Journal of Invertebrate Pathology 214 (2026) 108497

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Diet quality improves survival and mediates larval response to densovirus infection in the Melissa blue butterfly

Kelli J. McKeegan [a,b], Matthew L. Forister [a,b], Taylor Bradford [a,b], Mike B. Teglas [a,c], Angela M. Smilanich [a,b,*]

[a] Program in Ecology, Evolution, and Conservation Biology, University of Nevada, 1664 North Virginia Street, Reno, NV 89557, USA
[b] Department of Biology, University of Nevada, Reno, NV, USA
[c] Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, Nv, USA

ARTICLE INFO

Keywords:
Virus
Infection
Diet
Host plant
Parvovirus

ABSTRACT

Entomopathogenic viruses affect insect populations by reducing fitness and influencing other measures of performance such as development and longevity. Given the huge diversity of viruses, studies are needed to understand the mechanisms underlying how viruses affect their insect hosts. The Junonia coenia densovirus (JcDV) infects a wide range of Lepidoptera and can cause death in the larval and pupal stages. In this study, we used the Melissa blue butterfly (*Lycaeides melissa*: Lycaenidae) to describe infection progression at the individual level, the effects of infection on development, survival, and longevity, and whether plant diet influenced these factors. Caterpillars were infected with JcDV and reared on either a low-quality host plant (*Medicago sativa*) or one of two high-quality host plants (*Astragalus canadensis* or *Lotus nevadensis*). We found that average viral load was similar across six days post-inoculation with a slight peak on day four. Viral load was highest among individuals reared on the low-quality host plant. Infection resulted in faster development time compared to uninfected individuals, and diet-specific effects were evident in survival to adulthood, with the native host plant (*A. canadensis*) offering optimal conditions for survival. None of the infected individuals that reached adulthood successfully eliminated JcDV, but viral loads were much lower in adults than larvae, indicating individuals reduced or maintained low viral loads between life stages. These results suggest diet-dependent response to JcDV infection and have implications for understanding the mechanisms that facilitate or impede the colonization of novel host plants.

1. Introduction

Insects are part of the global biodiversity crisis, as has been increasingly recognized in recent years (Wagner, 2020), motivating research into the factors that threaten insect populations, including disease (Myers and Cory, 2016). However, given the range of pathogens causing disease in insects, our knowledge of insect-specific viruses outside of a few model systems (e.g., baculoviruses) is still lacking (Keating and Yendol, 1987; Williams, 2018). Field-based, observational approaches at the population or community level can be complemented by laboratory experiments to answer questions related to host resistance and tolerance, disease cycling, and pathogen transmission (Myers and Cory, 2016). Investigations into interactions at a physiological level, such as the impacts of sublethal infection on host development and variation in viral burden throughout infection, have the potential to improve our understanding of broader-scale patterns and the consequences of disease for individuals and populations (Rothman and Myers, 1996; Sood et al., 2010; Cabodevilla et al., 2011).

One mechanism by which individuals respond to infection is resistance, defined as the reduction of pathogen load usually by means of limiting the spread of a pathogen within the host, or eliminating it entirely by mounting an immune response or with the aid of endosymbionts (Roy and Kirchner, 2000; Prigot-Maurice et al., 2022). Organisms might alternatively preserve their fitness by investing in other strategies to reduce the damaging effects of disease without necessarily clearing infection, which is called tolerance (Miller and Cotter, 2018; Schneider and Ayres, 2008). While it is often difficult to distinguish between these types of strategies – especially in non-model, insect-host-pathogen systems – their influence on disease transmission and population dynamics are certainly different (Roy and Kirchner, 2000). For instance, because

* Corresponding author at: Program in Ecology, Evolution, and Conservation Biology, University of Nevada, 1664 North Virginia Street, Reno, NV 89557, USA.
E-mail addresses: kelli.mckeegan@gmail.com (K.J. McKeegan), mforister@unr.edu (M.L. Forister), taylorbradford@live.com (T. Bradford), mteglas@unr.edu (M.B. Teglas), asmilanich@unr.edu (A.M. Smilanich).

https://doi.org/10.1016/j.jip.2025.108497
Received 25 August 2025; Received in revised form 27 October 2025; Accepted 13 November 2025
Available online 16 November 2025

disease resistance is costly, individuals could instead allocate resources to other strategies such as development (Paraskevopoulou et al., 2022), overcoming fitness costs by reaching the reproductive stage earlier in what can be called "life history tolerance." This has implications for transmission, either horizontally, as disease-tolerant individuals are harboring and potentially shedding infectious particles for other individuals to encounter, or vertically if mothers can pass pathogens to the next generation. Moreover, hosts that undergo complete metamorphosis may experience life-stage dependent disease effects and transmission due to differences in encounter probability and life history traits between adult and juvenile hosts (Moerbeek and Van Den Bosch, 1997).

The Junonia coenia densovirus (JcDV, *Protoambidensovirus lepidopteran1: Densovirinae*) appears to be a widespread pathogen of Lepidoptera, with varying effects across its multiple known host species (Rivers and Longworth, 1972; Muchoney et al., 2022, 2023, 2024; McKeegan et al., 2024; Yoon et al., 2025). As evidenced in studies of Baltimore checkerspot butterflies (*Euphydryas phaeton*), transmission of JcDV can occur both horizontally (i.e., uninfected larvae become infected when reared in natal groups with JcDV-positive larvae) and vertically, between mother and offspring (Christensen et al., 2024). A possible route of horizontal transmission is through the consumption of JcDV particles on contaminated plant surfaces by larvae, after which the virus crosses the host midgut and replicates in the tracheae and epidermis (Mutuel et al., 2010). Infection may result in host death in the larval or pupal stages due to detrimental effects on molting, but not in every case, and effects vary with dose (Muchoney et al., 2024) and multiple host-specific factors such as diet (Muchoney et al., 2022, 2023; Christensen et al., 2024). In a prior study, JcDV was found in wild-caught Melissa blue butterfly adults (*Lycaeides melissa*: Lycaenidae), at a prevalence as high as 70 % of samples in a few locations (McKeegan et al., 2024). This suggests that at least in Melissa blues, JcDV may exist at sublethal levels in host populations, or that some individuals are resistant or tolerant to infection and can successfully reach adulthood.

Melissa blue butterflies undergo multiple, overlapping generations in a season and exist in discrete populations based on host plant occupancy, with little-to-no movement between populations (Gompert et al., 2014). They are considered dietary specialists in that they only utilize members of the legume family (Fabaceae) for oviposition and larval diet, and native host plants for Melissa blue populations include members of the *Astragalus*, *Glycyrrhiza*, and *Lupinus* genera. With the agricultural introduction and subsequent escape of alfalfa (*Medicago sativa*) in Western North America in the last 200 years, Melissa blue populations have established on alfalfa as a novel host (Chaturvedi et al., 2018). Alfalfa has been shown to be a nutritionally inferior diet resulting in lower pupal mass, survival, and even reduced mating frequency for Melissa blue butterflies (Forister et al., 2009; Forister and Scholl, 2012; Yoon et al., 2019), compared to their native plant hosts in various genera, some of which are utilized by other members of the *Lycaeides* genus (Scholl et al., 2012). Because Melissa blue butterflies are non-migratory, multivoltine, and demonstrate fitness differences based on diet, they represent a particular suite of life history traits that can be useful in understanding mechanisms behind viral pathogen effects.

The goals in the present study were to explore patterns of viral infection over the course of six days post-inoculation, and to investigate how infection affects host condition and longevity in order to lay the groundwork for understanding sublethal JcDV effects and possible resistance or tolerance in Melissa blue butterflies. We also investigated the effects of larval host plant diet on the response to infection using three host plants in the family Fabaceae that differ in nutritional quality: two high-quality native plants, *Astragalus canadensis* and *Lotus nevadensis* (the former a host of the Melissa blue, the latter a host of another species in the same genus; both plants are known to support high survival and performance), and a low-quality novel host plant, *Medicago sativa* that is associated with reduced larval performance. Research into the influence of host plants on herbivores' ability to defend themselves from entomoviruses has yielded many insightful studies showing that specialized metabolites in plants can influence viral burden in herbivorous insects (Muchoney et al., 2022; Wang et al., 2024, 2025). For example, Wang et al., 2024 found that consumption of particular specialized metabolites from host plants caused a down-regulation of genes associated with immunity, thus increasing larval susceptibility to a nucleopolyhedrovirus. We measured the relative abundance of viral DNA (i.e., viral titer or load; Schmittgen and Livak, 2008) in individuals across the larval, pupal, and adult stages to determine whether individuals eliminate or reduce pathogen load before emerging as adults. We also measured the amount of virus shed in larval frass (fecal matter), which has implications for horizontal transmission of JcDV because densovirus particles in waste could contaminate plant matter in the local community (Johnson and Rasgon, 2018).

