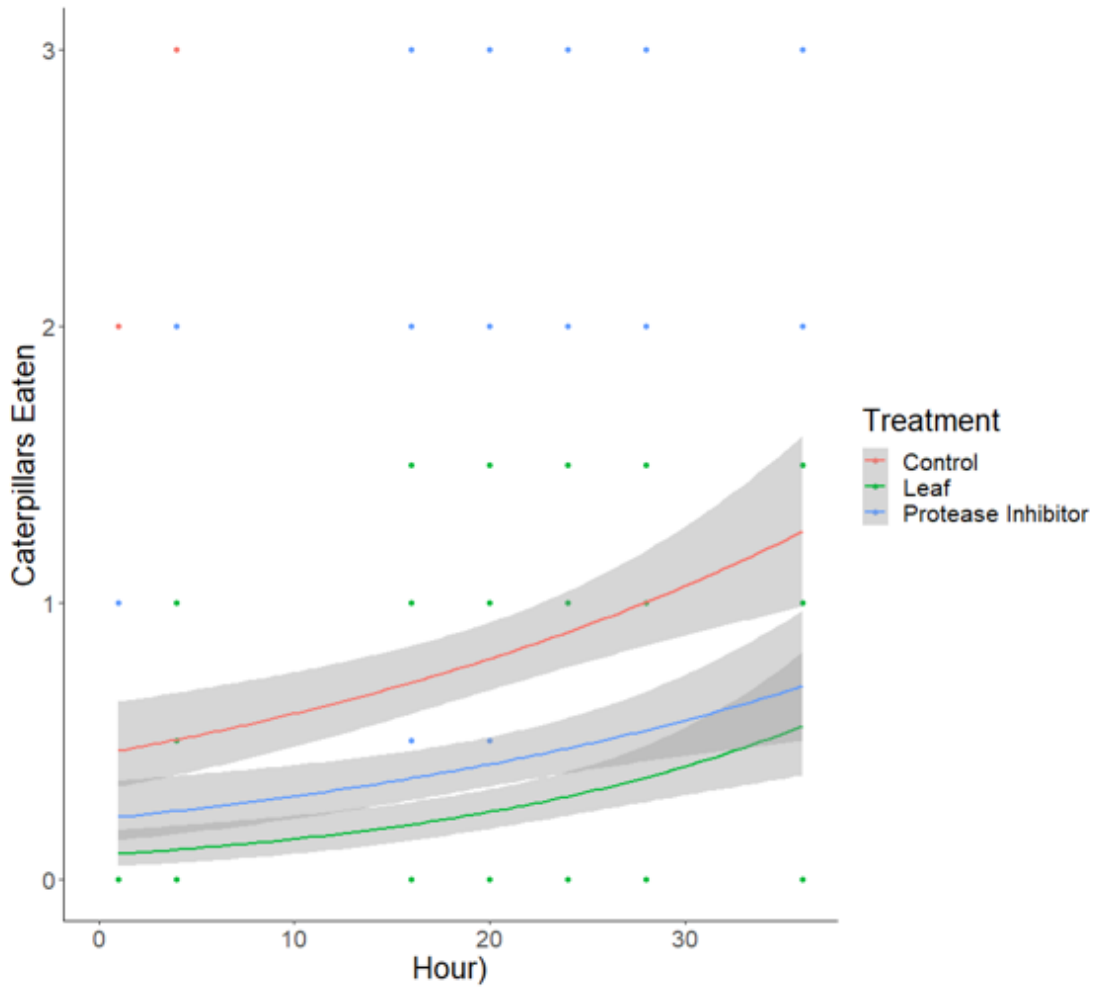
Figure 2: A comparison of the average number of caterpillars eaten per treatment across the 36-hour observation period. Dish and plant individual were included in the model as random effects. Confidence intervals are indicated by the grey bands surrounding the trend lines. The points indicate individual cannibals from each of the treatment groups. The control diet treatment exhibited significantly heightened cannibalistic consumption in comparison to both the leaf and protease inhibitor treatments. The leaf and protease inhibitor treatments were comparable to one another, although they differed mildly between the hours 16-24 observations.

