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Dose-dependent dynamics of densovirus infection in two nymphalid butterfly species utilizing native or exotic host plants

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ABSTRACT

Insects are attacked by a diverse range of microbial pathogens in the wild. In herbivorous species, larval host plants frequently play a critical role in mediating susceptibility to infection. Characterizing such plant-mediated effects on herbivore-pathogen interactions can provide insight into patterns of infection across wild populations. In this study, we investigated the effects of host plant use by two North American butterflies, *Euphydryas phaeton* (Nymphalidae) and *Anartia jatrophae* (Nymphalidae), on entomopathogen infection across a range of three doses. Both of these herbivores recently incorporated the same exotic plant, *Plantago lanceolata* (Plantaginaceae), into their host range and are naturally infected by the same entomopathogen, Junonia coenia densovirus (*Parvoviridae*), in wild populations. We performed two factorial experiments in which *E. phaeton* and *A. jatrophae* were reared on either *P. lanceolata* or a native host plant [*Chelone glabra* (Plantaginaceae) for *E. phaeton*; *Bacopa monnieri* (Plantaginaceae) for *A. jatrophae*] and inoculated with either a low, medium, or high dose of the virus. In *E. phaeton*, the outcomes of infection were highly dose-dependent, with inoculation with higher viral doses resulting in faster time to death and greater mortality. However, neither survival nor postmortem viral burdens varied depending upon the host plant that was consumed. In contrast, host plant use had a strong effect on viral burdens in *A. jatrophae,* with consumption of the exotic plant appearing to enhance host resistance to infection. Together, these results illustrate the variable influences of host plant use on herbivore resistance to infection, highlighting the importance of investigating plant-herbivore relationships within a tritrophic framework.

1. Introduction

Pathogens are a ubiquitous and integral component of ecosystems worldwide, exerting powerful influences on the ecology and evolution of the organisms that they infect (Gulland, 1995), along with the broader communities that they inhabit (Dobson and Hudson, 1986; French and Holmes, 2020; Paseka et al., 2020). In insects, pathogens play a major role in regulating population cycles (Dwyer et al., 2004; Myers and Cory, 2013) and have been linked to declines and/or management concerns in economically significant species (Cameron et al., 2011; Cox-Foster et al., 2007; Eilenberg and Jensen, 2018). Though insects are attacked by a diverse range of pathogens, including bacteria, fungi, viruses, and protozoa (Anderson and May 1981; Briggs et al., 1995), research on entomopathogens has historically been concentrated on their potential applications as biological control agents (Lacey et al., 2015; Moscardi, 1999), with less attention given to their influence in "natural" or unmanaged systems (but see Alger et al., 2018; Altizer et al., 2000; Cory and Myers, 2009 for examples). Such systems offer unique opportunities for insight into the ecological factors that shape insect-pathogen interactions (Cory, 2010; de Roode et al., 2008), with applications to both basic and applied entomological research.

Much research on ecological sources of variation in insect-pathogen relationships has highlighted the importance of larval host plants in mediating infection in herbivorous species (reviewed in Cory and Hoover, 2006). Both inter- and intraspecific variation in host plant quality, which may include aspects of nutritional and/or secondary chemistry, can impact susceptibility to infection in the herbivores that consume them (Duffey et al., 1995; Resnik and Smilanich, 2020; Shikano et al., 2010). In well-studied entomopathogens, including baculoviruses and the bacterium *Bacillus thuringiensis,* it has been repeatedly demonstrated

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that host mortality rates, pathogen replication, and speed of kill can vary markedly depending on the chemistry and/or identity of the host plant consumed by the herbivore (Ali et al., 1998; Duffey et al., 1995; Kouassi et al., 2001; Raymond et al., 2002). In baculovirus systems, much attention has been given to the potential for direct inhibition of pathogens by host plant constituents within the midgut lumen (e.g., Felton and Duffey, 1990; Hoover et al., 1998). However, host plants may also mediate herbivore-pathogen interactions through indirect effects, including impacts of macronutrients and phytochemicals on herbivore immune responses (Smilanich and Muchoney, 2022) or overall body condition (Shikano et al., 2010) both prior to and following infection. Though it is clear that host plant use can impact herbivore susceptibility to infection in many cases, the majority of research on this subject has focused on a small suite of pathogens and insect host species (Cory and Hoover, 2006). Non-model systems offer exciting opportunities to investigate the generalizability of these patterns across diverse taxa and gain insight into the broader role of bottom-up effects in regulating insect-pathogen interactions in wild populations.

