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Host Plant Chemistry and Infection Status Alters the Immune Response and Development Time in Vanessa cardui

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Justine Resnik

Dr. Smilanich, Thesis Advisor

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Angela Smilanich, Ph.D., Thesis Advisor

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Tamara Valentine, Ph.D., Director, Honors Program

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Abstract

Studies investigating the interplay between the animal immune response and life history traits fall under the umbrella of ecoimmunology. An important goal of ecoimmunology is to understand the tritrophic interactions influencing insect immunity and survival. This study focuses on the effects of diet and pathogen infection on the immune response of the polyphagous butterfly, *Vanessa cardui* (Nymphalidae). Specifically, this study aims to understand the life history tradeoffs *V. cardui* experiences when fighting pathogens and whether these tradeoffs are diet dependent. I hypothesized that *V. cardui* larvae infected with the entomopathic virus, *Junonia coenia densovirus* (JcDNV), would have two avenues for surviving the virus: invest in development or mount an immune response. I demonstrate that the ability to invest in one or the other is dependent on diet and infection status. I found that *V. cardui* reared on plant diets responded to the JcDNV infection by developing quicker than uninfected individuals. Infected individuals also had a lower immune response compared to the uninfected group. However, when larvae were reared on a more nutrient rich diet (artificial diets) there was no effect of virus infection on development time, suggesting that the impact of the virus on development time is nutrient related. I also found that other dietary factors (iridoid glycosides found in the novel host plant *Plantago lanceolata*) can provide additional protections (faster development and higher immunocompetence) compared to the native host plants *Lupinus argenteus* and *Lupinus albifrons*. Therefore, I suggest that diet impacts the effects of JcDNV and influences the course of action caterpillars take when infected with the virus. Overall, I found that viral infection and diet simultaneously impact the lepidopteran immune response and influence the life history tradeoffs in *V. cardui*.

1. Introduction

Understanding the tritrophic interactions between host plants, insect herbivores, and pathogens is important for understanding the evolutionary pressures exerted on insect populations (Corey and Hoover, 2006; Shikano 2017). By analyzing how diet and pathogens affect insect development and survival, new inferences can be made to help understand how these factors influence diet breadth evolution. Previous studies have demonstrated that plant chemistry can have a large impact on the development, survival, and immunity of insect herbivores (Dyer and Bowers,1996; Smilanich et al., 2009a; Smilanich et al., 2009b; Smilanich et al., 2010; Triggs and Knell, 2012; Smilanich et al., 2018), while several other studies have demonstrated how pathogens can also impact insect physiology and cause significant changes in development (Nakai et al, 2002; El-Sheikh, 2015; Saito et al., 2015; Karlhofer et al., 2012; Nakai et al., 2016). Together, these two factors impact the development and survival of insect herbivores by altering the biochemistry of the insect which may ultimately influence insect diet breadth.

One key factor in understanding insect evolution is understanding what influences insect diet breadth. One focus of this study is to understand how plant secondary metabolites can exert selection pressure that shape the diet breadth of *Vanessa cardui* (Nymphalidae: Lepidoptera). Recently, populations of *V. cardui* have incorporated a novel host plant, *Plantago lanceolata* (Plantaginaceae), into their diets. *P. lanceolata* contains high amounts iridoid glycosides (2-9% dry weight total IG) (Bowers and Stamp, 1992) which has been demonstrated to positively and negatively impact insect performance (Dyer and Bowers, 1996; Smilanich et al., 2009a; Smilanich et al., 2009b; Lampert et al., 2014; Smilanich et al., 2018). Past research demonstrates that iridoid

glycosides can have varying impacts on the lepidopteran immune response depending on the species and pathogen (Smilanich et al., 2009a; Lampert et al. 2014; Smilanich et al., 2018). For instance, when caterpillars sequester iridoid glycosides at high concentration, the immune response is decreased in *Junonia coenia* (Nymphalidae) (Smilanich et al., 2009a). However, feeding on *P. lanceolata* enhanced survival and immunity when *J. coenia* were infected with a virus (Smilanich et al., 2018). These results demonstrate that both trophic levels could be influencing diet breadth evolution in *J. coenia*, and that plant chemistry may have varying effects depending on the pathogen infecting the larvae. However, previous studies with *J. coenia* differ from the current study because *J. coenia* is a specialist herbivore, feeding only on plants containing iridoid glycosides (Bowers, 1984). In contrast, *V. cardui* is a generalist feeder, which means it will feed on a large range of host plants (Robinson et al. 2002; Nylin et al., 2014); therefore, understanding what drives the incorporation of a new host plant in *V. cardui* is not as intuitive. To address this question, this research asks whether there is a host associated enhancement of the immune response when *V. cardui* larvae are reared on *P. lanceolata* compared to one of its native hosts: *Lupinus argenteus* or *Lupinus albifrons*.

Previous studies analyzing the tritrophic interactions between viruses, host plants and lepidopterans have demonstrated that pathogenicity can be influenced by diet. These differences can lead caterpillars to change their behavior and exhibit self-medicating or compensatory behaviors when infected with a pathogen. Previous research with gypsy moths and nucleopolyhedroviruses (NPV) (Baculoviridae) demonstrated that viral susceptibility was dependent on host plant (lower on oak compared to aspen) and that outbreaks of NPV may be attributed to the proportion of oak trees found in an area

(Myers and Cory, 2016). This demonstrates that viral susceptibility can be influenced by diet. Another study demonstrated that infection with *Autographa californica* multiply-enveloped NPV (AcMNPV) caused *Trichoplusia ni* (cabbage looper) larvae to compensate by increasing their proportional protein intake (Shikano and Cory, 2016). This demonstrates that larvae seek out certain diets based on their infection status which may help explain host plant selection and diet breadth. In another study, *Grammia incorrupta* (Erebidae) were shown to self-medicate when infected with a fly parasitoid by increasing their pyrrolizidine alkaloids (PAs) consumption (Singer et al., 2009; Smilanich et al., 2011). This is particularly interesting because PAs have been demonstrated to be harmful to healthy *G. incorrupta* larvae (Singer et al., 2009; Smilanich et al., 2011). This study clearly demonstrates that host plant preferences can be heavily influenced by infection status. Together, these studies highlight that larval behavior pre and post infection can contribute to the survival of larvae. They also demonstrate that caterpillar behavior (especially host plant selection) can be altered based on the type of pathogen in the environment. Therefore, it is important that a tritrophic approach is taken when analyzing lepidopteran diet breadth. To thoroughly understand host plant selection in *V. cardui*, this study aims to identify how the effects of *Junonia coenia densovirus* (JcDNV) in *V. cardui* larvae are altered by diet. We do this by accessing survival, immunity and development time changes in larvae reared on four different diets: *P. lanceolata*, *L. argenteus*, *L. albifrons* and an artificial diet.

Another key factor in this study is understanding how host-prey relationships influence development and survival in *V. cardui*. One type of host-prey relationship is host-pathogen interactions. These interactions are unique because the pathogens

simultaneously rely on the survival of the host species while causing stress on the host's ability to survive. Several studies that focus on host-pathogen relationships demonstrate that pathogens can impact the development of lepidopteran species (El-Sheikh, 2015; Saito et al., 2015; Karlhofer et al., 2012; Nakai et al., 2016, Smilanich et al., 2018). For example, when J. coenia was infected with JcDNV, it resulted in a quickening of development time (Smilanich et. al, 2018). One proposed explanation for this result is that the caterpillars are trying to reach pupation before the full effects of the virus are realized (Smilanich et al., 2018). This is an interesting result because many other studies have demonstrated that viruses can cause a prolonged larval development in order to increase viral replication (Nakai et al., 2015; Nakai et al., 2002). The prolonged development that occurs post inoculation is caused by a decrease in the activity of juvenile hormone esterase (JHE): an enzyme that breaks down juvenile hormone (Nakai et al., 2015). Under normal conditions, juvenile hormone esterase activity is required to break down juvenile hormone and trigger metamorphosis (Furuta et al., 2013). Without a high activity of juvenile hormone esterase, caterpillars take a longer time to reach pupation. Conversely, we hypothesize that if caterpillars were to experience an increased activity of juvenile hormone esterase sooner in their development, they would pupate faster. A third facet of this research addresses whether the latter scenario is occurring in caterpillars infected with JcDNV. By analyzing JHE activity in caterpillars after they are inoculated with JcDNV, inferences can be made about what pathway is being impacted. This analysis, along with analyzing if a shortened development time impacts the survival of caterpillars infected with JcDNV, can provide new insights into how caterpillars respond to pathogens. This information can help explain whether it is the caterpillars or

JcDNV that gains from the quickening of development time.

2. Methods:

2.1 Insects, Plants and Viruses

This study focuses on the diet breadth of *Vanessa cardui* (the painted lady butterfly). *V. cardui* is known for its long-distance migrations throughout the spring and summer months (Stefenescu et al.; 2013) and is commonly found in North America, Northern Africa and Europe (Robinson et al., 2002; Stefenescu et al., 2013). Phylogenetic research predicts that the Vanessa group originally specialized in the Urticaceae family (Rosales) (Nylin et al., 2014). The Vanessa group has since incorporated a large range of host plants into its diet breadth (Nylin et al., 2014). The most polyphagous butterfly in the Vanessa group (and perhaps the most polyphagous butterfly in general) is *V. cardui* which has been recorded feeding on several different species in several different families (Robinson et al., 2002; Nylin et al., 2014). The fact that *V. cardui* is a highly polyphagous species makes understanding what drives *V. cardui* to incorporate new host plants not immediately clear.