We hypothesized that for individuals unable to resist or tolerate infection, viral load would increase with time after inoculation, and death would occur in larval or pupal stages. In addition, we predicted that some infected individuals would exhibit viral resistance and survive to adulthood by successfully eliminating the virus or reducing viral burden. We hypothesized that these patterns might at least partially be explained by host plant diet (Resnik and Smilanich, 2020; Muchoney et al., 2022, 2023; Wang et al., 2024, 2025), with larvae reared on the low-quality novel alfalfa (*M. sativa*) having the highest viral loads upon death, and those reared on *A. canadensis* and *L. nevadensis* able to resist infection and survive to adulthood due to their higher nutritional quality (Scholl et al., 2012). In line with the findings of Muchoney et al. (2023), we predicted that individuals infected with JcDV would develop faster, but have lower final masses and therefore reduced condition, compared to the uninfected control group. Again, we expected to see an effect of diet on these metrics, such that regardless of infection status, larvae reared on the high-quality diets would develop more quickly and have larger masses than those reared on the nutritionally inferior, novel host plant. Finally, virus particles have been detected in the frass of Baltimore checkerspot larvae infected with JcDV, and they had high amounts of virus in the frass day one post-inoculation. Virus loads decreased on subsequent days with the lowest amount on the final day of measurement, day five (Muchoney et al., 2024). Based on these findings and the description of the infection route of JcDV (Mutuel et al., 2010), we predicted that virus would be detected at high concentrations in frass shortly after inoculation, as virus inoculum particles pass along the gut without crossing into the hemocoel but become reduced in viral concentration with time following infection as the virus replicates in the target tissues or when individuals resist infection. Describing the effects of sublethal infection and patterns of tolerance to emerging diseases on the individual level facilitate predictions about disease dynamics in nature (Ren et al., 2014; Carson et al., 2025). By understanding how plant diet quality and JcDV infection affect *L. melissa* survival and condition, we lay the groundwork for exploring responses in similar systems to predict which hosts might fare better in the challenge of infection. Additionally, we answer important questions about the dynamics of JcDV, which can be useful to future population-scale studies of transmission and persistence of endemic pathogens within insect host communities.

2. Materials and methods

2.1. Rearing protocol

Melissa blue (*Lycaeides melissa*) females were collected in the third week of July from a cluster of wild populations in Verdi, Nevada, USA (latitude: 39.5 N, longitude: −120.0 W). Individual females were placed with *Astragalus canadensis* stems in 480-ml plastic cups, which were covered with tulle mesh and set in an outdoor cage with natural light and air. Females were allowed to oviposit in these chambers for three days, during which the mesh covers were misted with water 3–4 times/day and brushed lightly with a sugary electrolyte beverage (fruit punch flavored Gatorade) 1–2 times/day for hydration. On the fourth day, eggs

were collected from plants and kept separate by maternal line. We extracted whole-specimen DNA from ovipositing females (N = 34) and tested it for JcDV DNA with qPCR using the protocol detailed in McKeegan et al. (2024).

Neonate first-instar caterpillars (N = 235) were distributed systematically, to ensure even representation of each maternal line and hatch order, onto the following three plants: *Medicago sativa* (novel alfalfa), *Astragalus canadensis* (hereafter referred to as the "native" host), and *Lotus nevadensis* (a native host for the closely related species *Lycaeides anna*, but which we call "naïve" because *L. melissa* do not use it in nature). Before being fed to larvae, plants were surface-sterilized by being rinsed in a 5 % household bleach (0.25 % hypochlorite) and then distilled (DI) water. All larvae were reared in individual petri dishes in a growth chamber set to a constant temperature of 24 °C, with a 12-hour light/12-hour dark cycle. Upon molting into the third instar, eleven individuals from each host plant were randomly assigned as controls (total N = 34) while the rest were designated to be inoculated. Groups (control and treatment) were transferred to separate growth chambers to avoid cross-contamination, both thereafter were maintained on the same cycle of 27 °C for 12-hour light, 22 °C for 12-hour dark. Based on our previous work in this system (e.g., Forister et al., 2009; Scholl et al., 2012), the three host plants differ in quality as it relates to development and fitness with a clear hierarchy. The novel alfalfa confers the lowest *L. melissa* mass and fitness, the native host is of intermediate quality, and *L. nevadensis* results in improved development and fitness when consumed by *Lycaeides* in a laboratory setting.

2.2. Inoculation

Once larvae were confirmed to be third-instar by the appearance of conspicuous dorsal and marginal lines, and before molting into fourth (final) instar, larvae were starved for 24 h, then a 5 mm leaf disc was cut from the host plant being used by larvae, and 1 µl of 1 µM sucrose solution was pipetted onto each disc as a feeding stimulant (Singer et al., 2009). Once the solution was dry, we pipetted 1 µl of 1×10^9 viral genome units (purified JcDV stock supplied by the Ogliastro Laboratory at the University of Montpellier, France) to each leaf disc, using the pipette tip to spread the inoculum over the surface. The treated leaf discs were then placed in petri dishes as the only food source for the larvae for 24 h. The amount eaten from each leaf disc was visually assayed on a qualitative scale of 0–5, with 5 being the entire disc was consumed and 0 being no evidence of consumption (Fig. S1). After inoculation, larvae were placed on their designated host plant again, on which they continued to feed until sacrifice or death.

2.3. Infected groups

For each host plant, all larvae that had eaten at least some of their virus-contaminated leaf disc (assigned scores of 1–5 based on qualitative, visual assessment, N = 58) were divided into four groups: three groups to be sacrificed on the second, fourth, and sixth day post-inoculation (d.p.i.), and a no sacrifice group which was reared until individuals succumbed to disease or survived to adulthood. All individuals that did not consume their leaf disc (N = 35) were assigned to the no sacrifice group, while still being monitored in case they were already infected or had not consumed the inoculation (Fig. S1). Every two days after inoculation, the mass of each larva was recorded on a precision balance. To measure JcDV in larval fecal material, all frass (at least 0.1 mg) from each infected larva was collected from the petri dish every two days, and frozen at −4°C until viral screening (Semberg et al., 2019). Sacrifice was performed by freeze-killing individuals on their designated days (second, fourth, and sixth d.p.i.). To measure adult longevity, individuals in the no sacrifice group that survived to adulthood were placed in glassine envelopes, stored in the growth chamber at the same conditions specified above for larvae, and checked daily by partially opening and tapping on the envelope. For the no sacrifice group, dates of pupation, adult emergence, and death were recorded. If the pupal or adult stage was reached, mass was recorded on the day pupae were fully formed and within 24 h of emergence as adults. Viral loads for individuals in all groups were measured following the virus detection protocol below.

2.4. Control group

All individuals in the control group (N = 34) were kept in a different laboratory building, but at the same growth chamber parameters as the infected group. Controls underwent a 24-hour starvation and then were given a clean leaf disc for 24 h to replicate similar conditions as the virus infected group. Larval mass was measured every two days, and dates of pupation and adult emergence were recorded. Controls that survived to adulthood were treated the same as the no sacrifice, infected group in order to measure longevity and adult mass in the absence of viral infection.

2.5. JcDV measurement

To determine JcDV presence and load, qPCR with a melt curve was conducted on all samples with primers for a segment of the viral capsid gene (VP4; see McKeegan et al., 2024 for primer sequences and qPCR parameters). We used the comparative Ct method (Schmittgen and Livak, 2008) with primers for arthropod 28 s rDNA (Nice et al., 2009) to calculate viral load of whole cadavers, or dead specimens (Muchoney et al., 2022). Briefly, all threshold cycle (Ct) values of both VP4 and 28S qPCRs, that were at or below the cutoff of 40 cycles, were used in the formula $2^{-\Delta Ct}$ (Schmittgen and Livak, 2008). This produced the relative abundance of viral DNA compared to host DNA $[\Delta Ct = mean\ Ct(threshold\ cycle) for\ VP4 - mean\ Ct\ for\ 28S]$, which was scaled using log-transformation. Due to the presence of many inhibitors in caterpillar frass, which produce unclear reads of 28S, we used the regression equation from a standard curve (McKeegan et al., 2024) to calculate the absolute number of JcDV genome copies shed in the frass samples.