In this study, we examined the role of host plant use in mediating interactions between insect herbivores and an entomopathogenic virus, Junonia coenia densovirus (hereafter, JcDV). JcDV is a nonenveloped, single-stranded DNA virus in the family *Parvoviridae* (*Densovirinae: Protoambidensovirus lepidopteran1*) that infects hosts within the order Lepidoptera. Insects become infected with JcDV during the larval stage through ingestion of viral particles on host plants, which enter the hemocoel by crossing the midgut epithelium (without replication) and replicate in tracheae, hemocytes, visceral muscles, and epidermis (Mutuel et al., 2010; Pigeyre et al., 2019; Wang et al., 2013). Infection results in hypoxia and disruptions to molting and metamorphosis, with mortality depending upon the viral dose that is ingested (Mutuel et al., 2010; Smilanich et al., 2018). Though capable of infecting Lepidoptera in multiple families in a laboratory setting (Mutuel et al., 2010; Resnik and Smilanich, 2020; Rivers and Longworth, 1968), the prevalence, host range, and impact of this pathogen in wild herbivore populations is only recently beginning to be characterized (McKeegan et al., 2023; Muchoney et al., 2023, 2022).

Our research has documented the occurrence of JcDV across wild populations of two North American butterfly species, *Euphydryas phaeton* Drury (Nymphalidae), the Baltimore checkerspot (Muchoney et al., 2022), and *Anartia jatrophae* Linnaeus (Nymphalidae), the white peacock (Muchoney et al., 2023). Both of these herbivores recently incorporated the same exotic plant, *Plantago lanceolata* L. (Plantaginaceae) (hereafter, *Plantago*), into their dietary ranges (Knerl and Bowers, 2013; Stamp, 1979). Both herbivores also exhibit differences in growth and performance when utilizing this exotic species, compared to native host plants (Bowers et al., 1992; Brown et al., 2017; Knerl and Bowers, 2013; Lampert et al., 2014). Notably, *Plantago* contains iridoid glycosides (IGs), a class of terpene-derived secondary metabolites that are toxic and/or deterrent to many herbivores (Bowers and Puttick, 1988) and play an important role in mediating tritrophic interactions (Bowers, 1991; Dyer and Bowers, 1996; Smilanich et al., 2009; Theodoratus and Bowers, 1999). Both *E. phaeton* and *A. jatrophae* are able to consume and sequester these phytochemicals; however, *E. phaeton* specializes on plants containing IGs and sequesters these compounds in high concentrations (Bowers et al., 1992), whereas *A. jatrophae* consumes plants with or without IGs, and sequesters lower concentrations (Knerl and Bowers, 2013; Lampert et al., 2014).

The relatively recent colonization of *Plantago* by both *E. phaeton* (first reported in 1979; Stamp, 1979) and *A. jatrophae* (reported in 2013; Knerl and Bowers, 2013) has provided the opportunity to investigate how interactions with the focal viral pathogen, JcDV, vary depending on host plant use. In a field survey of *E. phaeton* caterpillars, we found that JcDV loads were higher in naturally infected individuals using *Plantago,* compared to the primary native host plant for this species, *Chelone glabra* L. (hereafter, *Chelone*; Plantaginaceae) (Muchoney et al., 2022), presenting the question of whether consuming the exotic host plant increases this species' susceptibility to the viral pathogen. In contrast, *A. jatrophae* caterpillars that were experimentally infected with JcDV in a laboratory setting exhibited reduced viral loads and higher survival when using *Plantago*, compared to a native host plant that does not contain iridoid glycosides, *Bacopa monnieri* L. Pennell (hereafter, *Bacopa*; Plantaginaceae) (Muchoney et al., 2023). Thus, consuming the exotic plant may enhance resistance to viral infection in this species.

In this study, we investigated whether the effects of host plant use on herbivore-virus interactions are dose-dependent. We performed two laboratory experiments in which the focal herbivore species were reared on either the exotic host plant, *Plantago,* or a native host plant (*Chelone* for *E. phaeton; Bacopa* for *A. jatrophae*) and orally challenged with one of three doses of JcDV. Our specific goals differed slightly between the two herbivore species, based on previous findings (Fig. 1). In *E. phaeton,* we aimed to determine the effects of host plant use on survival and postmortem viral burdens (i.e., viral yield) following ingestion of different viral doses in a controlled laboratory setting. As the influence of host plant use on survival and postmortem viral burdens was previously characterized in *A. jatrophae* in a laboratory setting, and found to be substantial (Muchoney et al., 2023), we aimed to gain insight into the timing and dose-dependence of these effects by examining how viral infection varied over five days post-inoculation with different doses. By evaluating variation in the dynamics and outcomes of infection in two herbivores using either native or exotic host plants, we provide insight into the role of diet in mediating the impacts of a naturally occurring pathogen on its insect hosts.