Using a pairwise comparison, a contrast was also conducted between each treatment group, and a corresponding P-value was generated for each pair to explain how much variation between the treatment groups could be attributed to chance. This analysis again revealed significant difference between the control-leaf treatments and control-PI treatment. These P-values and other statistics are in Table 4.

Table 4: Statistical output of a pairwise comparison of treatment groups, represented through standard error, degrees of freedom, T-ratio, and P-value. Significant P-values are indicated in bold. Both the control-leaf pair and the control-protease inhibitor pair exhibit a significantly different relationship. The leaf-protease inhibitor P-value, 0.6508, indicates that the difference between these two treatments is not statistically significant.

Contrast	Standard Error	Degrees of Freedom	T-Ratio	P-Value
Control-Leaf	0.457	625	3.425	**0.0019**
Control-Protease Inhibitor	0.441	625	2.614	**0.0248**
Leaf-Protease Inhibitor	0.468	625	-0.884	0.6508

Experiment 3a: Effect of Induction and Varying Resource Toxicity on Rates of Cannibalistic Consumption

For this portion of the experiment, the three food types were arranged essentially as a gradient: nutritionally complete artificial diet, complex leaf diet, and induced leaf diet with heightened toxicity. Thus, all three food types were analyzed together so as to reveal the most accurate effects of the treatments. Analysis of the three food types all together revealed no

significant difference between the treatments in terms of overall cannibalism rate. However, time had a significant effect, indicating that the amount of victims cannibalized for all treatments increased over time. Additionally, significant two-way interactions were present between time and virus inoculation, time and leaf substrate, as well as time and MeJA leaf substrate. The significant interaction between time and virus inoculation indicates that both variables together had a unique impact on the cannibalism rate on all food substrates. Specifically, as time increased, treatments with *Sf*MNPV-inoculated victims tended to cannibalize less on the leaf substrate and more on the diet, and cannibalism on the MeJA leaf remained relatively the same as for healthy victms.

In addition, a three-way interaction was present between time, virus inoculation, and uninduced leaves, but not time, virus, and MeJA leaves. This interaction implies that the uninduced leaf treatment was significantly less affected by the inherent increase in cannibalism rate as a result of progressing time when *Sf*MNPV infection was present in the victims. The number of caterpillars cannibalized on the non-induced leaf treatment at the end of the observation period was visually lower in the presence of virus, and the rate of cannibalization was slower, as is indicated by the less-steep curve in Figure 3.

Additionally, while not statistically significant, the data showed a very strong trend (P-value 0.0547) that virus-infected victims decreased the cannibalism rates regardless of the varying food treatments. A relatively strong trend toward impact on canibalism was also present in the differing substrates individually, with JA-leaf substrates exhibiting a P-value of 0.0649, and leaf substrates exhibiting a P-value of 0.0851. The results of the food-type analysis are visible in Table 5.

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Table 5: Statistical GLM analysis of variables in which all three substrate types were analyzed together. A P-value of less than 0.05 indicates statistical significance. Interactions between variables are indicated by the presence of a colon (:). Significant P-values are indicated in bold. Trending P-values are underlined.

Variable(s)	Estimate	Standard Error	Z-Value	P-Value
Intercept	-1.732	0.2343	-7.392	**1.45e-13**
Time	0.0464	0.0063	7.312	**<2.64e-13**
Virus	-0.6758	0.3517	-1.921	<u>0.0547</u>
JALeaf	-0.6571	0.3560	-1.846	<u>0.0649</u>
Leaf	-0.5940	0.3441	-1.722	<u>0.0851</u>
Time: Virus	0.0271	0.0094	2.893	**0.0038**
Time: MeJALeaf	0.0229	0.0096	2.375	**0.0175**
Time: Leaf	0.0276	0.0092	2.944	**0.0032**
Virus: MeJALeaf	0.3408	0.5312	0.642	0.5212
Virus: Leaf	0.7526	0.5054	2.489	0.1365
Time: Virus: JALeaf	-0.0182	0.0142	-1.280	0.2005
Time: Virus: Leaf	-0.0332	0.0135	-2.466	**0.0137**