2.6. Statistical analyses

Data were initially organized with the aid of OpenRefine (v3.4.1, Google Inc.), and manipulated with the "dplyr" package (v1.0.10, Wickham et al., 2022) for R (v4.2.1). We visualized trends of viral load and produced figures with "ggplot2" (v3.3.6, Wickham et al., 2022) and the R package "ggforce" (v0.4.1, Pederson, 2022). The "lubridate" package was used to transform dates to ordinal numbers (Spinu et al., 2021). Generalized linear models with a normal error structure were constructed to analyze the relationship of viral load with plant diet, amount of leaf disc eaten, adult longevity, and life stage at death (Table 1). To check for linearity of relationships and homogenous variance, histograms and Q-Q plots were generated as well as Shapiro-Wilks tests conducted in R. Viral load, given its wide range of values across orders of magnitude, was log-transformed and other variables were scaled (z-transformed) in regressions to normalize residuals, facilitate interpretation and satisfy the assumptions of linear models.

Due to the existence of multiple dependent variables and our need to evaluate their influence on each other, viral load, and ultimately survival, we conducted path analyses to further investigate these multilayer relationships. For these analyses, we manually created a design matrix for two of the host plants (naïve *L. nevadensis* and novel alfalfa), compared to native *A. canadensis* as the reference, and treated them as separate variables. Models including plant diet, mass, and viral load effects on survival (as a variable with binomial error structure) were built based upon a priori hypotheses and analyzed using "piecewiseSEM" with the Menard.OE option to correct for different scales (Lefcheck, 2016), "lavaan" (Rosseel, 2012), and "lavaanPlot" packages

Table 1
Linear model parameters and statistical outputs for analyses of JcDV load (i.e., viral burden) in *L. melissa* cadavers. For all models, the native host plant, *A. canadensis*, was the reference level. The reference level for sacrifice day was 2 d.p.i. and for stage the reference level was larval.

a) Effects of host plant diet and day sacrificed on *Lycaeides melissa* cadaver log10 viral load, with final sample mass as covariate ($R^2 = 0.180$, p = 0.012*)

Fixed effect	Estimate (± SE)	*t*-value	p-value
Mass before DNA extraction (g)	−23.45 (23.16)	−1.142	0.259
Host: High quality (*Lotus nevadensis*)	0.343 (1.335)	0.257	0.798
Host: Novel alfalfa (*Medicago sativa*)	2.348 (1.183)	1.985	0.053
Sacrifice Day (d.p.i.)	−1.13e-3 (0.194)	−0.006	0.995
Interaction: Final mass × High quality	9.947 (30.79)	0.323	0.748
Interaction: Final Mass × Novel alfalfa	−97.01 (52.73)	−1.840	0.072

b) Effects of host plant diet and life stage at death on un-sacrificed *L. melissa* viral load, with sample mass as covariate ($R^2 = 0.684$, p = 2.74e-11*)**

Fixed effect	**Estimate (± SE)**	***t*-value**	**p-value**
Mass before DNA extraction (g)	138.6 (34.66)	4.000	2.33e-4***
Host: High quality (*Lotus nevadensis*)	−0.301 (0.754)	−0.400	0.691
Host: Novel alfalfa (*Medicago sativa*)	0.991 (0.956)	1.037	0.306
Stage: Pupa	−2.620 (0.958)	−2.735	0.009**
Stage: Adult	−3.428 (0.978)	−3.505	0.001***

c) Development time (days from starvation to adult emergence) of *L. melissa* by treatment and host plant ($R^2 = 0.457$, p = 1.93e-6*).**

Fixed effect	**Estimate (± SE)**	***t*-value**	**p-value**
Treatment: JcDV Infected	−3.306 (0.686)	−4.822	1.53e-05***
Host: High quality (*Lotus nevadensis*)	−1.850 (0.737)	−2.512	0.016*
Host: Novel alfalfa (*Medicago sativa*)	2.400 (1.332)	1.801	0.078
Interaction: Infected × High quality	2.445 (1.022)	2.391	0.021*
Interaction: Infected × Novel alfalfa	2.306 (1.713)	1.346	0.185

d) Pupal mass (mg) by treatment and host plant ($R^2 = 0.740$, p < 2.2e-6*).**

Fixed effect	**Estimate (± SE)**	***t*-value**	**p-value**
Treatment: JcDV Infected	−0.906 (2.850)	−0.318	0.752
Host: High quality	−6.008 (3.008)	−1.997	0.051*
Host: Novel alfalfa	−29.36 (3.917)	−7.496	4.29e-10***
Interaction: Infected × High quality	3.752 (3.999)	0.938	0.352
Interaction: Infected × Novel alfalfa	−7.018 (5.184)	−1.354	0.181

Significance cutoff values: 0.05*; 0.01**; 0.001***.

(Lishinski, 2024). Prior to analyses of mass, we corrected for the effects of diet and life stage by adding the residuals of a linear model with these variables (i.e., mass predicted by host plant and life stage) to mean final mass. This step simplified models because the connections between host plants, life stage and mass were of secondary interest and thus (following residual correction) did not need to be included in path analyses. Path analysis models were compared using the Akaike Information Criterion (AIC) and Fisher's C fit statistics.

The probability of infected individuals surviving to adulthood based on diet was visualized with a Kaplan-Meier survival estimate, fit with a Cox proportional hazard regression model (Table 3), employing the "survival" package in R (v3.3–1, Therneau et al., 2022). The effect of host plant diet and infection status (infected or control) on development time and pupal and adult masses were analyzed using linear models (Table 1). For the frass portion of the study, viral load of cadaver at time of death, mass of frass collected, and time (d.p.i.) were compared to viral load shed in larval frass in linear models (Table 2). In all models with viral load, sample mass was added as a covariate to the model. Models were compared using AIC.

Table 2
Linear model parameters and output of the effects of cadaver JcDV load and time (d.p.i.) on viral load shed in frass (adjusted $R^2 = 0.431$, p = 3.78e-8***).

Fixed effect	Estimate (± SE)	*t*-value	p-value
Mass of frass collected	−57.35 (8.768)	−6.541	1.41e-8***
Cadaver JcDV load on day of frass collection	0.139 (0.118)	1.184	0.241
Time (d.p.i.)	−0.060 (0.080)	−0.750	0.456

Significance cutoff values: 0.05*; 0.01**; 0.001***.

3. Results

3.1. Viral prevalence and amount of leaf disc eaten

Upon death, qPCR confirmed that none of the individuals in the control group (N = 34) were positive for JcDV. For the infected group (sacrificed and no sacrifice), JcDV was detected in all individuals upon death, regardless of life stage or host plant diet. This confirmed that even for instances where larvae did not appear to consume any of the leaf disc (level 0), they were still exposed and became infected with the virus. In a linear regression analysis, the amount of leaf disc eaten (levels 0–5, Fig. S1) did not have a clear impact on viral burden ($F = 0.996$, df = 5 and 102, p = 0.424, Fig. 1). Those that did not eat their leaf disc (level 0) had the lowest average loads, while those that ate their entire disc (level 5) had the highest load (Fig. 1); however, this relationship was not a linear increase over the six categories of leaf disc consumption. Nonetheless, a Cox proportional hazard regression found that eating the whole leaf disc increased the risk of death before emergence more than other levels of consumption (β = 2.506 ± 0.975, p = 0.010, Table S2).