In this study we analyze the fitness of *V. cardui* on three separate host plants and an artificial diet. The two native host plants that were chosen as controls were *Lupinus argenteus* (Silvery Lupine) and *Lupinus albifrons* (Silver Lupine or White-Leaf Bush Lupine). All plants in the lupinus genesis contain varying amounts of alkaloids (Wink and Witte, 1995). Despite both being from North America, *L. argenteus* and *L. albifrons* differ in their alkaloid composition with *L. argenteus* having a larger variety of alkaloids (Wink and Witte, 1995). The alkaloid composition in *L. argenteus* is also highly variable

with separate populations of *L. argenteus* often differing in their alkaloid composition (Wink and Witte, 1995). The plant of interests in this study, *Plantago lanceolata* (Ribwort Plantain), came to the Americas about 200 years ago (Cavers et al., 1980) and has since been incorporated into the diet breadth of *V. cardui*. This novel host plant has been extensively studied in the context of lepidopterans (Bowers, 1984; Bowers and Stamp, 1992; Dyer and Bowers, 1996; Singer et al., 2009; Smilanich et al., 2009a; Smilanich et al., 2009b; Smilanich et al., 2010; Lampert et al., 2014; Smilanich et al., 2018). *P. lanceolata* contains high amounts iridoid glycosides (2-9% dry weight total IG) (Bowers and Stamp, 1992) and one hypothesis is this class of secondary metabolites contributes to changes in the larval immune response (Singer et al., 2009; Smilanich et al., 2009a; Smilanich et al., 2009b; Smilanich et al., 2010; Lampert et al., 2014; Smilanich et al., 2018). The artificial diet was purchased from Southland Products and prepared according to the manufacturer's instructions. To our knowledge, this diet is not lacking in any essential nutrients and is nitrogen rich.

To challenge and test the larval immune response, larvae were inoculated with Junonia coenia densovirus (JcDNV). JcDNV is non-enveloped single stranded DNA virus that effects lepidopteran species (Mutuel et al., 2010). The effects of JcDNV peak 4 days after infection and results in either hypoxia during the larval stage or a failure to molt (Mutuel et al., 2010). Previous research with JcDNV and J. coenia demonstrated that larvae infected with JcDNV experienced a decrease in development time (Smilanich et al., 2018). The reason for this effect is still not understood. The prevalence or concentration of JcDNV found in wild populations of *V. cardui* is also not known.

2.2 Experimental Overview

Three experiments were conducted over two years. The first experiment (‘*L. argenteus* as native’ experiment) was conducted in Fall 2016 using *Vanessa cardui* eggs obtained from Carolina Biological. Caterpillars were reared in an incubator with a photoperiod of 16:8 L:D with a 25˚C daytime temperature and a 20˚C nighttime temperature. Caterpillars were selected at random to be reared on either the novel host plant *Plantago lanceolata* (N= 97), or *Lupinus argenteus* (N= 93). *Plantago lanceolata* was collected from Idlewilde Park in Reno, NV. *Lupinus argenteus* was collected from Lake Forest Beach in Tahoe City, CA. Approximately half of the caterpillars from each plant group were then randomly chosen to be infected with JcDNV at a concentration of 10^7 viral particle per µl. Caterpillars chosen to be infected were infected on the 1st day of their 5th instar (see Bioassay). JcDNV was sent to our lab after being isolated and purified in the M. Ogliastro Lab (Mutuel et al., 2010). Four days after each caterpillar’s 5th instar molt, hemolymph was collected to access the phenol-oxidase activity. Caterpillars were then reared to adulthood to assess for survival. All individuals who emerged from their pupae were recorded as individuals who survived.

The second experiment (‘*L. albifrons* as native’ experiment) was conducted in Spring 2017 and followed the same methods used for the ‘*L. argenteus* as native’ experiment except for a few differences: the native host plant and viral concentration. In the ‘*L. albifron*ss as native’ experiment, caterpillars were selected at random to be reared on either the novel host plant *Plantago lanceolata* (N= 83), or *Lupinus albifrons* (N= 85). This change was made because previous populations of *Lupinus argenteus* used in the first experiment were not robust enough to be used in the second experiment. Like the ‘*L.*

argenteus as native' experiment, *Plantago lanceolata* was collected from Idlewilde Park in Reno, NV. *Lupinus albifrons* was collected in Humboldt-Toiyabe National Forest near Verdi, CA. After observing a 24% survival rate in the '*L. argenteus* as native' experiment, the viral concentration was lowered to 10^6 viral particles per µl for the '*L. albifrons* as native' experiment in order to rear more individuals to adulthood and assess for survival.

The third experiment (artificial diet experiment) was conducted in Fall 2017. The purpose of this experiment was to measure the difference in JHE activity in infected and non-infected caterpillars. Caterpillars and virus were obtained in the same way as the previous experiments. The same temperature and light conditions used in previous the experiments were also used in this one. Caterpillars were reared on an artificial diet obtained from Southland Products. Caterpillars were randomly separated into three viral groups and a control. Caterpillars were infected with JcDNV on the same day as their 5th instar molt (see Bioassay). The highest viral group was infected with JcDNV at a concentration of 10^7 viral particle per µl (N=60). The median viral group was infected with JcDNV at a concentration of 10^5 viral particle per µl (N=60). The lowest viral group was infected with JcDNV at a concentration of 10^3 viral particle per µl (N=60). The control group did not receive any virus (N=60). In each group, caterpillars were randomly assigned a time stamp for hemolymph collection (Day 0, Day 1, Day 2, Day 3). The day represents the days following the caterpillars 5th instar molt. For example, a caterpillar chosen for hemolymph collection on Day 2, had hemolymph collected two days after their 5th instar molt. For each day, 12 caterpillars were chosen from each viral concentration group for hemolymph collection. Hemolymph was used to measure PO

activity and JHE activity. Caterpillars were then reared to adulthood to assess for survival.

2.3 Bioassay

For the experiments utilizing plants, newly molted 5th instar *V. cardui* larvae were inoculated with JcDNV at a concentration of 10^7 viral particle per µl for the '*L. argenteus* as native' experiment and 10^6 viral particles per µl for the '*L. albifrons* as native' experiment. The larvae were inoculated with JcDNV by placing 1 µl of JcDNV solution on a small leaf disk obtained by hole punching a leaf with a hole puncher. A virus coated leaf disk was placed in an empty 1 oz container with one individual. To ensure that the leaf disk was consumed, and the caterpillars were inoculated, the individuals were restricted from additional food for 24 hours. Survival and mortality were analyzed daily.

For the artificial diet experiment, the diet was prepared according to the manufacturer's directions: 81g of powder was added to 465 ml of boiling water and agar was allowed to solidify overnight. Caterpillars were inoculated on the day they reached their 5th instar. Caterpillars were randomly chosen to be inoculated with JcDNV using three different concentrations 10^7, 10^5 , and 10^3 viral particles per µl (High, Medium and Low respectively). Caterpillars were inoculated the same as above except uniform, small cubes of artificial diet were used instead of leaf disks.

2.4 Hemolymph Collection

For all experiments hemolymph samples were collected. For experiments utilizing

plants, hemolymph samples were collected from larvae four days after their 5th instar molt. For the artificial diet experiment hemolymph samples were obtained from the caterpillars during the four days following their 5th instar molt. Each of the viral concentration groups were randomly split into 4 groups (N= 15), and each group was assigned a different time day for hemolymph collection (Day 0, Day 1, Day 2, Day 3). Day 0 refers to the day that the caterpillar had its 5th instar molt (the day the caterpillars were infected with JcDNV). Caterpillars that were selected to be in the Day 0 group had hemolymph collected right before they were infected with the virus (see Bioassay).

For all experiments, hemolymph was collected by gently piercing the cuticle of the A1 abdominal segment with a hand-pulled Pasteur pipette needle (Smilanich et al., 2009a). Caterpillars were then squeezed until a total of 20 µl (split into two samples of 10 µl) was collected using a micropipette. A total of 10 µl was added to 500 µl of ice cold phosphate buffered saline in a 1.5 mL Eppendorf tube. This sample was set aside for the PO assay. The other 10 µl sample was added to a separate 1.5 mL Eppendorf tube to be used for the hemocyte count (for '*L. argenteus* as native' and '*L. albifrons* as native' experiments) or the JHE assay (for the artificial diet experiment). All solutions and supplies (including pipette tips) were kept on ice during this process to prevent degradation.

2.5 Immune Assay

Two separate approaches were used to assess the caterpillar immune response. A colorimetric assay was used to measure phenol-oxidase(PO) activity in 5th instar caterpillars (Adamo, 2004a). Hemocyte counts were also collected as an additional

measure of the immune response (Triggs and Knell, 2012).

PBS-bound hemolymph solution (see hemolymph collection) was stored in the refrigerator until the assay was ready to be conducted. Samples were stored for no longer that 8 hours. Once all samples were prepped, they were vortexed and 100 µl of each sample was loaded into a 96 well plate in duplicate. 200 µl of L-DOPA (0.118g L-DOPA in 30ml deionized water) was added to each well and the reading was recorded immediately using and an iMark Microplate Absorbance Reader (Bio-Rad). The absorbance was read at 490 nm every 30s for 45m. The slope of the resulting line was used to analyze PO activity. For analyses, only the linear portion of the curve was utilized. All samples were linear until 30min, so the graphs were truncated at 30min.
To conduct the hemocyte counts, hemolymph was collected as described above. For each sample, 10 µL was placed into 20 µl anticoagulant (0.684g EDTA, 0.346g citric acid in 180 ml PBS). Samples were stored in at - 20˚C up to two weeks before being counted. To count the hemocytes, 10µl of each hemolymph-anticoagulant solution was examined under a light microscope using a Neubauer Bright-Line hemocytometer. All cells within the full grid were counted and recorded. Samples were analyzed in duplicate and the average between them was used for analysis.

2.6 JHE Activity

To understand if JcDNV infection alters the levels of juvenile hormone by altering the juvenile hormone esterase activity, JHE activity was measured according to a previously described method (Hammock and Sparks 1977). Modifications to the original method were made as described by another study (El-Sheikh et al., 2016). Hemolymph

was collected as described above. After collection, hemolymph was pooled by taking 10µl from four individuals and combining them in a micro-centrifuge tube. Samples were stored at - 20˚C prior to JHE assay. The pooled samples were then diluted 1:19 with a sodium phosphate buffer solution (50 mM sodium phosphate buffer at a pH7.4 containing 0.1 mg/ml of BSA) (SPB: 38.7 ml of 1 M Na2HPO4 and 11.3 ml of 1M NaH2PO4 added to 1 liter with dd H2O).