It is clearly visible in Figure 3 that cannibalism for all treatments increased over time. While the cannibalism rate for the MeJA-leaf treatment remained relatively unaffected by the presence of *Sf*MNPV-inoculated victims, virus-inoculation was associated with a significant decrease in cannibalism over time for the uninduced leaf treatment, and an increasing trend in cannibalism for the control diet. Although cannibalism over time visually exhibited similar rates across all food types when victims were *Sf*MNPV-infected, interestingly, this differed for non-infected victims. In the case of non-infected victims on the non-induced leaf substrate, cannibalism over time exhibited a strong trend that was much higher than that of the infected victims, achieving ~2.5 healthy victims consumed at hour 42, in comparison to ~2 infected victims consumed at hour 42. Contrarily, while the presence of virus overall decreased cannibalism on the leaf substrates, it intensified cannibalism on the diet treatments and increased

the rate of cannibalization (a steeper curve). Cannibalism over time for the MeJA leaf substrate remained very similar regardless of infection status of victim.
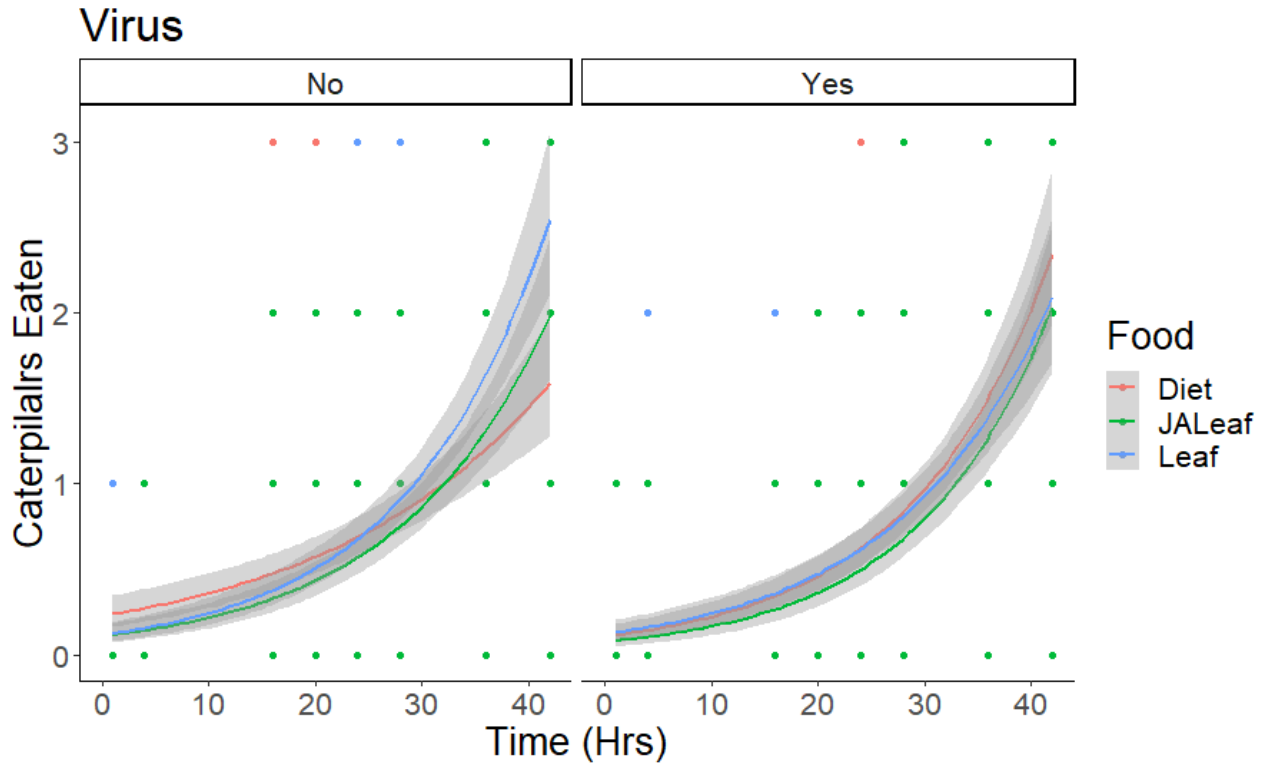