3.2. Sacrifice experiment to track infection

Average log-transformed JcDV load was −1.793 (±1.974 sd), and viral burden was highest in larvae sacrificed at 4 days post inoculation (d.p.i.), compared to those sacrificed 2- and 6-d.p.i. (−2.018 ± 1.183 and −2.764 ± 2.341, respectively), but only by a small margin (p = 0.995, Table 1a; Fig. 2). Individuals reared on the native host (*A. canadensis*) had the lowest viral load (mean = −3.13 ± 1.124), while the high-quality naïve host (*L. nevadensis*) group had intermediate viral loads (mean = −2.36 ± 2.140). The highest viral burden was present in sacrificed larvae reared on the low-quality host plant, alfalfa (*M. sativa*),

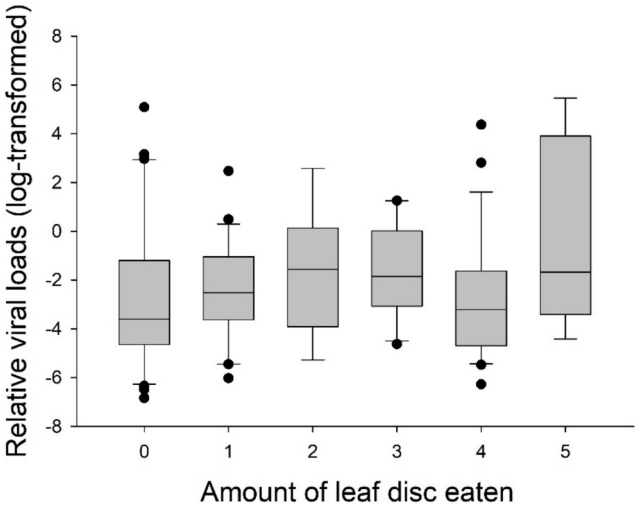

Fig. 1. Viral load of infected individuals upon death, based on the amount of leaf disc consumed (qualitative scale 0–5).

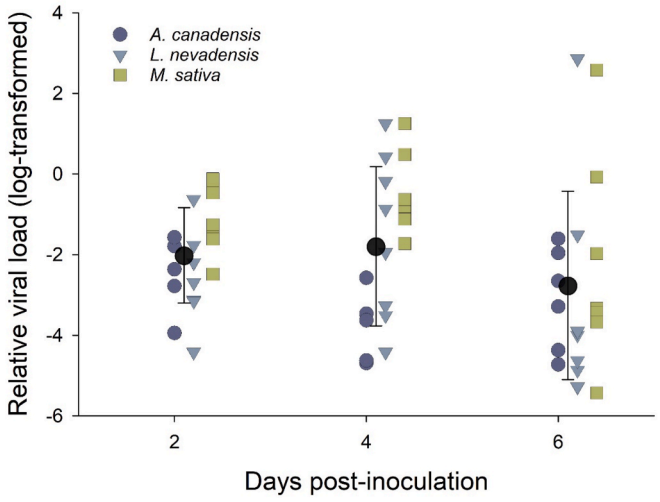

Fig. 2. Mean log-transformed relative viral load (+-S.D.) (calculated by the comparative ΔCt formula; Schmittgen and Livak, 2008) by sacrifice day (2-, 4-, and 6-days post-inoculation). Although individuals sacrificed 4 d.p.i. had the highest average loads, this was not a strong relationship (p = 0.993). Data points colored by host plant diet (*Astragalus canadensis* = native host, circles; *Lotus nevadensis* = naïve, inverted triangles; *Medicago sativa* = novel alfalfa, squares).

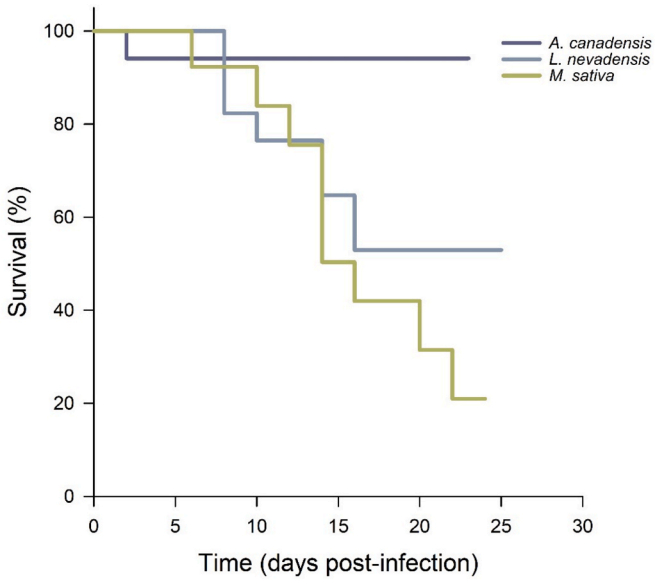

Fig. 3. Survival to adulthood for JcDV-infected *L. melissa*, colored by host plant diet.

which had two-fold higher viral loads on a log scale compared to individuals reared on *A. canadensis*, which was the only statistically significant difference in loads between individuals sacrificed (mean = −1.29 ± 1.830; β = 2.480; p = 0.041). There were no interactive effects between host plant diet and the day individuals were sacrificed on viral burden (Table S2).

3.3. Host plant effects on survival and JcDV load in unsacrificed group

Out of 34 individuals in the control (uninfected) group, 25 survived to adulthood (71 %). Of these adults that successfully emerged, there were slightly more males (N = 14) than females (N = 11). In the infected group, 31 successfully emerged as adults (out of 113, for 27 % survival), with most of these being female (N = 20); only eleven infected males survived to adulthood. Although this appears to be a reverse of sex ratios based on treatment, because of the low survival in the infected group, this was not a robust pattern ($X^2 = 1.6$, p = 0.206). Therefore, we cannot confidently say whether survival of JcDV infection depends on sex based on these data.

Cox proportional hazard regression analysis of control and infected individuals determined that while JcDV infection impacted survivorship to adulthood (β = 0.7381, p = 0.068), the effect of host plant was much stronger regardless of infection status (Fig. 3). Specifically, those reared on the naïve *L. nevadensis* were over ten times more likely to die before adulthood (β = 2.378, p = 0.024) and those reared on alfalfa over 25 times greater risk of failing to survive to adulthood (β = 3.256, p = 0.002) compared to those consuming the native *A. canadensis* diet (Cox proportional hazard, Table 3, Fig. 3). To measure the effects of host plant and viral infection on survival within the infected group, a subset of larvae was not sacrificed and allowed to live until they succumbed to the virus or reached adulthood (N = 51). Survival to adulthood was high in individuals reared on the native host plant (N = 17) with only one individual dying as a larva. In individuals reared on the naïve host plant, *L. nevadensis,* survival to adulthood was 53 % (N = 9), while four individuals died as pupae and four as larvae (Fig. 3). Larvae reared on alfalfa (*M. sativa*, the lowest-quality diet) performed worse than the groups on the higher quality hosts in all metrics (Table 1b, Fig. 3). Twelve individuals that were reared on alfalfa died as larvae or pupae; the probability of individuals surviving to adulthood (N = 4) dropped below 20 % after 24 days of infection (Fig. 3).

Table 3
Cox proportional hazard regression model, showing the increase of hazard (i.e., risk of death before emergence as adult, or survival = 0) depending on host plant diet (reference: *Astragalus canadensis,* likelihood ratio test coefficient: 26.44 on 2 df, p = 2.0e-6***).

Covariate	Coefficient (±SE)	Exp (coef)	z-value	Pr(>|z|)
Host: High quality (*Lotus nevadensis)*	2.312 (1.056)	10.092	2.190	0.029*
Host: Novel alfalfa (*Medicago sativa)*	3.267 (1.026)	26.245	3.185	0.002**

Significance cutoff values: 0.05*; 0.01**; 0.001***.

There was a robust difference in viral burden by life stage (between larvae, pupae, and adults; corrected for mass $F = 58.3$, df = 1, p < 0.001, Table 1b, Fig. 4). The JcDV loads were highest in infected larvae (mean = 0.933), followed by pupae (mean = 0.057, β = −2.620), and those that survived to adulthood had the lowest average viral burden (mean = −4.50, β = −3.428; Table 1b). There was a slight decline in viral load in larval cadavers with time post-infection (β = −0.166), and none of the individuals eliminated JcDV.

To better understand how host plant, viral load, and sample mass affected survival, we used path analysis which allows for testing the influence of multiple dependent variables on each other. (Fig. 5; p = 0.445, Fisher's $C = 3.723$, df = 4). We found that final mass had positive, direct effects of similar magnitude on viral load (γ = 0.318 ± 0.125) and survival to adulthood (γ = 0.311 ± 0.789). Viral load was also directly influenced by larval host plant diet (Fig. 5), such that individuals had higher loads (compared to the native host plant as reference) if they were reared on the novel alfalfa (γ = 0.392 ± 0.303) or the naïve *L. nevadensis* (γ = 0.409 ± 0.298) relative to the reference condition (the native host). Viral load had a strong, negative effect on survival (β = −0.860 ± 1.524), corroborating the Cox proportional hazard analyses (Table 3) showing that individuals reared on the higher quality hosts had lower viral loads and therefore higher chances of surviving to adulthood. The indirect effect of mass on survival as mediated by JcDV load (γ = −0.274) was of similar magnitude of the direct effects of mass, but in the opposite direction, therefore elucidating the complexity of the tripartite interactions of mass, viral burden, and diet on survival.