The JHE assay was prepared in triplicate by reacting 100µl of hemolymph solution with 1 µl of tritium-labeled juvenile hormone III solution (1:4 tritium-labeled JH III:JH III). The reaction mixture was incubated at 30°C for 30 minutes. To stop the reaction 100 µl of basic methanol (10:9:1 methanol:water:ammonium hydroxide) was added to each solution. After that, 250 µl of iso-octane was added to all tubes and the tubes were vortexed repeatedly (21 times) so that the aqueous and organic layers were thoroughly mixed. To separate the aqueous and organic layers, samples were centrifuged for 5 minutes at 4,000 rpm, 1787 xg. Scintillation vials were prepped by adding 1ml of scintillation cocktail and 50µl of the aqueous layer from each sample into a scintillation vial. Counts per minute were analyzed using a liquid scintillation counter.

2.7 Statistical Analysis

All statistical analysis was performed in the free programing software R 3.4.1 and RStudio. To analyze hostplant/diet effects and viral effects on PO activity logistic regression analysis was performed using the GLM model with “binomial” as the family and “logit” as the link. PO values less than zero were excluded in this analysis. Either host plant/diet (*P. lanceolata, L. argenteus, L. albifrons* or artificial) or viral infection

status (yes or no) were used as predictor variables. PO activity was used as the dependent variable.

To analyze hostplant/diet effects and viral effects on development time a standard t-test was ran. This demonstrates whether the means for the two conditions were significantly different but does not identify any predictor variables. Pre-infection development time was counted from hatch date to date of infection (first day of the caterpillar's 5th instar). Post-infection development time was counted from date of infection to date of pupation ('*L. albifrons* as native' experiment and 'artificial diet' experiment) or date of eclosion ('*L. argenteus* as native' experiment). Host plant/diet (*P. lanceolata*, *L. argenteus*, *L. albifrons* or artificial) or viral infection status (yes or no) were used as the grouping variables.

To analyze the interaction between host plant (novel or native) and viral infection status (yes or no) on PO activity a two-way ANOVA was used with a linear model. Both host plant and viral infection status were used as the independent variables while PO activity was used as a dependent variable. Data was Log transformed so residuals could be distributed more normally. One drawback from this analysis is that PO activity residuals did not satisfy the Shapiro Wilk test for normality (null hypothesis is data is normal, $P = 0.024$). However, the residuals looked normal when graphed. Since there is evidence that ANOVAs can still be accurate with moderate deviances from normality (Glass et al. 1972, Harwell et al. 1992, Lix et al. 1996) we decided to include the two-way ANOVA data.

Linear regression models were performed to draw comparisons between development time, weight and PO activity. In Figures 9, 10 and 15 the variable labeled on

the x-axis was used as the predictor variable, while the variable labeled on the y-axis was used as the dependent variable.

3. Results

3.1 *L. argenteus* as native experiment

Host plant and infection status influenced the larval development time. Caterpillars reared on *P. lanceolata* reached their 5th instar sooner than caterpillars reared on *L. argenteus* (t-test: $t = 24.570$, $df = 158$, $P < 0.001$, $N = 189$, Figure 1). The development period from hatching to the start of the 5th instar was used for analysis to avoid including the effects of the virus (larvae were inoculated with JcDNV in their 5th instar). On average, the caterpillars feeding on *P. lanceolata* reached their 5th instar 5.65 days (30%) sooner than those feeding on *L. argenteus*. Interestingly, viral infection status also had an impact on development time. Caterpillars infected with JcDNV had a shorter development period and reached adulthood sooner than the control caterpillars (t-test: $t = 6.779$, $df = 26$, $P < 0.001$, $N = 73$, Figure 2). The post-infection development time included the days from infection (start of the 5th instar) until the day of eclosion. On average, caterpillars infected with JcDNV reached adulthood 4.23 days (22%) sooner than those not infected with the virus.

Caterpillars reared on *P. lanceolata* had a higher average absorbance for the PO assay compared to caterpillars reared on *L. argenteus*, suggesting that caterpillars feeding on *P. lanceolata* had increased immunocompetence compared to those feeding on *L. argenteus* (logistic regression: $Z = 2.578$, $P = 0.010$, $N = 121$, *P. lanceolata* mean = 32.835, *L. argenteus* mean = 18.945, Figure 3). The average PO activity of caterpillars

feeding of *P. lanceolata* was 73% higher than caterpillars feeding on *L. argenteus*. There was also a clear interaction between host plant and viral infection status on PO activity. Caterpillars infected with JcDNV and feeding on *L. argenteus* had suppressed PO activity compared to caterpillars feeding on *P. lanceolata* (anova: F = 7.890, P = 0.006, N = 156, DF = 1, Figure 4). One possible limitation in this experiment is that PO activity was not a significant predictor of survival (logistic regression: Z = 0.775, P = 0.439, N = 121). This result may have been influenced by the small sample size caused by high mortality. Only 24% of the infected individuals survived to adulthood. This lowered the sample size in the virus infected group (N=17) and hampered the ability to run accurate models predicting survival.

3.2 *L. albifrons* as native experiment

Host plant and infection status impacted development time in the '*L. albifrons* as native' experiment. Caterpillars reared on *P. lanceolata* reached their 5th instar 2.03 days (14%) sooner than caterpillars reared on *L. albifrons* (t-test: t = 9.024, df = 143, P < 0.001, N = 168, Figure 5). Caterpillars infected with JcDNV reached pupation sooner than controls; however, the total effect of JcDNV infection was lower in the second experiment compared to the first. The effect of JcDNV on development was only observed between infection and pupation with caterpillars infected with JcDNV reaching pupation 0.89 days (13%) sooner (t-test: t = 2.945, df = 73, P = 0.004, N = 77, Figure 6). This impact was negligible when looking at the post infection to adult eclosion development time. When measuring the time of infection to day of eclosion, caterpillars infected with JcDNV reached adulthood only 0.68 days (5%) sooner than control

caterpillars. This difference was insignificant since JcDNV infected caterpillars' development time was not significantly different from the controls (t-test: t =1.310, df = 43, N = 45, P = 0.191).

Host plant did not impact immunocompetence in the '*L. albifrons* as native' experiment. There were no significant differences in PO activity between *L. albifrons* and *P. lanceolata* (logistic regression: Z = -0.634, P = 0.526, N = 121, *P. lanceolata* mean = 41.639, *L. albifrons* mean = 47.246, Figure 7). The mean PO activity was higher for both the native and novel host plant in this experiment compared to the '*L. argenteus* as native' experiment. Although the average PO activity was higher for caterpillars reared on *P. lanceolata* in this experiment compared to the first, the difference was not significant (logistic regression: Z = 1.28, P = 0.216, N = 180). This demonstrates that the PO values for caterpillars reared on *P. lanceolata* were not inconsistent between the two experiments. However, there was a significant interaction between host plant and viral status on PO activity in this experiment (anova: F = 6.441, P = 0.012, N = 143, DF = 1, Figure 8). Caterpillars that were not infected with JcDNV and were reared on *P. lanceolata* expressed higher PO activity than those reared on *L. albifrons*. Caterpillars that were infected with JcDNV and were reared on *P. lanceolata* experienced a suppressed immune response compared to those reared on *L. albifrons*. This was opposite of what was observed in the '*L. argenteus* as native' experiment where caterpillars reared on *P. lanceolata* had increased immunocompetence when infected with JcDNV.

Development time was a predictor of PO activity as well as weight. Linear regression analysis was used to identify a positive linear relationship between development time and PO activity (linear regression: P = 0.022, Adjusted R-squared =

0.137, Figure 9). This result shows that caterpillars that grew more quickly had a lower PO activity. Linear regression analysis was also used to identify a negative linear relationship between development time and larval weight (linear regression: P = 0.001, Adjusted R-squared = 0.272, Figure 10). This result shows that caterpillars who grew more quickly tended to have a larger weight. Host plant also impacted larval weight with caterpillars reared on *L. albifrons* having a lower weight than those reared on *P. lanceolata* (logistic regression: Z = 5.442, P < 0.001, N = 148, *L. albifrons* mean = 0.331, *P. lanceolata* mean = 0.412, Figure 11).

3.3 Artificial diet experiment

Infection with JcDNV did not cause caterpillars to develop quicker when caterpillars were reared on an artificial diet. Control caterpillars had a shorter post-infection development time than all three viral conditions (t-test: t = -5.47, df = 87, P < 0.001, N = 163, Figure 12). Post-infection development time was measured from day of infection (the first day of the caterpillar's fifth instar) to pupation. Post-infection time means were 5.479 days for the control group and 6.179 for the combination of the viral group. This suggests that the impact of the virus was limited (less than a day difference). Caterpillars did gain more weight when infected with JcDNV compared to controls (logistic regression: Z = 4.881, P < 0.001, N = 160, Figure 13). On average, the control caterpillars had a weight gain of 1.7% from the beginning of their fifth instar to their pupal stage. JcDNV infected caterpillars had a weight gain of approximately 30.5% from the beginning of their fifth instar to their pupal stage.

The average absorbance in the PO assay was low for all conditions with the

control, high, medium and low condition groups having an average absorbance of 1.619, 1.366, 1.495, and 1.724 respectively. The PO activity of caterpillars reared on the artificial diet was significantly lower than caterpillars reared on the plant diets (logistic regression: Z = -3.318, P = 0.009, N = 214, Figure 14). On average the PO activity was 2.6 - 9.8% of the activity observed by any of the plant diets. PO activity was also a predictor for larval weight with higher PO activity indicating a lower larval weight (linear regression: t = -2.807, Adjusted R- Square = 0.157, P = 0.008, Figure 15).

Upon noticing that the changes in development time were not consistent with the '*L. argenteus* as native' and '*L. albifrons* as native' experiments we chose to only measure JHE activity for caterpillars infected with the high concentration of JcDNV (10^7 viral particle per µl) and controls. The results were inconclusive since JHE activity spiked before the effects of the virus could be realize (Figure 16). To properly interpret whether JcDNV had an impact on JHE activity, Day 0 for the JcDNV infected caterpillars should have been similar to controls since Day 0 hemolymph collection occurred right before the caterpillars were infected with JcDNV. Therefore, the results from Figure 16 indicate that there may be a lot of individual variation between the caterpillars. More replicates are needed to obtain more accurate results and draw meaningful conclusions.