2. Methods

2.1. Overview of experiments

To examine the effects of host plant use on herbivore-virus interactions across a range of doses, two factorial laboratory experiments were performed, the first focusing on *E. phaeton* and the second focusing on *A. jatrophae* (Fig. 1). In both experiments, herbivores were reared on either the exotic host plant, *Plantago,* or a native host plant (*Chelone* for *E. phaeton; Bacopa* for *A. jatrophae*) and orally inoculated with one of three doses of purified JcDV [low dose: 1.0 x 10^7 viral equivalent genomes (veg), medium dose: 1.0 x 10^9 veg, or high dose: 5.1 x 10^{10} veg]. The lowest viral dose employed in these experiments was found to result in 25 % mortality of *A. jatrophae* in a previous study (Muchoney et al., 2023); therefore, sequentially higher doses were selected for the medium and high treatments in order to examine the outcomes of more severe JcDV infections. Notably, the highest dose used in these experiments was similar to a dose that resulted in 80 % pre-adult mortality of fifth-instar larvae in another lepidopteran, *Spodoptera frugiperda* (5.0 x 10^{10} veg; Mutuel et al., 2010), and was expected to result in high mortality.

In both experiments, caterpillars were orally inoculated with their assigned viral dose on the first day following molting to the final (sixth) larval instar (see inoculation protocol below). In the *E. phaeton* experiment (Experiment 1), JcDV-challenged caterpillars were subsequently reared until death in the larval, pupal, or adult stage to assess the effects of host plant species on survival, development, and postmortem viral loads (i.e., viral yield) at different doses (Fig. 1). In contrast, JcDV-challenged individuals in the *A. jatrophae* experiment (Experiment 2) were sacrificed at varying time points following inoculation in order to gain insight into the time course of viral infection within herbivores reared on the two host plant species (Fig. 1).

2.2. Experiment 1: Euphydryas phaeton

Euphydryas phaeton caterpillars used in this experiment were the offspring of individuals collected from wild populations located in Massachusetts and Vermont in May 2017. Caterpillars were reared in a laboratory at the University of Colorado, Boulder on either the native