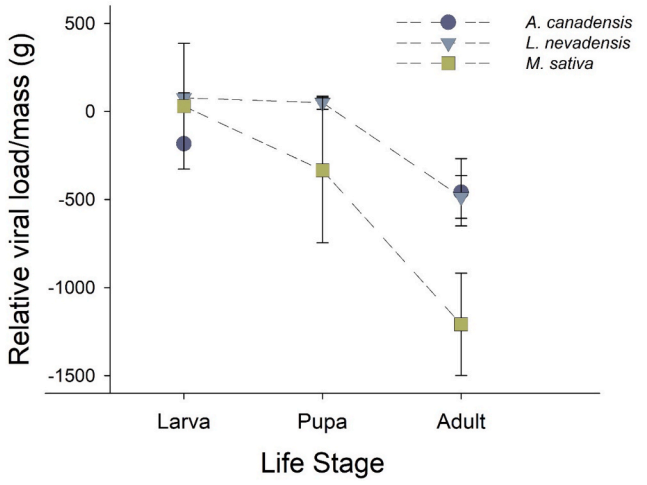

Fig. 4. Mean relative viral load (+-S.D.) (log-transformed and adjusted for mass) at time of death for each life stage, colored by host plant diet (*Astragalus canadensis* = native host, circles; *Lotus nevadensis* = naïve, inverted triangles; *Medicago sativa* = novel alfalfa, squares). Regardless of host plant, load differed significantly between life stages, with adults having the lowest loads ($p = 0.001$) and pupae with intermediate loads ($p = 0.009$). Although not many larvae reared on novel alfalfa survived to adulthood, that group experienced the largest effect of life stage on viral load ($p = 4.0e\text{-}4$).

3.4. Effects of JcDV infection on adult longevity and body condition

Adults in the control group survived an average of 3.9 (±0.99 sd) days after emergence if reared on the native host plant, 3.0 (±0.85) days for the high-quality naïve host, and those reared on the low-quality alfalfa lived an average of 2.0 (±1.73) days. While these host plant differences in adult longevity were notable in the control group, adult longevity of the infected group was not determined by host plant ($\beta = 0.327 \pm 0.336$, $F = 0.944^{1,24}$, $df = 1$, $p = 0.341$) and instead strongly influenced by viral load ($\beta = -0.293 \pm 0.126$, $F = 5.398^{1,28}$, $df = 1$, $p = 0.028$; Fig. 6). Inclusion of an interaction term between host plant and viral load in our analysis of adult longevity in infected individuals revealed a complex relationship. Individuals reared on *L. nevadensis* showed a negative relationship between viral load and adult longevity, while individuals reared on *A. canadensis* lived slightly longer as adults with lower viral load, but remarkably did not decrease in adult longevity even at higher viral loads (Fig. 6; note that a linear relationship is not shown for the novel host because of the small number of individuals that survived).

Development time was measured as the number of days between pre-inoculation starvation (since both treatment groups underwent that phase) and adult emergence. Overall, JcDV-infected individuals developed an average of two days quicker than controls ($\beta = -3.306$, $p = 1.53e\text{-}5$, Fig. 7a). However, this pattern was mediated by an interaction between JcDV infection and plant diet (Table 1c), such that individuals

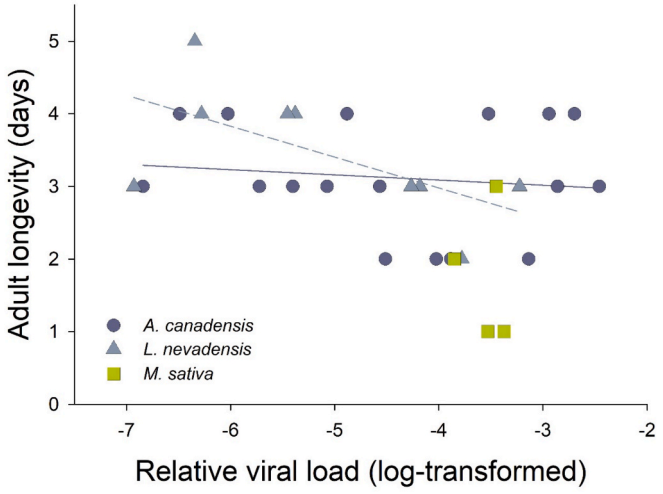

Fig. 6. Effects of relative viral load on adult survival time (d.p.i.), colored by larval plant diet. Mean longevity of control groups for each host plant were as follows: native *A. canadensis* (circles) = 3.9 days; naïve *Lotus nevadensis* (triangles) = 3.0 days; novel *M. sativa* (squares) = 2.5 days. Overall, higher log-loads conferred shorter survival time for the infected groups (closed circles; $p = 0.028$), but this pattern was dependent on larval diet.

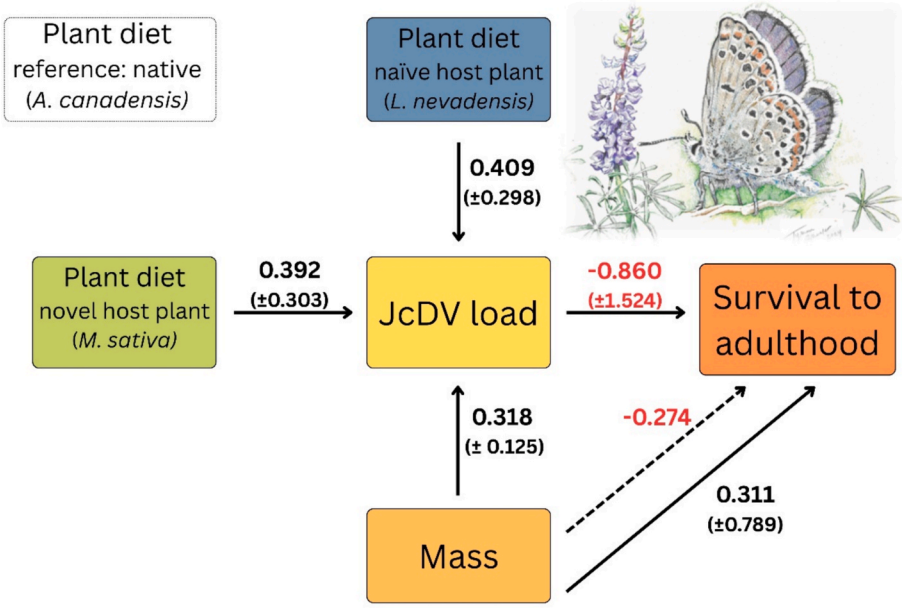

Fig. 5. Piecewise path analysis of host plant diet (with native *Astragalus* as reference) and sample mass (after correcting for differences in diet and life stage) effects on relative viral load (endogenous variable), and survival to adulthood (binomial: 0 or 1). Numbers are path coefficients with standard errors of each variable effect (fit: Fisher's C = 3.723, p-value = 0.445 on 4 df). Solid lines represent direct effects, while dotted lines show indirect effects. *Lycaeides melissa* illustration credit: Tynan Wheeler.

reared on the naïve host (*L. nevadensis*) developed faster than those on the other host plants (β = −1.850, p = 0.016, Fig. 7a), but when infected, individuals reared on *A. canadensis* developed the fastest (β = 2.445, p = 0.021, Fig. 7a). There was one infected individual from the alfalfa group that took 36 days to emerge; upon removal of this outlier from the dataset, the patterns of longevity and development time as they related to treatment and host plant remained the same. Average pupal mass between the infected and control groups were similar, within 0.30 mg of each other (p = 0.756, Fig. 7b). Diet quality, however, still had a strong effect on average pupal masses (Table 1d; Fig. 7b) with 51.13 mg for those reared on the native host, 47.12 mg for those reared on the highest quality host, and only 18.02 mg for the alfalfa group.