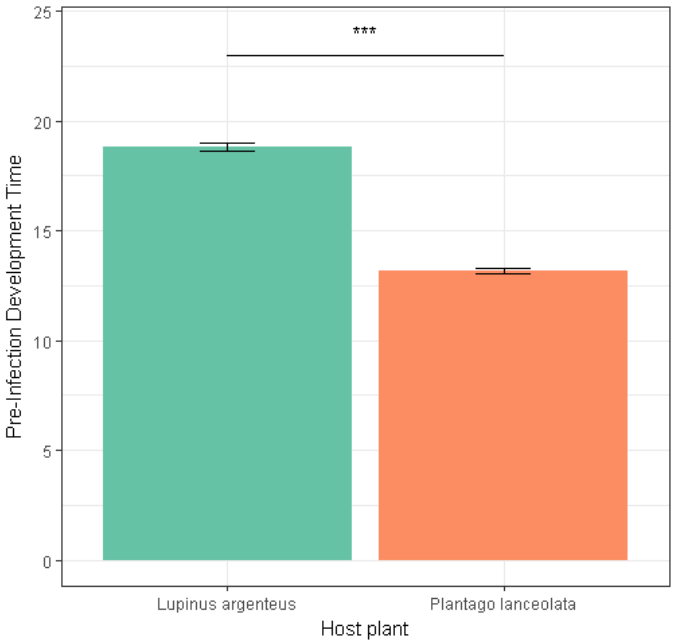

Figure 1: Data were taken from the '*L. argenteus* as native' experiment. Graph depicts the average pre-infection development time (day of hatch to day of infection) of caterpillars reared on the native hostplant, *L. argenteus* and the novel hostplant, *P. lanceolata*. Caterpillars were infected on the day they reached their 5th instar. There was a significant difference between the host plants. Error bars represent the standard error of the mean.

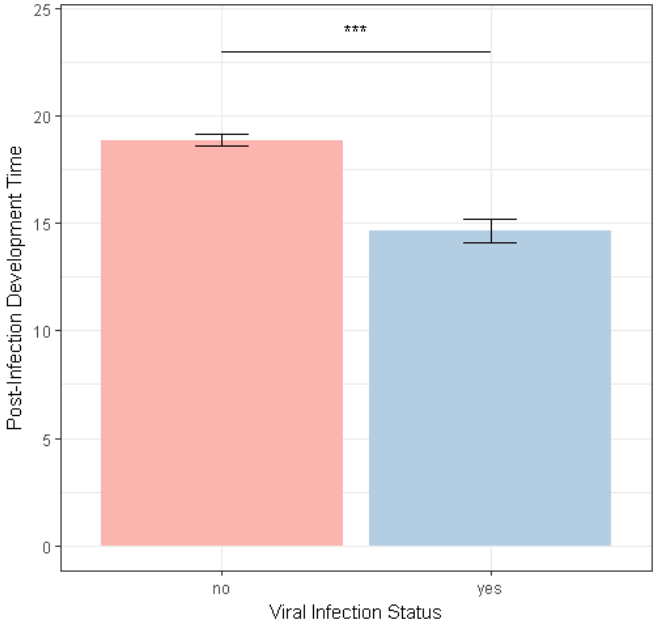

Figure 2: Data were taken from the '*L. argenteus* as native' experiment. The graph depicts the average post-infection development time (day of infection to day of emergence from pupae) of caterpillars infected with JcDNV and controls. Caterpillars were infected on the day they reached their 5th instar. There was a significant difference between caterpillars infected with JcDNV and control caterpillars. Error bars represent the standard error of the mean.

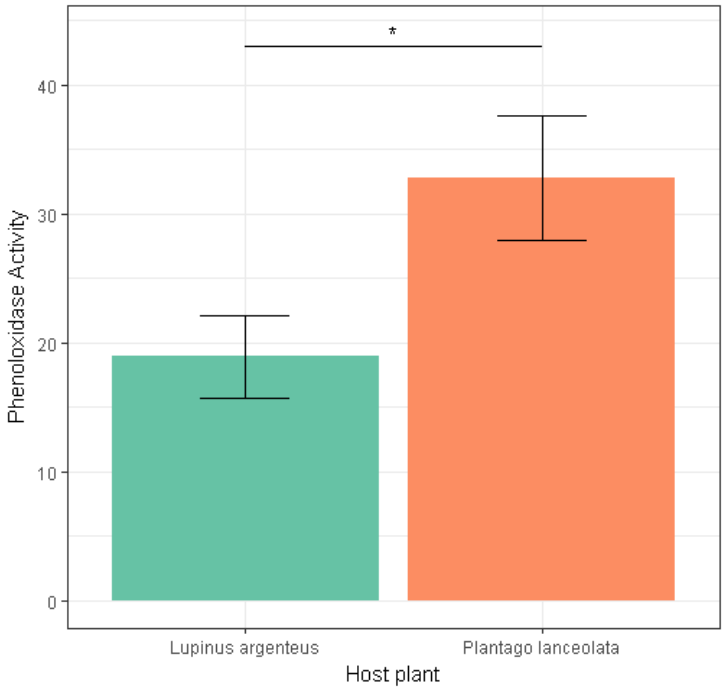

Figure 3: Data were taken from the '*L. argenteus* as native' experiment. The graph depicts the average phenol-oxidase activity between caterpillars reared on the native hostplant, *L. argenteus* and the novel hostplant, *P. lanceolata*. Includes caterpillars infected with JcDNV and controls. There was a significant difference between the host plants. Error bars represent the standard error of the mean.

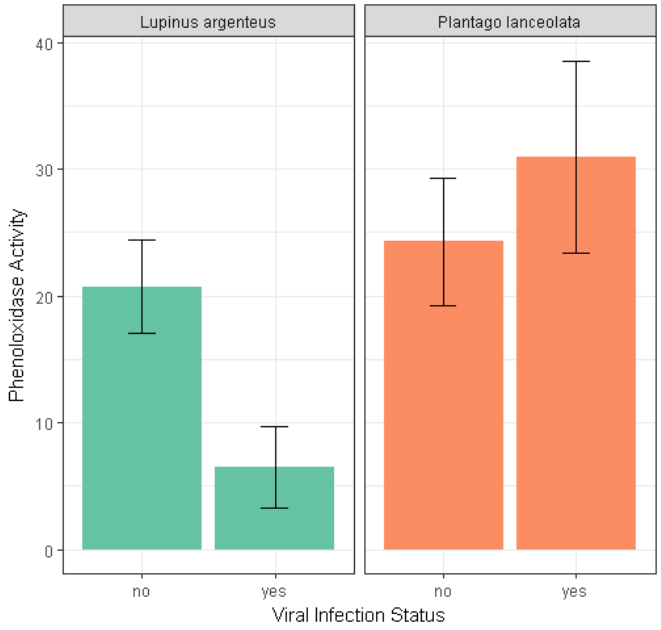

Figure 4: Data were taken from the '*L. argenteus* as native' experiment. The graph depicts the average phenoloxidase activity grouped by host plant and infection status. There was a significant interaction between infection status (virus vs. no virus) and host plant (*L. argenteus* vs. P.lanceolata). Error bars represent the standard error of the mean.

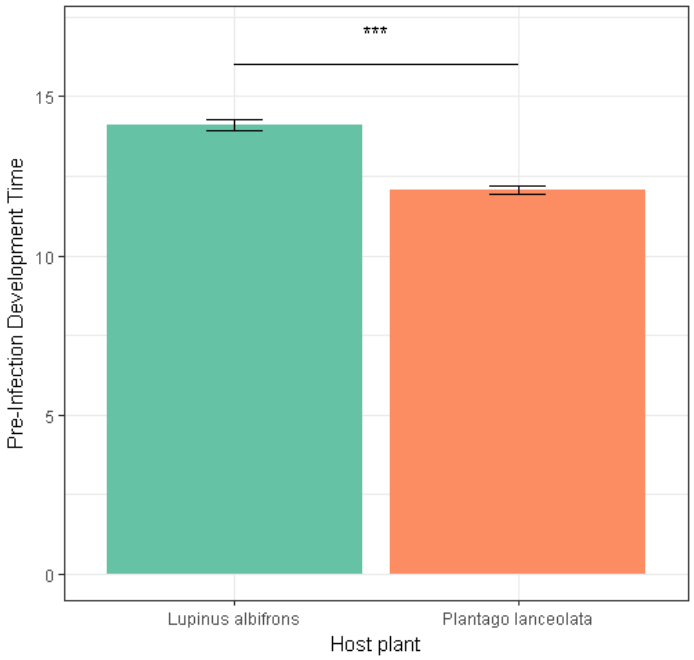

Figure 5: Data were taken from the '*L. albifrons* as native' experiment. The graph depicts the average pre-infection development time (day of hatch date to day of infection) of caterpillars reared on the native hostplant, *L. albifrons* and the novel hostplant, *P. lanceolata*. Caterpillars were infected on the day they reached their 5th instar. There was a significant difference between the host plants. Error bars represent the standard error of the mean.

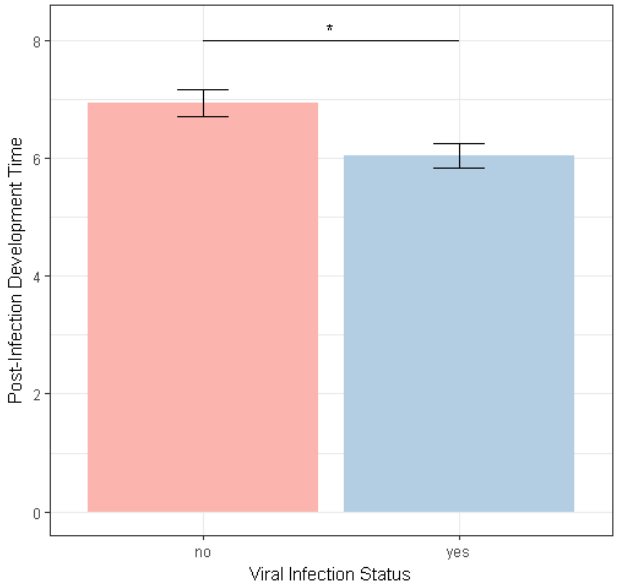

Figure 6: Data were taken from the '*L. albifrons* as native' experiment. The graph depicts the average post-infection development time (day of infection to day of pupation) of caterpillars infected with JcDNV and controls. Caterpillars were infected on the day they reached their 5th instar. There was a significant difference between caterpillars infected with JcDNV and control caterpillars. Error bars represent the standard error of the mean.