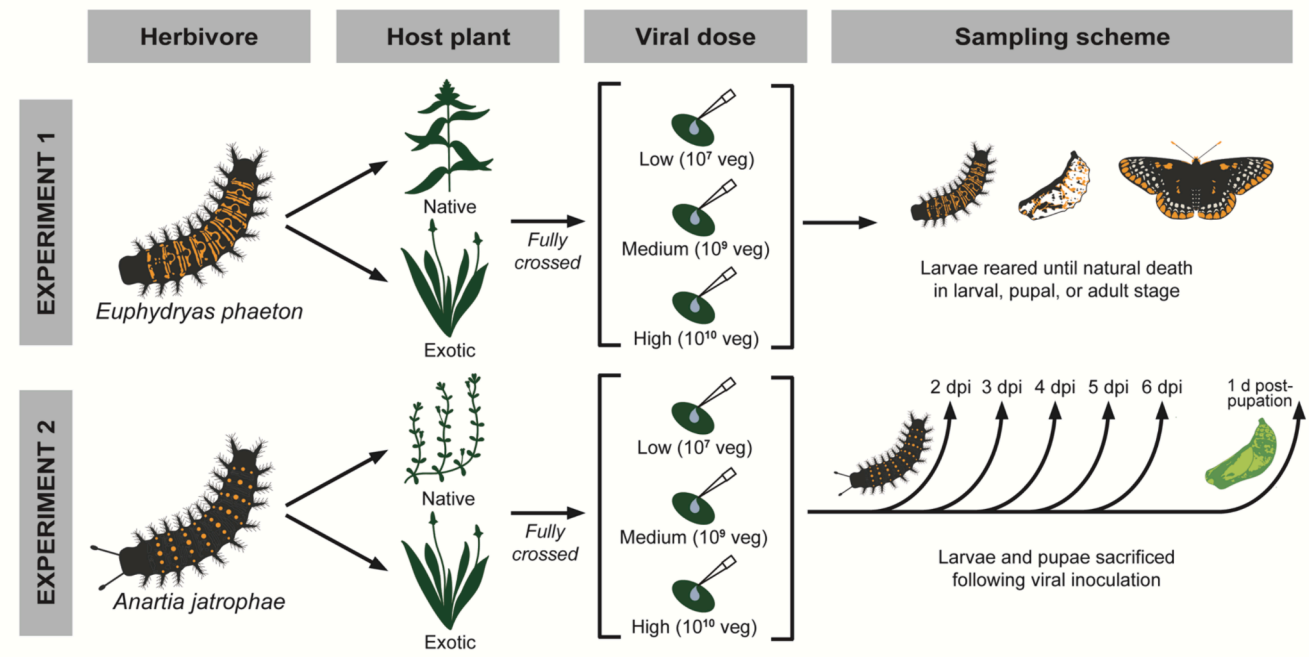

Fig. 1. Factorial experiments investigating the effects of host plant use on viral infection in the herbivores *Euphydryas phaeton* (Experiment 1) and *Anartia jatrophae* (Experiment 2). In both experiments, herbivores were reared in the laboratory on either an exotic host plant, *Plantago lanceolata,* or a native host plant (*Chelone glabra* for *E. phaeton; Bacopa monnieri* for *A. jatrophae*). Upon reaching the final larval instar, caterpillars were orally inoculated with one of three doses of Junonia coenia densovirus [low: 1.0 x 10^7 viral equivalent genomes (veg), medium: 1.0 x 10^9 veg, or high: 5.1 x 10^{10} veg]. In Experiment 1, individuals were subsequently reared until natural death in the larval, pupal, or adult stage to assess survival and postmortem viral loads. In Experiment 2, individuals were sacrificed on days 2–6 post-inoculation (dpi) and day 1 following pupation to evaluate viral burdens more immediately following inoculation.

host plant, *Chelone,* or the exotic host plant, *Plantago,* throughout pre-diapause development (larval instars 1–3) and underwent obligate overwintering diapause at this laboratory during the fourth instar. During this period, caterpillars were placed on damp paper towels to prevent desiccation and maintained at a constant temperature of 4 °C to simulate winter conditions (see also Christensen et al., 2024; Muchoney, 2022). In late May 2018, caterpillars were transferred to the University of Nevada, Reno, where they emerged from diapause and resumed feeding on their respective host plant species. From this point onward, caterpillars were reared individually in 2 oz plastic cups in incubators using a 16 h photoperiod (day temperature: 25 °C, night temperature: 20 °C) and fed ad libitum with *Chelone* or *Plantago* foliage. *Plantago* leaves were collected from the wild in Reno, NV, while *Chelone* leaves were collected from Montpelier, VT and stored in a refrigerator. Leaf surfaces were sterilized prior to feeding by soaking in 5 % bleach solution for 10 min and rinsing thoroughly.

Caterpillars reared on each host plant species were randomly assigned to one of four treatment groups: the low, medium, or high viral dose ($n = 9$–11 per host plant for each dose) or a control group (no virus) ($n = 11$ per host plant; not shown in Fig. 1). On the first day following molting to the sixth instar, caterpillars in the low-, medium-, and high-dose groups were orally inoculated with their assigned dose of JcDV. Each caterpillar was presented with an 8 mm leaf disc (*Chelone* or *Plantago,* according to host plant group) with 1.0 x 10^7, 1.0 x 10^9, or 5.1 x 10^{10} viral equivalent genomes suspended in 1 μl of DI water pipetted onto the surface and allowed to dry. Caterpillars were given 24 h to consume the leaf disc, and those that did not were either re-inoculated the following day following the same protocol or excluded from the experiment.

Following inoculation, caterpillars continued to be fed according to their host plant group and were checked daily to monitor development and survival time (defined as *n* days between inoculation and death in either the larval, pupal, or adult stage). For individuals that died as pupae, the date of death was defined as the pupation date. Control insects were maintained in an incubator at a separate location from JcDV-challenged insects to avoid cross-contamination between groups. In addition, frass was collected from each JcDV-challenged caterpillar on days 1–5 following inoculation to determine the amount of viral DNA that was excreted over time following each dose and the extent to which viral excretion in frass was representative of the administered dose. Sterile technique was used between handling of each insect and frass sample, which entailed soaking instruments in DNA-Erase (MP Biomedicals, Santa Ana, CA, USA), a 30 % solution of bleach, and a 70 % solution of ethanol. Individuals that reached the pupal stage were weighed and transferred to 32 oz plastic containers with mesh lids for eclosion, and butterflies were maintained on a diet of 10 % honey water until death. After death in the larval, pupal, or adult stage, all individuals were promptly frozen for use in viral screening.