3.5. JcDV in larval frass

JcDV was detectable in frass throughout infection (virus detected in frass for a total of 22 d.p.i.) but steadily decreased in load over time (Fig. 8a). Viral loads of whole-cadaver tissue were compared to viral loads in frass collected for 2-, 4-, and 6 d.p.i. Frass and tissue samples were taken from the same individual to test whether frass viral loads were a good predictor of tissue viral loads. Samples were corrected for differences in starting mass and log-transformed for analysis and visualization. We found that overall viral load in frass was a strong predictor of tissue viral load, especially for days 4 and 6 post-inoculation (Pearson's product-moment correlation = 0.338, p = 0.006; Fig. 8b).

4. Discussion

In this study, we provide a basis for understanding Junonia coenia densovirus (JcDV) infection of *Lycaeides melissa* at the individual level. Our hypotheses regarding the patterns of viral burden throughout the course of infection were partially supported: there was a slight decrease of JcDV load over the six days post-inoculation. However, overall patterns of load were more dependent upon host plant quality and the ability of the individual to successfully reach adulthood (which was also explained by plant diet). We did not detect strong differences in viral load between sacrifice groups (2-, 4-, and 6-d.p.i.), and many larvae in the no sacrifice group survived longer than 6 d.p.i., which suggests JcDV infection in *L. melissa* is maintained throughout their development and into adulthood.

Lycaeides melissa performance was strongly influenced by host plant identity for the no sacrifice individuals. As predicted, individuals reared on novel alfalfa (*M. sativa*) fared the worst, having lower final masses and higher mortality from infection than those reared on the higher-quality diets. Many infected larvae reared on alfalfa experienced a type of stunted growth: they developed more slowly and had lower survival to adulthood than the other groups. Individuals reared on *L. nevadensis,* a host plant which other butterflies in the genus *Lycaeides* perform well on but is naïve for *L. melissa* (Scholl et al., 2012), had intermediate survival with approximately half of the infected group dying in the larval or pupal stage. Within the infected group, caterpillars reared on the native host (*A. canadensis*) had the highest survival – only one individual died in the larval stage and the rest survived to adulthood. Indeed, the high survival probabilities of individuals reared on the native plant after being inoculated with $1x10^9$ viral genome units/μL, was compelling, considering studies of JcDV in larger-bodied hosts (e.g., Nymphalidae) resulted in 50 % mortality at lower viral concentrations (Muchoney et al., 2023). Prior studies have documented both positive and negative impacts of plant specialized metabolites on immune function (Garvey et al., 2021; Wang et al., 2021; Muchoney et al., 2023; Ghosh et al., 2023), which can subsequently affect viral loads in the host insect (Wang et al., 2020; Muchoney et al., 2022). Even though, we did not measure the immune response in our caterpillars, it is possible that variation in immunity on the three host plants explains the variation in viral loads (Yoon et al., 2025).

Although we predicted some individuals would be able to completely resist infection by eliminating the virus, this was not the case: JcDV was detected in every cadaver from the infected group, regardless of life stage or host plant diet. This conflicts with findings in other host systems (*Spodoptera frugiperda* and *S. littoralis*: Noctuidae; *Anartia jatrophae:* Nymphalidae) that suggests JcDV infection can be cleared before adult emergence (Mutuel et al., 2010; Muchoney et al., 2023). Having JcDV-positive adults in *L. melissa* populations increases the rate and likelihood of vertical transmission between generations (Christensen et al., 2024). However, despite the 100 % JcDV prevalence in *L. melissa* cadavers, adults overall had significantly lower viral burden than larvae, which may indicate that these individuals were capable of resisting JcDV by reducing the viral burden they experienced (Muchoney et al., 2023). There were too few individuals that survived to adulthood in the alfalfa (*M. sativa,* lowest quality host) group to reveal patterns, but adults reared on *L. nevadensis* (high quality) showed increased longevity and lower viral loads, while increasing viral load did not change adult longevity for individuals reared on the native host plant, possibly showing tolerance to the virus at the adult stage for this group.

In other studies investigating JcDV load and mortality over time using *S. frugiperda*, analyses do not go beyond 10 d.p.i. (Mutuel et al., 2010; Pigeyre et al., 2019), and several *L. melissa* spent much more time than this as larvae when infected with the virus. Because JcDV can be transmitted perorally, and we were able to detect JcDV presence in frass, viral particles shed through frass onto plant material (if at high enough levels to remain viable in the environmental; Xu et al., 2014) may allow for disease transmission by the fecal-oral route (Muscio et al., 2000). Further, differences in larval diet impacted the progression of infection through life stages, such that while many reared on the alfalfa died as

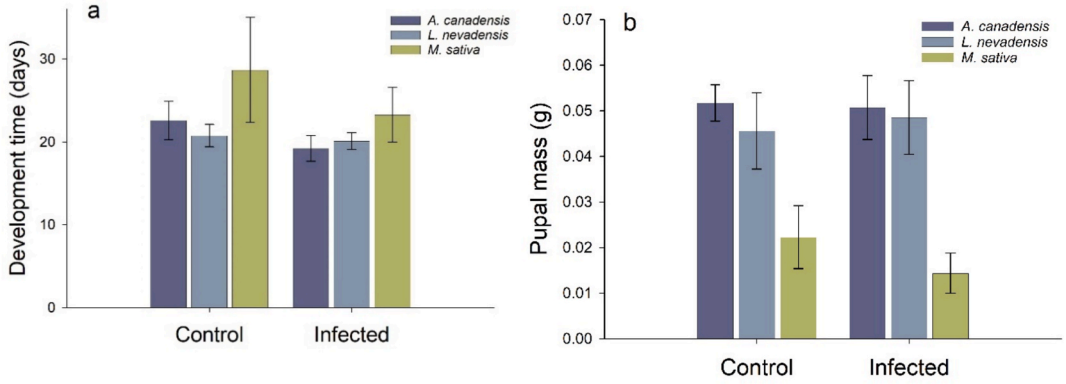

Fig. 7. Development time and pupal mass for control and no sacrifice, infected groups based on host plant diet (*M. sativa* = novel alfalfa, green; *A. canadensis* = native, dark blue; *L. nevadensis* = naïve, light blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

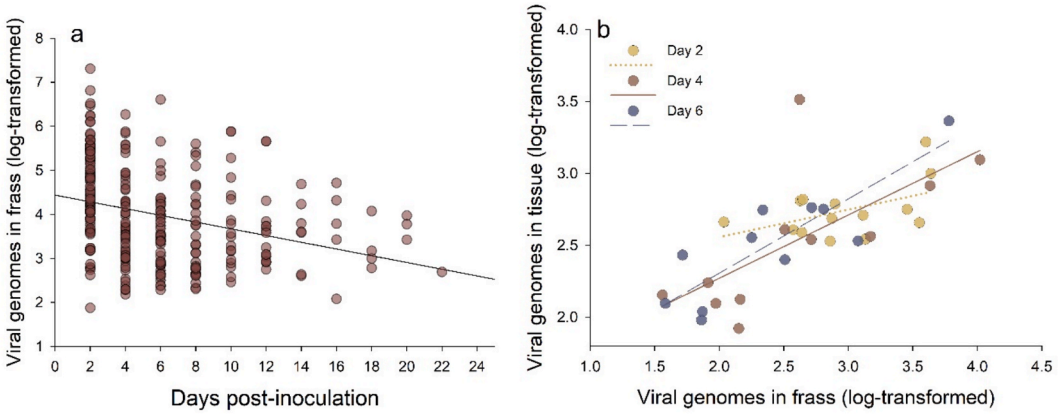

Fig. 8. a) Jcdv load (viral genomes, calculated with standard curve equation) in larval waste across time(d.p.i.); b) Average viral load (viral genomes) in frass compared to viral load in cadaver tissue for 2- (yellow), 4- (brown), and 6 d.p.i. (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

larvae, those that successfully emerged as adults had much lower viral burden than larval tissue, or cadavers of any life stage reared on the higher quality host plants. Those reared on the high-quality plants (*L. nevadensis* and *A. canadensis*) also showed reduced loads in adults and had much higher survival than those reared on novel alfalfa. It is worth noting that McKeegan et al. (2024) found a relatively high JcDV prevalence in natural *L. melissa* populations utilizing alfalfa, which could mean that although JcDV-infected larvae have lower survival on the novel host, it may not hinder population success since many adults that were collected harbored the virus. In the current study, having higher mass resulted in higher viral burden, but also increased probability of surviving to adulthood (perhaps due to better body condition), ultimately suggesting that *L. melissa* have different strategies for coping with JcDV infection depending on the larval host plant diet.