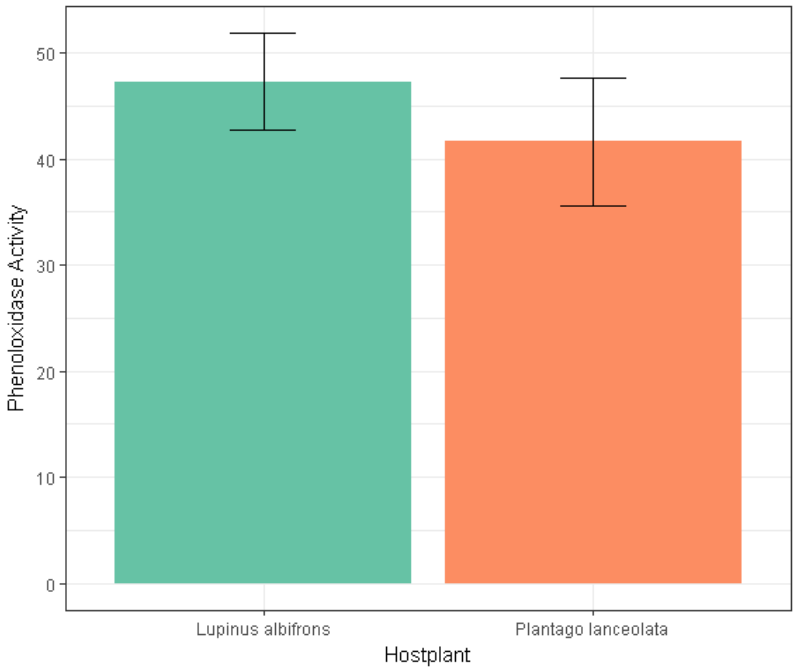

Figure 7: Data were taken from the '*L. albifrons* as native' experiment. The graph depicts the average phenoloxidase activity between caterpillars reared on the native hostplant, *L. albifrons* and the novel hostplant, *P. lanceolata*. Includes caterpillars infected with JcDNV and controls. There was no significant difference between the host plants. Error bars represent the standard error of the mean.

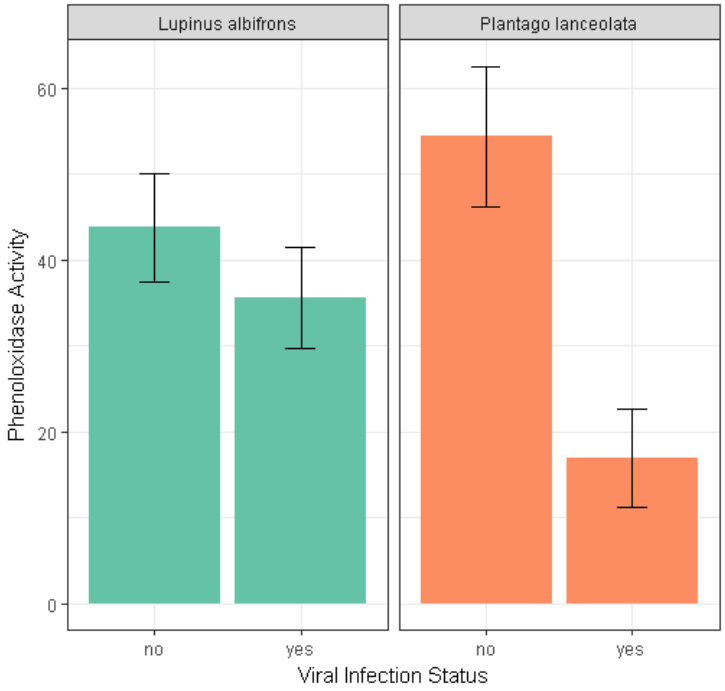

Figure 8: Data were taken from the '*L. albifrons* as native' experiment. The graph depicts the average phenol-oxidase activity grouped by host plant and infection status. There was a significant interaction between infection status (virus vs. no virus) and host plant (*L. albifrons* vs. P. lanceolata). Error bars represent the standard error of the mean.

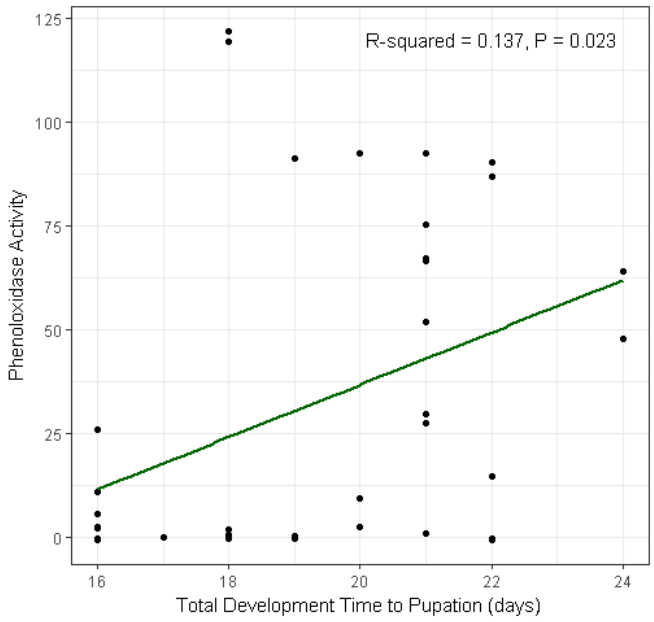

Figure 9: Data were taken from the '*L. albifrons* as native' experiment. Graph shows a scatterplot of phenol-oxidase activity split by development time (day of hatch to day of pupation). Only caterpillars that were infected with JcDNV were included. The line that overlays the scatterplot was determined using linear regression analysis. Development time was a significant predictor of phenoloxidase activity.

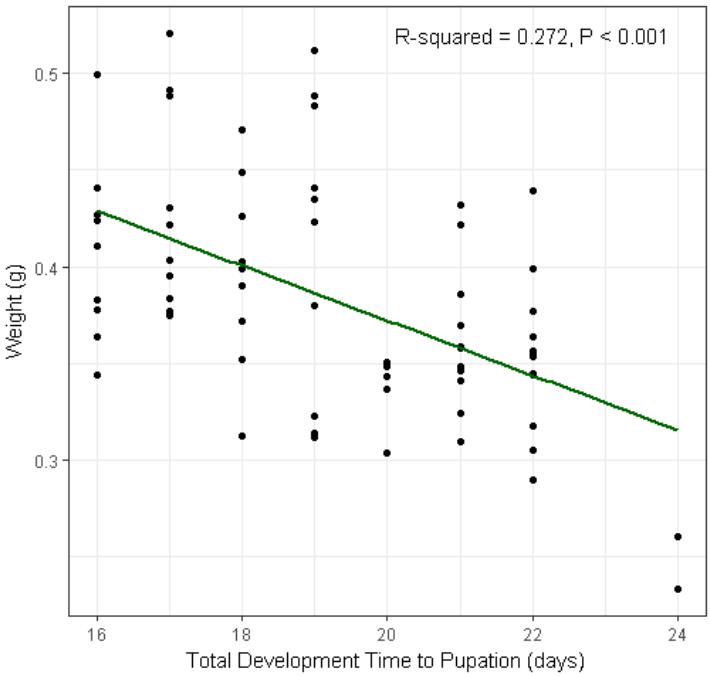

Figure 10: Data were taken from the '*L. albifrons* as native' experiment. Graph shows a scatterplot of weight activity split by development time (day of hatch to day of pupation). All caterpillars were included. The line that overlays the scatterplot was determined using linear regression analysis. Development time was a significant predictor of weight.

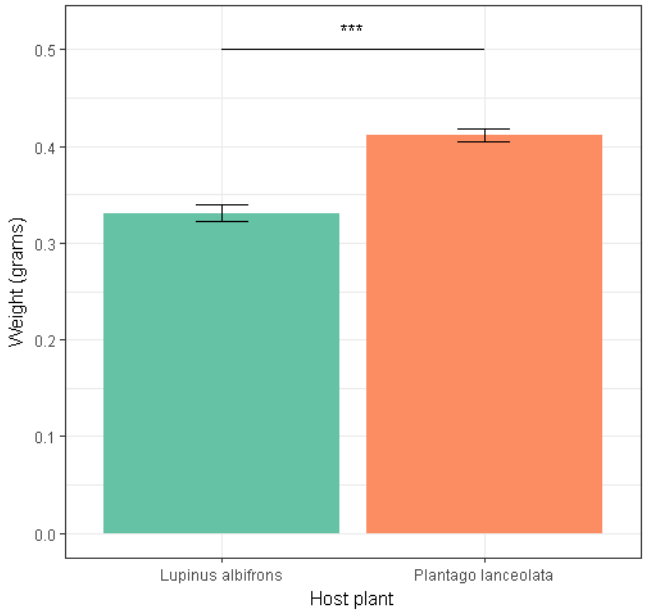

Figure 11: Data were taken from the '*L. albifrons* as native' experiment. The graph depicts the average larval weight of caterpillars reared on *L. albifrons* and *L. argenteus*. Weight was measured on the first day of the caterpillar's 5th instar before caterpillars were infected with JcDNV. There was a significant difference between the host plants. Error bars represent the standard error of the mean.

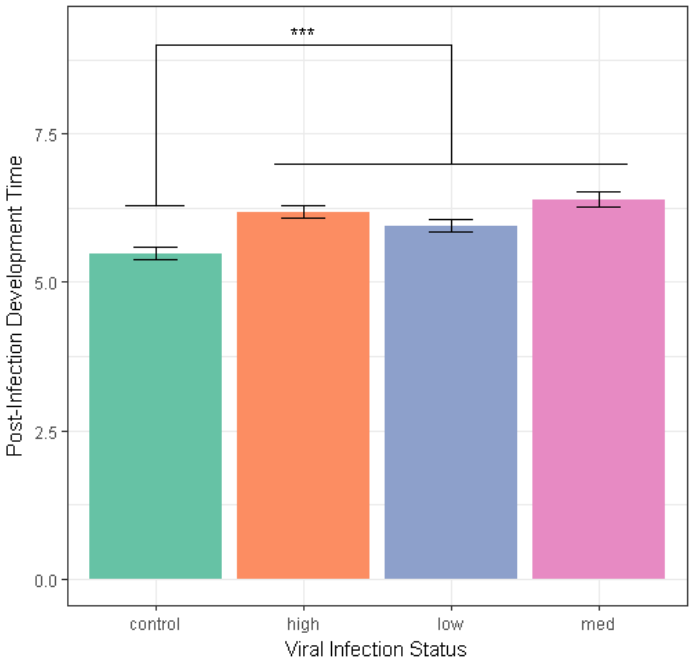

Figure 12: Data were taken from the 'artificial diet' experiment. The graph shows the average post-infection development time (days from infection to pupation) between caterpillars at a high, medium, low and control JcDNV virus condition. The was a significant difference between control and viral conditions. Error bars represent the standard error of the mean.