Figure 3: A comparison of cannibalism over time across the varying food/ substrate types with virus inoculation overlayed. Dish and plant individual were included as random effects. Confidence intervals are indicated by the grey bands surrounding the trend lines. Dots on the graph represent individual data points. All food types exhibited similar cannibalism rates when the victims were infected. Cannibalism rates of non-infected victims was noticeably faster for the leaf and JA-leaf treatments than for the control diet.

Experiment 3b: Examination of Cannibal Mortality Due to Viral Infection

After assessment of the cannibalism rates across the varying treatment groups, mortality of the cannibals due to viral infection was investigated. Visual inspection of viral mortality among treatment groups revealed zero of the cannibals in the no-virus control group died due to viral infection. This indicates that there was no cross-contamination between the control and virus groups. Furthermore, a total of 15 out of 30 cannibals from the virus-positive artificial diet treatment, 18 out of 30 cannibals from the virus-positive leaf treatment, and 19 out of 30 cannibals from the virus-positive MeJA treatment died due to viral infection. One cannibal was removed from data analysis in the virus treatment because it ate 0 victims but was infected with virus, thus it did not acquire viral infection from cannibalistic consumption. Additionally, a Wilcoxon rank sum test indicated that cannibal mortality was significantly impacted by virus-infected victims (P-value <1.595e-15).

Lastly, all food types were analyzed in relation to cannibal virus mortality. A GLM statistical model of mortality and food type revealed significant statistical difference in the relationship between the total number of infected caterpillars consumed and cannibal virus mortality (P-value 0.0470) (Table 6). This indicates that the number of caterpillars consumed significantly impacted the proportion of cannibal death by viral infection— specifically, as the number of infected victims consumed increased, the likelihood of cannibal viral mortality also increased. This relationship was observed for all treatment groups. Also notably, the model showed presence of a strong trend between viral mortality and the methyl-jasmonate leaf treatment (P-value 0.0873) (Table 6). This specifically implies that the MeJA leaf treatment appeared to show a trend of higher rates of viral mortality in comparison to that of the artificial diet and non-induced leaf substrates.

Table 6: GLM analysis of variables in binomial distribution in which food type (including induction for the MeJA treatment) was examined in relation to cannibal virus mortality. P-values that suggest strong trends are underlined, whereas significant P-values are indicated in bold.

Variable	Estimate	Standard Error	Z-Value	P-Value
Intercept	-1.411	0.7127	-0.001	**0.0477**
Total Eaten	0.5421	0.2729	1.815	**0.0470**
Leaf	0.7529	0.5649	1.459	0.1826
MeJA Leaf	0.9913	0.5797	1.680	0.0873