We found that JcDV infection reduced average development time by approximately two days, which corroborates findings by Muchoney et al. (2023) and Smilanich et al. (2018). Because individuals from the infected group reached adulthood (the reproductive stage) more quickly than controls, it is possible that *L. melissa*, particularly those reared on the native host plant, may receive conditional benefits from infection with JcDV (e.g., because of reduced exposure time to natural enemies in the vulnerable juvenile stage) as has been shown in other host-densovirus systems (Xu et al., 2014, 2020); however, our study was not designed to test this question but could be the focus of future studies. While pupal masses (as another measure of host condition) were indistinguishable between treatments (infected and control), pupal masses and the ability to survive to adulthood were dependent on host plant, suggesting strong diet-dependence of the interaction (Sternberg et al., 2012; Roberts et al., 2020; Yoon et al., 2019). Therefore, we confirm that there is intraspecific variation in response to JcDV infection based on host plant quality, as is shown in other lepidopteran pathogens and densovirus systems (Keating and Yendol, 1987; Li et al., 2019; Ren et al., 2014; Smilanich and Muchoney, 2022). Considering additional evidence from the JcDV-*L. melissa* system, Yoon et al. (2025) found that the immune response as measured by phenoloxidase activity and expression of immune genes were both elevated in *L. melissa* larvae reared on the native host plant, *A. canadensis*, while viral infection alone did not have a significant impact on immunity, again supporting a strong impact of host plant quality on larval health.

4.1. Conclusion

In this study, we demonstrated how larval plant diet influences survival to adulthood and response to the challenge of JcDV infection. Moreover, our comparison between JcDV load in frass and cadavers on day-of-death has implications not only for viral transmission, but also as a non-destructive method for viral detection in future studies. Non-destructive methods of viral detection could allow for explicit hypotheses about resistance and tolerance to JcDV. Future studies could also investigate the viability or infectivity of viral particles shed in waste to understand the rate of JcDV transmission by the fecal-oral route.

Funding statement

Funding for this research was supported by a National Science Foundation grant DEB-1929522 (to AMS, MLF, and MBT), DEB-2128243 (to AMS) and the UNR Graduate Student Association Travel, Research, and Materials Grant (to KJM).

CRediT authorship contribution statement

Kelli J. McKeegan: Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Matthew L. Forister:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Taylor Bradford:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Mike B. Teglas:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Angela M. Smilanich:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thank you to Tara Christensen, Su'ad Yoon, Dominic Zullo, and Samantha DiFulvio for assistance with caterpillar rearing and maintenance, data and plant collection, and consultation on protocols. We thank the Ogliastro Lab at the University of Montpelier, France, for providing viral stock used for inoculation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2025.108497.

References

Cabodevilla, O., Villar, E., Virto, C., Murillo, R., Williams, T., Caballero, P., 2011. Intra- and intergenerational persistence of an insect nucleopolyhedrovirus: adverse effects of sublethal disease on host development, reproduction, and susceptibility to superinfection. Appl. Environ. Microbiol. 77 (9), 2954–2960.

Carson, B.D., Orians, C.M., Crone, E.E., 2025 Oct. Context-dependent effects of a cryptic virus on butterfly population dynamics. Ecology 106 (10), e70199.

Chaturvedi, S., Lucas, L.K., Nice, C.C., Fordyce, J.A., Forister, M.L., Gompert, Z., 2018. The predictability of genomic changes underlying a recent host shift in Melissa blue butterflies. Mol. Ecol. 27 (12), 2651–2666. https://doi.org/10.1111/mec.14578.

Christensen, T., Dyer, L.A., Forister, M.L., et al., 2024. Host plant-mediation of viral transmission and its consequences for a native butterfly. Ecology 12, e4282. https://doi.org/10.1002/ecy.4282.

Forister, M.L., Nice, C.C., Fordyce, J.A., Gompert, Z., 2009. Host range evolution is not driven by the optimization of larval performance: the case of Lycaeides melissa (Lepidoptera: Lycaenidae) and the colonization of alfalfa. Oecologia 160 (3), 551–561. https://doi.org/10.1007/s00442-009-1310-4.

Forister, M.L., Scholl, C.F., 2012. Use of an exotic host plant affects mate choice in an insect herbivore. Am. Nat. 179 (6), 805–810.

Garvey, M., Bredlau, J., Kester, K., Creighton, C., Kaplan, I., 2021. Toxin or medication? Immunotherapeutic effects of nicotine on a specialist caterpillar. Funct. Ecol. 35 (3), 614–626.

Ghosh, E., Paul, R.L., Ode, P.J., 2023. Plant species with higher chemical defences enhance herbivore cellular immunity with differential effectiveness against two parasitoid species. Funct. Ecol. 37 (5), 1492–1503.

Gompert, Z., Lucas, L.K., Buerkle, C.A., Forister, M.L., Fordyce, J.A., Nice, C.C., 2014. Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Mol. Ecol. 23 (18), 4555–4573. https://doi.org/10.1111/mec.12811.

Johnson, R.M., Rasgon, J.L., 2018. Densonucleosis viruses ('densoviruses') for mosquito and pathogen control. Cur. Opinion Insect Sci. 28, 90–97.

Keating, S.T., Yendol, W.G., 1987. Influence of selected host plants on gypsy moth (Lepidoptera: Lymantriidae) larval mortality caused by a baculovirus. Environ. Entomol. 16 (2), 459–462. https://doi.org/10.1093/ee/16.2.459.

Lefcheck, J.S., 2016. piecewiseSEM : Piecewise structural equation modelling in r for ecology, evolution, and systematics. Freckleton R, ed. Methods Ecol Evol. 7 (5), 573–579. https://doi.org/10.1111/2041-210X.12512.

Lishinski, A., 2024. lavaanPlot: Path Diagrams for "Lavaan" Models via "DiagrammeR.'. Published online 2024.

Li, J., Dong, Y., Sun, Y., Lai, Z., Zhao, Y., Liu, P., Gao, Y., Chen, X., Gu, J., 2019 Jul. Frontiers in Microbiology 10, 1549.

McKeegan, K.J., Muchoney, N.D., Teglas, M.B., Forister, M.L., Smilanich, A.M., 2024. Exploring spatial and temporal patterns of viral infection across populations of the Melissa blue butterfly. Ecol. Entomol. 49 (1), 54–66. https://doi.org/10.1111/een.13280.

Miller, C.V.L., Cotter, S.C., 2018. Resistance and tolerance: The role of nutrients on pathogen dynamics and infection outcomes in an insect host. Ardia D, ed. J. Anim. Ecol. 87 (2), 500–510. https://doi.org/10.1111/1365-2656.12763.

Moerbeek, M., Van Den Bosch, F., 1997. Insect-pathogen dynamics: stage-specific susceptibility and insect density dependence. Math. Biosci. 141.

Muchoney, N.D., Bowers, M.D., Carper, A.L., Mason, P.A., Teglas, M.B., Smilanich, A.M., 2022. Use of an exotic host plant shifts immunity, chemical defense, and viral burden in wild populations of a specialist insect herbivore. Ecol. Evol. 12 (3). https://doi.org/10.1002/ece3.8723.

Muchoney, N.D., Bowers, M.D., Carper, A.L., Teglas, M.B., Smilanich, A.M., 2023. Use of an exotic host plant reduces viral burden in a native insect herbivore. Ecol. Lett. 26 (3), 425–436. https://doi.org/10.1111/ele.14162.

Muchoney, N.D., Watanabe, A.M., Teglas, M.B., Smilanich, A.M., 2024. Dose-dependent dynamics of densovirus infection in two nymphalid butterfly species utilizing native or exotic host plants. J. Invertebr. Pathol. 206, 108176.

Muscio, O., Bonder, M.A., La Torre, J.L., Scodeller, E.A., 2000. Horizontal transmission of triatoma virus through the fecal-oral route in *Triatoma infestans* (Hemiptera: Triatomidae). J. Med. Entomol. 37, 271–275. https://doi.org/10.1603/0022-2585-37.2.271.

Mutuel, D., Ravallec, M., Chabi, B., et al., 2010. Pathogenesis of Junonia coenia densovirus in Spodoptera frugiperda: A route of infection that leads to hypoxia. Virology 403 (2), 137–144. https://doi.org/10.1016/j.virol.2010.04.003.