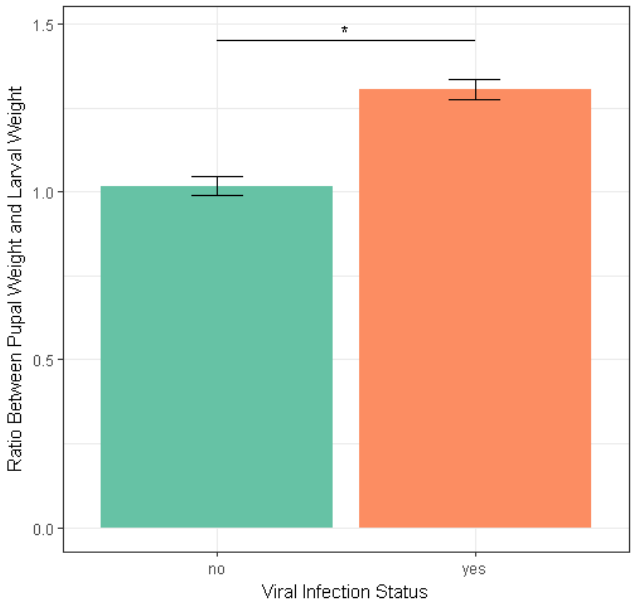

Figure 13: Data were taken from the 'artificial diet' experiment. The graph shows the average ratio between pupal weight and larval weight. Larval weight was measured on the first day of the caterpillar's 5th instar. The ratio between pupal weight and larval weight was significantly higher in caterpillars infected with JcDNV. Error bars represent the standard error of the mean.

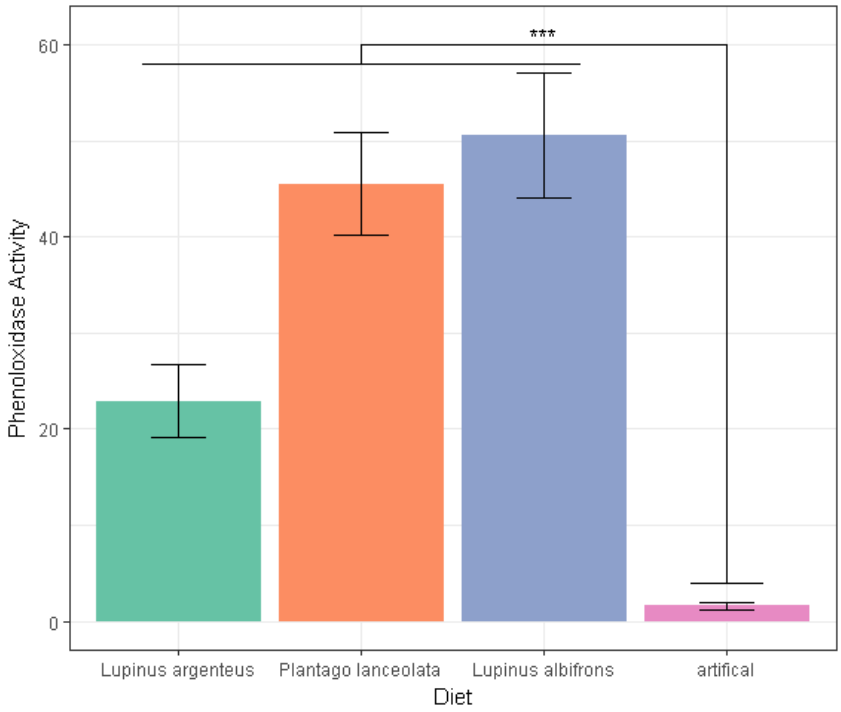

Figure 14: Data were taken from all three experiments: '*L. argenteus* as native', '*L. albifrons* as native', and the 'artificial diet' experiments. Only viral control caterpillars were used for analysis. Larvae reared on *P. lanceolata* from both the '*L. argenteus* as native' and '*L. albifrons* as native' experiments were combined into one group. There was a significant difference between the artificial diet and plant diets. Error bars represent the standard error of the mean.

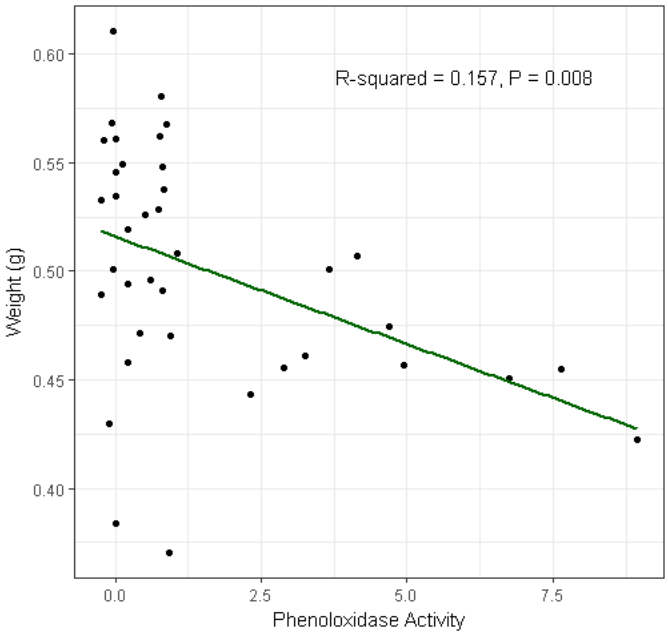

Figure 15: Data were taken from the 'artificial diet' experiment. The graph shows a scatterplot of pupal weight by phenol-oxidase activity. The line overlaying the scatterplot was determined using linear regression analysis. Phenol-oxidase activity was a significant predictor of pupal weight.

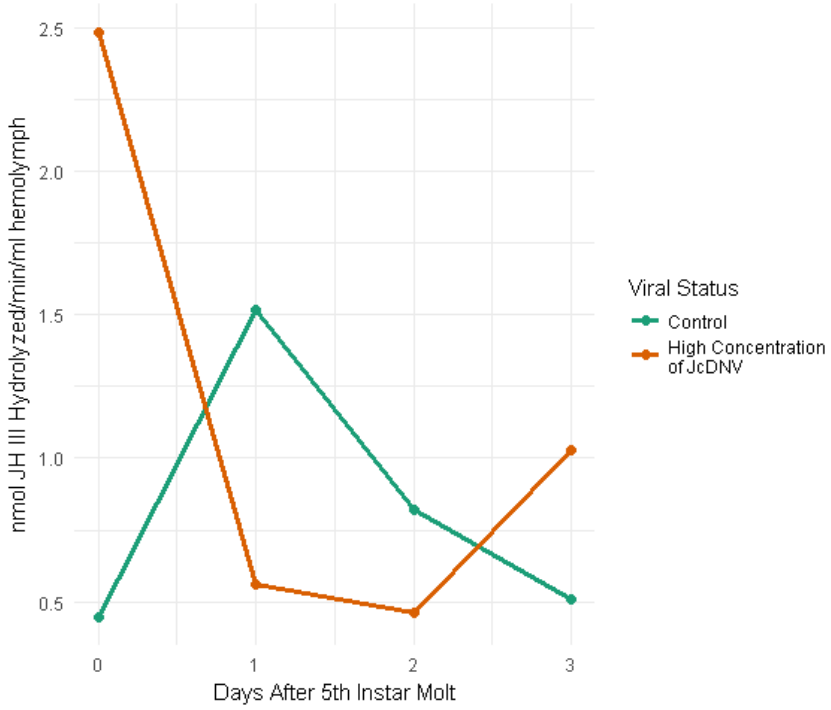

Figure 16: Data were taken from the 'artificial diet' experiment. Graph depicts JHE activity following infection with JcDNV. Day 0 is the day that the caterpillars were infected with JcDNV. Only caterpillars infected with the high concentration of JcDNV (10^7 viral particle per µl) and controls were analyzed for JHE activity to see if there were any differences.

4. Discussion

This study explored how diet and JcDNV infection influenced caterpillar development and immunity in *V. cardui*. We aimed to understand how both host plant chemistry and infection status drives *V. cardui* to incorporate certain host plants into its diet breadth. We also analyzed how JcDNV could alter the biochemistry of *V. cardui* to cause larvae to develop faster and how this effect is diet dependent. One of the main findings in this study is that the novel host plant, *P. lanceolata*, provides additional protections compared to the native host plants: *L. argenteus* and *L. albifrons*. These

protections include faster development time compared to larvae reared on *L. argenteus* or *L. albifrons*, and higher immunocompetence than larvae reared on *L. argenteus*. The other main finding is that the effects of JcDNV are diet dependent, and the changes in development time may be caused by altered levels of JHE activity or changes in larval weight.

In the experiments that utilized plants, development time was highly variable which can provide insight into some of the potential reasons why *P. lanceolata* has been incorporated into the diet breadth of *V. cardui*. In both the '*L. argenteus* as native' experiment and '*L. albifrons* as native' experiment, caterpillars feeding on the novel host plant, *P. lanceolata*, developed more quickly and reached adulthood in less time than individuals feeding on one of the native host plants, *L. argenteus* or *L. albifrons* (Figure 1 and Figure 5). By reaching adulthood sooner, it can be inferred that caterpillars feeding on *P. lanceolata* would also be able to mate and reproduce sooner than those reared on *L. argenteus* or *L. albifrons*. This will allow caterpillars feeding on *P. lanceolata* to have shorter generation times, suggesting that a population utilizing *P. lanceolata* will be able to supply more offspring during a mating season compared to a population feeding on *L. argenteus* or *L. albifrons*. This could help explain why we are observing populations of *V. cardui* utilizing *P. lanceolata*. One limitation to this inference is that it is still unclear whether caterpillars reared on *P. lanceolata* will have an oviposition preference for *P. lanceolata* as an adult. Previous research shows that *V. cardui* will lay eggs indiscriminately in nectar reach areas (Janz, 2005), which offers some evidence that *V. cardui* butterflies might not have any preference to lay eggs on *P. lanceolata*. However, further research is still needed to fully determine if *V. cardui* larvae reared on *P.*

lanceolata will preferentially oviposit on *P. lanceolata* as adults.