Visually, artificial diet substrate appeared to show the weakest relationship between increasing number of caterpillars eaten and cannibal death due to virus. Cannibal virus mortality for the artificial diet appeared to remain under 50% with three victims consumed. Both leaf substrates appeared to have visually stronger relationships between number of victims consumed and cannibal virus mortality, with the MeJA treatment exhibiting mildly higher mortality rates at two and three victims consumed, albeit within the confidence interval of the other treatments. Both leaf treatments exhibited approximately 75% cannibal virus mortality at three victims consumed. The graphed results are depicted in Figure 4.
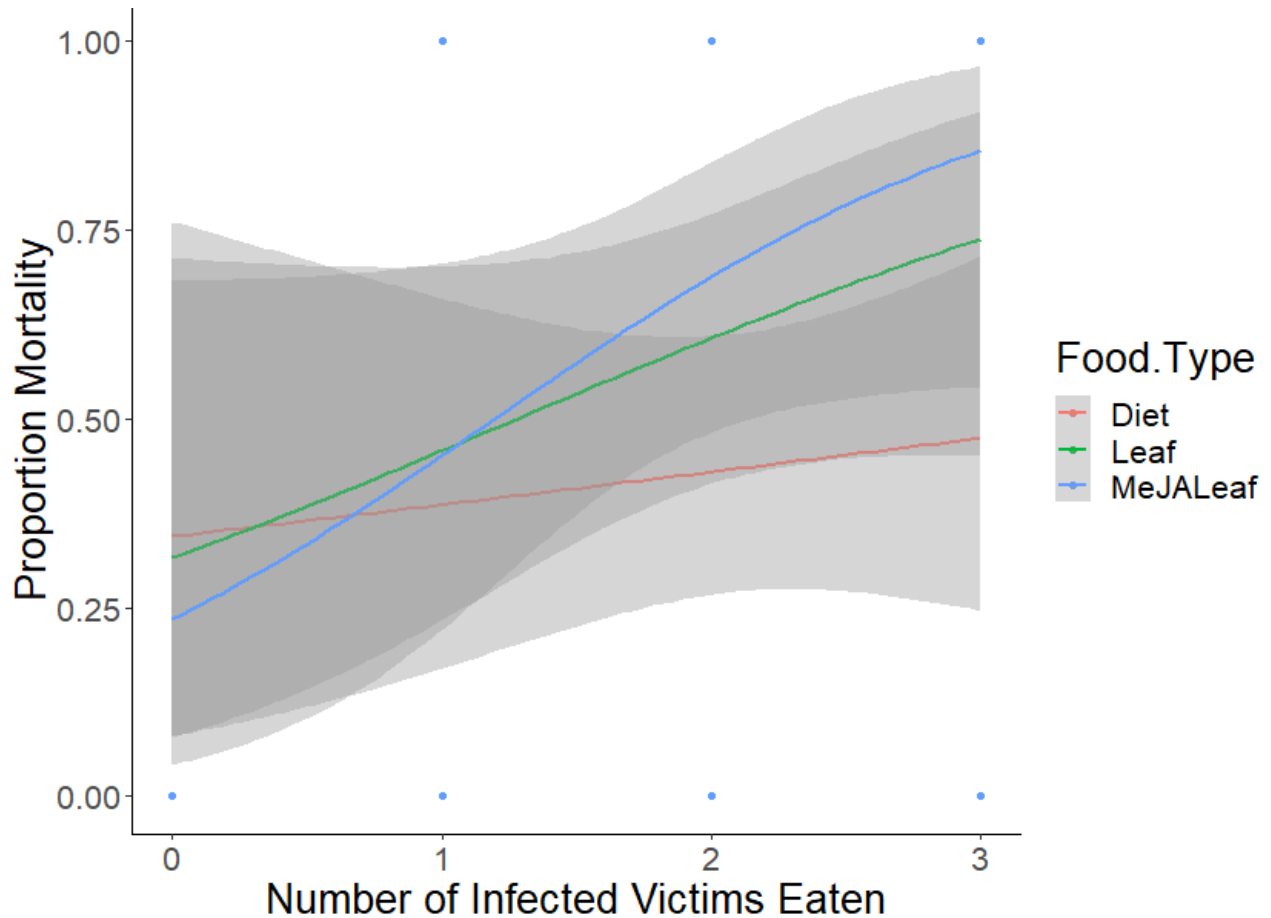
Figure 4: Binomial fit of cannibalism rate for the three food types when victims were infected with virus. Dish and plant individual were included as random effects. Confidence intervals are shown by the grey bands surrounding the trend lines. The dots indicate individual cannibals from their respective treatment groups. The MeJA substrate showed a strong trend on cannibal viral mortality.