Myers, J.H., Cory, J.S., 2016. Ecology and evolution of pathogens in natural populations of Lepidoptera. Evol. Appl. 9 (1), 231–247. https://doi.org/10.1111/eva.12328.

Nice, C.C., Gompert, Z., Forister, M.L., Fordyce, J.A., 2009. An unseen foe in arthropod conservation efforts: the case of Wolbachia infections in the Karner blue butterfly. Biol. Conserv. 142 (12), 3137–3146.

Paraskevopoulou, S., Gattis, S., Ben-Ami, F., 2022. Parasite resistance and parasite tolerance: insights into transgenerational immune priming in an invertebrate host. Biol. Lett. 18 (4), 20220018. https://doi.org/10.1098/rsbl.2022.0018.

Pederson, T., 2022. ggforce: Accelerating 'ggplot2. Published online 2022.

Pigeyre, L., Schatz, M., Ravallec, M., et al., 2019. Interaction of a densovirus with glycans of the peritrophic matrix mediates oral infection of the lepidopteran pest Spodoptera frugiperda. Viruses 11 (9), 870. https://doi.org/10.3390/v11090870.

Prigot-Maurice, C., Beltran-Bech, S., Braquart-Varnier, C., 2022. Why and how do protective symbionts impact immune priming with pathogens in invertebrates? Dev. Comp. Immunol. 126, 104245. https://doi.org/10.1016/j.dci.2021.104245.

Ren, X., Hughes, G.L., Niu, G., Suzuki, Y., Rasgon, J.L., 2014. *Anopheles gambiae* densovirus (AgDNV) has negligible effects on adult survival and transcriptome of its mosquito host. PeerJ 2, e584.

Resnik, J.L., Smilanich, A.M., 2020. The effect of phenoloxidase activity on survival is host plant dependent in virus-infected caterpillars. Jaronski S, ed. J. Insect Sci. 20 (5), 26. https://doi.org/10.1093/jisesa/ieaa116.

Rivers, C., Longworth, J.F., 1972. A nonoccluded virus of Junonia coenia (Nymphalidae: Lepidoptera). J. Invertebr. Pathol. 20 (3), 203–214.

Roberts, K.E., Meaden, S., Sharpe, S., et al., 2020. Resource quality determines the evolution of resistance and its genetic basis. Mol. Ecol. 29 (21), 4128–4142. https://doi.org/10.1111/mec.15621.

Rosseel, Y., 2012. An *R* package for structural equation modeling. J Stat Soft. 48 (2). https://doi.org/10.18637/jss.v048.i02.

Rothman, L.D., Myers, J.H., 1996. Debilitating effects of viral diseases on host Lepidoptera. J. Invertebr. Pathol. 67, 1–10.

Roy, B.A., Kirchner, J.W., 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54 (1), 51–63. https://doi.org/10.1111/j.0014-3820.2000.tb00007.x.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C (T) method. Nat. Protoc. 3 (6), 1101–1108.

Schneider, D.S., Ayres, J.S., 2008. Two ways to survive infection: What resistance and tolerance can teach us about treating infectious diseases. Nat. Rev. Immunol. 8, 889–895.

Scholl, C.F., Nice, C.C., Fordyce, J.A., Gompert, Z., Forister, M.L., 2012. Larval performance in the context of ecological diversification and speciation in *Lycaeides* Butterflies. Int. J. Ecol. 2012, 1–13. https://doi.org/10.1155/2012/242154.

Semberg, E., De Miranda, J.R., Low, M., Jansson, A., Forsgren, E., Berggren, Å., 2019. Diagnostic protocols for the detection of Acheta domesticus densovirus (AdDV) in cricket frass. J. Virol. Methods 264, 61–64. https://doi.org/10.1016/j.jviromet.2018.12.003.

Singer, M.S., Mace, K.C., Bernays, E.A., 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. May RC, ed. PLoS One 4 (3), e4796.

Smilanich, A.M., Langus, T.C., Doan, L., et al., 2018. Host plant associated enhancement of immunity and survival in virus infected caterpillars. J. Invertebr. Pathol. 151, 102–112. https://doi.org/10.1016/j.jip.2017.11.006.

Smilanich, A.M., Muchoney, N.D., 2022. Host plant effects on the caterpillar immune response. In: Marquis, R.J., Koptur, S. (Eds.), Caterpillars in the Middle. Fascinating Life Sciences. Springer International Publishing, pp. 449–484. https://doi.org/10.1007/978-3-030-86688-4_14.

Sood, P., Mehta, P.K., Bhandari, K., Prabhakar, C.S., 2010. Transmission and effect of sublethal infection of granulosis virus (PbGV) on *Pieris brassicae* Linn (Pieridae: Lepidoptera). J. Appl. Entomol. 134 (9–10), 774–780. https://doi.org/10.1111/j.1439-0418.2010.01514.x.

Spinu, V., Grolemund, G., Wickham, H., et al., 2021. ubridate: Make Dealing with Dates a Little Easier. Published online 2021.

Sternberg, E.D., LeFèvre, T., Li, J., et al., 2012. Data from: Food plant-derived disease tolerance and resistance in a natural butterfly-plant-parasite interaction. Published online April 30, 2012:144190 bytes. https://doi.org/10.5061/DRYAD.82J66.

Wagner, D.L., 2020. Insect declines in the anthropocene. Annu. Rev. Entomol. 65 (1), 457–480. https://doi.org/10.1146/annurev-ento-011019-025151.

Wang, J.Y., Jiang, J.X., Fan, N.N., Wu, H.W., Ji, X.Y., Wan, N.F., Hou, Y.M., 2025. Allicin impacts the susceptibility of Spodoptera exigua to nucleopolyhedrovirus. J. Pest. Sci. 98 (2), 747–757.

Wang, J.Y., Fan, N.N., Yuan, Y., Bass, C., Siemann, E., Ji, X.Y., Jiang, J.X., Wan, N.F., 2024. Plant defense metabolites influence the interaction between an insect herbivore and an entomovirus. Curr. Biol. 34 (24), 5758–5768.

Wang, J.Y., Zhang, H., Siemann, E., Fan, N.N., Ji, X.Y., Chen, Y.J., Jiang, J.X., Wan, N.F., 2021. Plants impact cellular immunity of caterpillars to an entomovirus. Pest Manag. Sci. 77 (5), 2415–2424.

Wang, J.Y., Zhang, H., Siemann, E., Ji, X.Y., Chen, Y.J., Wang, Y., Jiang, J.X., Wan, N.F., 2020. Immunity of an insect herbivore to an entomovirus is affected by different host plants. Pest Manag. Sci. 76 (3), 1004–1010.

Wickham, H., Chang, W., Henry, L., et al., 2022. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. Published online 2022.

Wickham, H., François, R., Henry, L., Müller, K., 2022. dplyr: A Grammar of Data Manipulation. Published online 2022.

Williams T. Viruses, 2018. In: Hajek, A.E., Shapiro-Ilan, D.I. (Eds.), Ecology of Invertebrate Diseases. John Wiley & Sons, pp. 213–285.

Xu, P., Liu, Y., Graham, R.I., Wilson, K., Wu, K., 2014. Densovirus Is a mutualistic symbiont of a global crop pest (Helicoverpa armigera) and protects against a baculovirus and Bt Biopesticide. Jiggins FM, ed. PLoS Pathog. 10 (10), e1004490. https://doi.org/10.1371/journal.ppat.1004490.

Xu, P., Yang, L., Yang, X., Li, T., Graham, R.I., Wu, K., Wilson, K., 2020. Novel partiti-like viruses are conditional mutualistic symbionts in their normal lepidopteran host, African armyworm, but parasitic in a novel host, Fall armyworm. PLoS Pathogens 16 (6), e1008467.

Yoon, S.A., Harrison, J.G., Philbin, C.S., et al., 2019. Host plant-dependent effects of microbes and phytochemistry on the insect immune response. Oecologia 191 (1), 141–152. https://doi.org/10.1007/s00442-019-04480-3.

Yoon, S.A., So, K., Harrison, J.G., Shastry, V., Urie, K., Gompert, Z., Miura, P., Smilanich, A.M., Forister, M.L., Chaturvedi, S., 2025. High quality diet enhances immune response and affects gene expression during viral infection in an insect herbivore. Mol. Ecol., e17694