JcDNV infection status also had an impact on development time. In both the '*L. argenteus* as native' and '*L. albifrons* as native' experiments, JcDNV infected caterpillars experienced a quickening in development time compared to non-infected controls (Figure 2 and Figure 6). This result is consistent with previously reported data which demonstrated that J. coenia larvae developed more quickly when infected with JcDNV (Smilanich et al., 2018). By comparing the first two experiments in this study, there is also some evidence that suggests this effect is dependent on viral concentration. In the '*L. argenteus* as native' experiment, JcDNV (10^7 viral particle per µl concentration) infected larvae emerged 4.23 days (22%) sooner than those not infected with the virus (Figure 2). In the '*L. albifrons* as native' experiment, JcDNV (10^6 viral particle per µl concentration) infected larvae emerged 0.68 days (5%) sooner than control caterpillars (this result was not significant). These results demonstrate that larvae developed quicker when given a high concentration of JcDNV compared to a lower concentration. For the '*L. albifrons* as native' experiment, the increased development time was only significant when looking at recently infected larvae (time from infection to pupation) (Figure 6) but the effect disappeared when looking at the total post-infection development time (time of infection to eclosion). This demonstrates that the effect JcDNV on development time may be temporary and occur only for a few days following JcDNV infection. Overall, these results provide evidence that the change in development time caused by JcDNV is concentration dependent with higher concentrations of JcDNV causing larvae to develop faster. However, this effect does not occur when larvae are reared on an artificial diet. For the 'artificial diet' experiment, JcDNV infected larvae experienced a slightly prolonged

development time compared to controls (Figure 12). This result is opposite of what was observed when larvae were reared on the plant diets. This demonstrates that the effects of JcDNV on development time is also dependent on diet.

What properties of the diet are causing the effects of JcDNV to vary is still unclear. Previous research done with *Spodoptera littoralis* (Boisduval) showed that larvae reared on a high-quality protein diet developed faster than larvae reared on a low-quality protein diet (Lee et al., 2008). Therefore, it is possible that when larvae are provided with a nutrient rich diet, like the artificial diet, they have abundant resources to utilize so they are not under stress of allocation costs. This may negate the usual effects of JcDNV since larvae can only develop so quickly. However, this does not explain why JcDNV infected larvae reared on the artificial diet had the opposite response of caterpillars reared on the plant diets (Figure 2, Figure 6 and Figure 12). Although the difference in development time was small between JcDNV infected larvae and controls in the 'artificial diet' experiment, it suggests that the effects of JcDNV can be switched based on diet. Overall, more research is needed to fully understand what dietary factors are causing the effect of JcDNV to change.

To help explain how JcDNV is impacting development time, we offer two possible explanations. First, it is possible that changes in JHE activity are contributing to the changes in development time; however, more research is needed to confirm this. We were able to demonstrate that JHE activity was not consistent between JcDNV infected and control larvae (Figure 16). However, it is unclear whether these differences were enough to change the development time since the changes in development time were small between JcDNV infected larvae and controls (Figure 12). The results from Figure

16 are also interesting because there was a substantial difference in JHE activity between infected and control individuals on Day 0. Since larvae were infected directly after hemolymph was extracted on Day 0, this difference cannot be contributed to JcDNV infection. This result may indicate that JHE activity is highly variable and more replicates are needed to accurately access JHE activity in *V. cardui*. Overall, to confirm whether JHE plays a major role during JcDNV infection, experiments should be ran using multiple plant diets and more replicates.

Another possible explanation for the change in development time is weight gain. In the 'artificial diet' experiment larvae infected with JcDNV experienced a 30.5% weight gain during their final instar; whereas healthy individuals only experienced a 1.7% weight gain during their final instar (Figure 13). One explanation for this observation is that JcDNV is causing the caterpillars to consume more so they can mount an immune response. Previous studies done with *S. littoralis* demonstrated that larvae had increased protein consumption during nucleopolyhedrovirus (NPV) infection (Lee et al., 2006; Lee et al., 2008). One potential reason for this response is that the larvae are consuming more to ensure they have enough resources to synthesis PO (a protein enzyme) and mount an immune response (Lee et al., 2006). However, during the 'artificial diet' experiment PO activity was severely suppressed (Figure 14). This suggests that an immune response was not being mounted. Therefore, it is possible that the larvae are allocating their resources towards development instead of immunity, so they can pupate sooner and escape the effects of the virus. This is supported by the fact that development time was a predictor variable for larval weight (Figure 10). Caterpillars that developed more quickly had a higher larval weight (Figure 10), indicating that larger

and heavier larvae develop more quickly. This suggests that caterpillars may be triggered to eat more during infection which can lead to them developing faster due to their weight gain. Both of these factors (JHE and weight gain) may simultaneously be influencing development time post JcDNV infection, which would help explain the variability in the results. Overall, more research is needed to fully understand how JcDNV is altering larval development and how that effect is dependent on diet.

We used PO activity an indicator of immunocompetence. Phenol-oxidase is responsible for converting phenols into melanin during melanogenesis (Gonzalez-Santoyo and Cordoba-Aguilar, 2012). This melanin can then be used for melanization: which is the process of depositing melanin around infected tissue (Gonzalez-Santoyo and Cordoba-Aguilar, 2012). In all the experiments, PO activity was highly variable suggesting that immunocompetence was highly dependent on diet. In the '*L. argenteus* as native' experiment, caterpillars reared on *L. argenteus* had a much lower immune response than the caterpillars reared on *P. lanceolata* (Figure 3). For the '*L. albifrons* as native' experiment there were no differences in PO activity between the two plants when looking at host plant alone (Figure 7). Since the average PO activity for the caterpillars reared on *P. lanceolata* was not significantly different between the two experiments, this demonstrates that *L. argenteus* is a poor host plant compared to both *L. albifrons* and *P. lanceolata*. This point is supported by the fact that caterpillars reared on *L. argenteus* and infected with JcDNV had a suppressed immune response compared to *P. lanceolata* (Figure 4). This suggests that the native host plant, *L. argenteus*, provides less protection against viral infection compared to *P. lanceolata*. In contrast, in the '*L. albifrons* as native' experiment, caterpillars reared on *L. albifrons* and infected with JcDNV had a

higher immunocompetence than those reared on *P. lanceolata* (Figure 8). This suggests that compared to some native hostplants, *P. lanceolata* will offer additional immune protection while compared to others, it will offer less protection. This conflicting result may also be due to other factors such as time of year the plants were collected. The '*L. argenteus* as native' experiment was conducted in the fall while '*L. albifrons*' as native experiment was conducted in the spring. The chemistry of *P. lanceolata* varies throughout the year with leaves having a higher iridoid glycoside concentration later in the season (Darrow and Bowers, 1997). Therefore, despite collecting the plants from the same location, it is likely the *P. lanceolata* used in the '*L. argenteus* as native' experiment contained higher levels of iridoid glycosides than the *P. lanceolata* used in the '*L. albifrons* as native' experiment. Since *V. cardui* has been shown to have a higher melanization response with increased iridoid glycoside sequestration (Lampert et. al, 2014), changes in PO activity between the two experiments could be caused by differing concentrations of iridoid glycosides found in *P. lanceolata* during different times of the season. Also, a lower concentration of the virus (10^6 viral particles of JcDNV per µl) was used in the '*L. albifrons* as native' experiment compared to the higher concentration (10^7 viral particles of JcDNV per µl) that was used in the '*L. argenteus* as native' experiment. This likely added to the variation between the experiments

The native host plants also had their own host plant chemistry. Both *L. argenteus* and *L. albifrons* contain alkaloids (Wink et al., 1995). Previous research with gypsy moth larvae (Lymantria dispa) showed that alkaloids can act as a deterrent for larvae (Shields et al., 2008). This suggests that alkaloids may impact feeding efficiency which could have negative effects on lepidopteran immunity and development. Therefore, alkaloid

concentrations may also be contributing to the discrepancies in PO activity and development time observed when larvae are reared on *L. argenteus* or *L. albifrons* and compared to *P. lanceolata*.

Interestingly, PO activity was severely suppressed in caterpillars reared on the artificial diet (Figure 14). With caterpillars only having a PO activity 2.6 - 9.8% that of the caterpillars reared on any of the plant diets, it suggests that diet has a huge impact on the larval immune response. Since the artificial diet was nutrient rich compared to the plant diets, this suggests that larvae may be triggered to mount their immune response based on the nutrient composition of their diet with less nutrient rich diets causing an increased PO response. Why this difference occurs is unclear. PO has been shown to be an energy costly enzyme (Gonzalez-Santoyo and Cordoba-Aguilar A, 2012), which is supported by the fact that we found higher PO activity to be predictive of a lower weight (Figure 15) and slower development to be a predictive of higher PO activity (Figure 9). This suggests that there are energy costs (lower weight and longer development) associated with high PO concentrations. Given this relationship, it would be expected that caterpillars receiving an excess of calories would be more equipped to synthesize this enzyme. However, the opposite is happening. This suggests that either larvae are lacking a necessary precursor to synthesize the enzyme (which would be found in the host plant) or that they are not receiving a necessary signal from their environment (which would be found in or on the hostplant) which would trigger them to produce PO at higher concentrations. Either way, there is evidence that there are diet dependent effects on PO activity.