Discussion

Initial determination of an effective protease inhibitor concentration was complicated by the batch effects from the different trials. While larvae fed protease inhibitor diet from Batch B

exhibited significantly different weights from the control diet from Batch A, this cannot be considered, as the data was likely affected by batch effects— that is, the larvae from Batch B were genetically different from Batch A, thus resulting in significantly different masses. Furthermore, while no significant difference was found between the 2% protease inhibitor spiked diet and its respective control diet, the 2% PI concentration was chosen because it exhibited the strongest trend of all PI-control comparisons (P-value 0.6928), and thus showed potential. Additionally, prior research has shown that a 1% (w/v) protease inhibitor concentration is effective in decreasing proper digestion of food substrates in Lepidopteran insects (Larocque and Houseman 1990). Thus, I concluded that 2% should have a similar if not greater effect.

Cannibalism rates on the artificial diet were similar between experiment 2 and experiment 3 when victims were uninfected. However, in experiment 3, artificial diet was found to have a lower cannibalization rate than the leaf and MeJA treatments, which contradicts the data from experiment 2. Despite having the same chemical makeup (in terms of the control and leaf treatments) and a heightened toxicity in the MeJA-leaf treatment, I found in experiment 3 that the cannibalism rate was higher and faster on both induced and non-induced leaf substrates than on the diet, with the non-induced leaf resulting in the highest cannibalism rates. This reversal in food-cannibalism rate could potentially be attributable to the difference in surface area of the substrate. In experiment 2, both control and PI-diet were available in small diet slices placed in the center of the petri dish, which was done due to constraints surrounding the amount of protease inhibitor available for use. This differed from the setup in experiment 3, in which the control diet covered the entirety of the bottom of the petri dish. Perhaps this large difference in resource availability and surface area resulted in higher cannibalism rates on the control diet in experiment 2, as the caterpillars may have more frequently encountered their conspecifics and

unintentionally avoided their small diet slice. It has been shown that cannibalism among fall armyworm larvae occurs at higher rates when food is scarce (Chapman et al 1999). Thus, the inconsistencies in my data between experiments 2 and 3 could very well be attributed to resource availability.

For all food substrates in which the potential victims were inoculated with *Sf*MNPV-baculovirus, cannibalism rate, as well as total cannibalism at the end of the observation period, was highly similar. However, control diet exhibited a mildly higher rate and leaf substrate exhibited a lower rate when victims were infected, in comparison to when victims were uninfected. I posit that part of this interaction may be due to the landscape of the resource. In the diet dish, there were no potential enclaves in which potential victims could hide from the cannibal, as were available in the folds and crevices of the leaf treatments. Thus, in open area such as the diet dish, it is more likely that cannibal and victim will encounter one another, especially if the victim is sluggish due to infection with virus. This potentially explains the increasing cannibalism rate as was seen in the diet-virus treatment. The overall similarity of cannibalism rates on all food sources with virus-inoculated victims, however, is likely attributable to the infection itself. Infection makes the victim more sluggish (Cuartas-Otalora et al 2019), thus potentially allowing for easier hunting and subsequent consumption by the uninfected cannibal.

Analysis of cannibal viral mortality across treatment groups overall consistently revealed a statistically significant impact of increasing number of victims eaten on viral mortality. This data is expected, as it logically follows that as more infected victims are consumed by the cannibal, more virus occlusion bodies are consumed, thus increasing the likelihood of death by viral infection. Furthermore, while all three treatment groups revealed similar cannibalism rates

in the presence of virus-inoculated victims, the mortality rates of the cannibals due to viral infection showed a strong trend of difference in the MeJA treatment in comparison to both the artificial diet and leaf treatments. While my results for this particular analysis were not statistically significant, these trends in relation to induced plants as a food substrate and increasing mortality suggests the potential existence of some relationship between the two variables.

In conclusion, my research has shown that differing rates of cannibalism can occur within populations of the fall armyworm, but that these differing rates are dependent on a number of factors, including a combination of resource quality and availability. Furthermore, I found that the primary influence of virus mortality in relation to cannibalism is the number of infected victims eaten, with increasing infected victims consumed resulting in increased likelihood of cannibal mortality due to virus. While my research did not show statistical significance of the impact of plant-induced defenses on increased viral transmission, my data did indicate a trend that this might be the case. Further research should be conducted, perhaps with more replicates and more consistency in experimental design, to determine whether this trend would become statistically significant.

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