Future studies should include both hostplant chemistry and pathogen infection

status when analyzing host plant diet breadth in lepidopterans. This study demonstrated the life history traits and immunocompetence of *V. cardui* were influenced by both diet and infection with JcDNV. When analyzing development time, we saw that *P. lanceolata* was a superior host to *L. argenteus* and *L. albifrons*. There is also evidence that healthy larvae reared on *P. lanceolata* had a higher immunocompetence than those reared on *L. argenteus* or *L. albifrons*. This suggests that healthy larvae utilizing *P. lanceolata* will perform better than those reared on *L. argenteus* or *L. albifrons*. This could help explain why populations of *V. cardui* are utilizing *P. lanceolata*. However, this result changes when introducing JcDNV infection. Larvae that were reared on *L. albifrons* and were infected with JcDNV had higher immunocompetence than those reared on *P. lanceolata*. This makes it difficult to determine which hostplant (*P. lanceolata* or *L. albifrons*) is superior. One host plant offers a decrease in development time (*P. lanceolata*) and the other offers increased immunocompetence when challenged with JcDNV (*L. albifrons*). It is likely that the answer to this question differs based on pathogens in the environment. This could help explain the wide diet breadth in *V. cardui*. Since host plant fitness varies based on the pathogens in the environment, it is likely that this migratory lepidopteran needs to adopt several different host plants in order to have optimal survival.

This study also demonstrated that the effects of JcDNV are diet dependent. When caterpillars were reared on one of the plant diets (*P. lanceolata, L. argenteus* or *L. albifrons*) they experienced a quickening in development after infection with JcDNV. This result disappeared when larvae were reared on the artificial diet. PO activity was also suppressed in larvae reared on the artificial diet. These results may be related. We demonstrated that faster development corresponds with a lower PO activity which may

help explain why caterpillars reared on the artificial diet experienced different patterns in their development times.

Overall, I propose that there are two larval modes at play here. When larvae are given a nutritionally dense diet that lacks plant chemistry (like the artificial diet) they are receiving a signal (or lacking a signal) that encourages them to invest their energy into development and not the immune response. When larvae are given diets that are less nutritionally complete and contain more complex chemistry (alkaloids or iridoid glycosides) they are receiving signals that encourage them to invest in immunity instead of development. Whether it is more beneficial to invest in development or immunity is not clear. Overall, both of these modes could be contributing to the fitness of *V. cardui* in different ways, but it is unlikely that V. carudi is able to allocate resources to both modes simultaneously. This could also help explain the variability between infected and non-infected caterpillars. When larvae are infected with JcDNV, PO activity tends to decrease, and development time tends to increase. This offers support that *V. cardui* larvae are required to choose between development and immunity. Whether *V. cardui* will invest in immunity or development is dependent on both diet and infection status suggests that a tritrophic approach is necessary when analyzing the effects of host plant and infection status on *V. cardui*.

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References

Adamo SA. 2004. Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket Gryllus texensis. Journal of Insect Physiology 50(2-3):209-216.

Bowers, M.D., 1984. Iridoid glycosides and hostplant specificity in larvae of the buckeye butterfly, Junonia coenia (Nymphalidae). Journal of Chemical Ecolody 10: 1567–1577.

Bowers MD, Stamp NE, 1992. Chemical variation within and between individuals of Plantago lanceolata (Plantaginaceae). J. Chem. Ecol. 18, 985–995.

Cavers PB, Bassett IJ, Crompton CW. 1980. The biology of Canadian weeds Plantago lanceolata. Canadian Journal of Plant Science 60: 1269-1282.

Cory JS, Hoover K. 2006. Plant-mediated effects in insect-pathogen interactions. Trends in Ecology & Evolution 21(5):278-286.

Darrow K, Bowers MD. 1997. Phenological and population variation in iridoid glycosides of Plantago lanceolata (Plantaginaceae). Biochemical Systematics and Ecology 25(1):1-11.

Dyer LA, Bowers MD. 1996. The importance of sequestered iridoid glycosides as a defense against an ant predator. Journal of Chemical Ecology 22(8):1527-1539.

El-Sheikh EA. 2015. Efficacy of spodoptera littoralis nucleopolyherdovirus on spodoptera frugiperda (je smith) and spodoptera exigua (hubner): Virulence, biological effects, and inhibition of juvenile hormone esterase. Egyptian Journal of Biological Pest Control. 25(3):587-595.

El-Sheikh ESA, Kamita SG, Hammock BD. 2016. Effects of juvenile hormone (JH) analog insecticides on larval development and JH esterase activity in two spodopterans. Pesticide Biochemistry and Physiology 128:30-36.

Furuta K, Ichikawa A, Murata M, Kuwano E, Shinoda T, Shiotsuki T. 2013. Determination by lc-ms of juvenile hormone titers in hemolymph of the silkworm, bombyx mori. Bioscience Biotechnology and Biochemistry. 77(5):988-991.

Gonzalez-Santoyo I, Cordoba-Aguilar A. 2012. Phenoloxidase: A key component of the insect immune system. Entomologia Experimentalis Et Applicata. 142(1):1-16.

Glass, G.V., P.D. Peckham, and J.R. Sanders. 1972. Consequences of failure to meet assumptions underlying fixed effects analyses of variance and covariance. Rev. Educ. Res. 42: 237-288.

Hammock BD, Sparks TC. 1977. Rapid assay for insect juvenile hormone esterase-activity. Analytical Biochemistry 82(2):573-579.

Harwell, M.R., E.N. Rubinstein, W.S. Hayes, and C.C. Olds. 1992. Summarizing Monte Carlo results in methodological research: the one- and two-factor fixed effects ANOVA cases. J. Educ. Stat. 17: 315-339.

Janz N. 2005. The relationship between habitat selection and preference for adult and larval food resources in the polyphagous butterfly Vanessa cardui (Lepidoptera : Nymphalidae). Journal of Insect Behavior 18(6):767-780.

Karlhofer J, Schafellner C, Hoch G. 2012. Reduced activity of juvenile hormone esterase in microsporidia-infected Lymantria dispar larvae. Journal of Invertebrate Pathology 110(1):126-128.

Lampert, E., L.A. Dyer, and M.D. Bowers. 2014. Dietary specialization and the effects of plant species on potential multitrophic interactions of three species of nymphaline caterpillars. Entomologia experimentalis et applicate 153(3): 207-216.

Lee KP, Cory JS, Wilson K, Raubenheimer D & Simpson SJ (2006) Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. Proceedings of the Royal Society of London Series B: Biological Sciences 273: 823–829.

Lee KP, Simpson SJ & Wilson K (2008) Dietary protein-quality influences melanization and immune function in an insect. Functional Ecology 22: 1052–1061.

Lix, L.M., J.C. Keselman, and H.J. Keselman. 1996. Consequences of assumption violations revisited: A quantitative review of alternatives to the one-way analysis of variance F test. Rev. Educ. Res. 66: 579-619.

Mutuel D, Rayallec M, Chabi B, Multeau C, Salmon JM, Fournier P, Ogliastro M. 2010. Pathogenesis of Junonia coenia densovirus in Spodoptera frugiperda: A route of infection that leads to hypoxia. Virology 403(2):137-144.

Myers JH, Cory JS. 2016. Ecology and evolution of pathogens in natural populations of Lepidoptera. Evolutionary Applications 9(1):231-247.

Nakai M, Goto C, Shiotsuki T, Kunimi Y. 2002. Granulovirus prevents pupation and retards development of Adoxophyes honmai larvae. Physiological Entomology 27(2):157-164.

Nakai M, Kinjo H, Takatsuka J, Shiotsuki T, Kamita SG, Kunimi Y. 2016. Entomopoxvirus infection induces changes in both juvenile hormone and ecdysteroid levels in larval Mythimna separata. Journal of General Virology 97:225-232.

Nylin S, Slove J, Janz N. Host plant utilization, host range oscillations and diversification in nymphalid butterflies: a phylogenetic investigation. Evolution. 2014;68(1):105-24.

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW & Hernandez LM (2002) Hostplants of the Moth and Butterfly Caterpillars of America North of Mexico. Memoirs of the American Entomological Institute, Gainesville, FL, USA.

Saito Y, Kamita SG, Hammock BD, Kunimi Y, Inoue MN, Nakai M. 2015. Juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity is downregulated in larval Adoxophyes honmai following nucleopolyhedroviruses infection. Journal of Insect Physiology 80:71-80.

Shields VDC, Smith KP, Arnold NS, Gordon IM, Shaw TE, Waranch D. 2008. The effect of varying alkaloid concentrations on the feeding behavior of gypsy moth larvae, Lymantria dispar (L.) (Lepidoptera: Lymantriidae). Arthropod-Plant Interactions 2(2):101-107.

Shikano I. 2017. Evolutionary Ecology of Multitrophic Interactions between Plants, Insect Herbivores and Entomopathogens. Journal of Chemical Ecology 43(6):586-598.

Shikano I, Cory JS. 2016. Altered nutrient intake by baculovirus-challenged insects: Self-medication or compensatory feeding? Journal of Invertebrate Pathology 139:25-33.

Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. Plos One 4(3): e4796. doi:10.1371/journal.pone.0004796

Smilanich AM, Dyer LA, Chambers JQ, Bowers MD. 2009a. Immunological cost of chemical defense and the evolution of herbivore diet breadth. Ecology Letters 12(7):612-621.

Smilanich AM, Dyer LA, Gentry GL. 2009b. The insect immune response and other putative defenses as effective predictors of parasitism. Ecology 90(6):1434-1440.

Smilanich AM, Mason PA, Sprung L, Chase TR, Singer MS. 2011. Complex effects of parasitoids on pharmacophagy and diet choice of a polyphagous caterpillar. Oecologia 165(4):995-1005.

Smilanich AM, Langus TC, Doan L, Dyer LA, Harrison JG, Hsueh J, Teglas MB. 2018. Host plant associated enhancement of immunity and survival in virus infected caterpillars. Journal of Invertebrate Pathology 151:102-112.

Stefanescu C, Paramo F, Akesson S, Alarcon M, Avila A, Brereton T, Carnicer J, Cassar LF, Fox R, Heliola J et al. . 2013. Multi-generational long-distance migration of insects: studying the painted lady butterfly in the Western Palaearctic. Ecography 36(4):474-486.

Triggs AM, Knell RJ. 2012. Parental diet has strong transgenerational effects on offspring immunity. Functional Ecology 26(6):1409-1417.

Wink M, Meissner C, Witte L. 1995. Patterns of quinolizidine idine alkaloids in 56 species of the genus lupinus. Phytochemistry 38(1):139-153.