2.3. Experiment 2: Anartia jatrophae

Anartia jatrophae caterpillars used in this experiment were obtained from a colony maintained at the University of Colorado, Boulder, which originated from wild butterflies collected from Florida in April 2017. Insects from this colony were shipped to the University of Nevada, Reno (UNR), and a mixture of first- and third-generation UNR offspring were used for the experiment. Approximately half of experimental insects were reared on the native host plant, *Bacopa* ($n = 178$) and half were reared on the exotic host plant, *Plantago* ($n = 195$), throughout larval development. Though *Bacopa*- and *Plantago*-fed individuals were present in both generation sets, the first generation primarily consisted of *Plantago*-fed larvae, while the third generation primarily consisted of *Bacopa*-fed larvae, due to variation in host plant availability. Caterpillars were reared in incubators under the same conditions described for Experiment 1 (above) and fed ad libitum with sterilized *Bacopa* or *Plantago* foliage. *Bacopa* was grown in a greenhouse at the University of Colorado, Boulder and stored in a refrigerator, whereas *Plantago* was either collected from the wild in Reno, NV or grown in a hydroponics

system at UNR.

Caterpillars reared on each host plant were randomly assigned to receive either the low, medium, or high dose of JcDV (n = 56–80 per host plant for each dose) on the first day after molting to the sixth instar. Oral inoculations were performed following the protocol described for Experiment 1 (above), after which larvae continued to be fed according to their host plant group. Beginning on day two following inoculation, subsets of caterpillars from each dose and host plant group were freeze-killed each day until day six post-inoculation (n = 8–14 per day). An additional subset of each group was sacrificed on the first day following pupation (n = 4–9) to examine viral burdens in pupae.

2.4. Viral screening and quantification

To detect and quantify JcDV infection in *E. phaeton* and *A. jatrophae*, total DNA was extracted from a tissue sample from each insect using Qiagen DNeasy 96 Blood and Tissue Kits (Qiagen, Hilden, North Rhine-Westphalia, Germany) following the Protocol for Purification of Total DNA from Animal Tissues. Whole caterpillars and pupae were homogenized using a Qiagen TissueLyser II and DNA was extracted from a 20 mg aliquot of tissue, whereas whole bodies (with wings removed) were used for butterflies.

Extracted DNA samples were screened for JcDV using quantitative PCR, with primers specific to the VP4 capsid protein gene of JcDV (Wang et al., 2013) and arthropod 28S rDNA primers (Nice et al., 2009) as an internal control. DNA samples were screened in duplicate for both VP4 and 28S using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) at a reaction volume of 10 µl. Reactions were run on a Bio-Rad CFX96 Optics Module with C1000 Thermal Cycler following the protocols of Muchoney et al. (2022). Viral loads were calculated as $2^{-\Delta Ct}$ (Schmittgen and Livak, 2008), representing the abundance of the JcDV VP4 gene relative to the abundance of the internal control gene [ΔC_t = mean C_t (threshold cycle) for VP4 – mean C_t for 28S], and log-transformed for analyses and visualization.

To detect and quantify JcDV in *E. phaeton* frass, total DNA was extracted from each sample following the protocol described above. For frass samples weighing less than 20 mg, whole samples were used, while a 20 mg aliquot of homogenized frass was used for larger samples. Extracted DNA was screened in duplicate for the JcDV VP4 gene using the qPCR protocol above, and viral quantity was estimated using a standard curve. Briefly, a stock solution of JcDV was serially diluted and used to generate a standard curve over seven orders of magnitude (from 1.0 x 10^3 to 1.0 x 10^9 viral equivalent genomes), which was used to calculate absolute quantity of JcDV in each sample of extracted DNA (elution volume: 100 µl) based on C_t values for the VP4 gene [expressed as log-transformed viral equivalent genomes (veg)].

2.5. Statistical analyses

2.5.1. Experiment 1: Euphydryas phaeton

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021). The dose-dependent effect of JcDV on *E. phaeton* survival was evaluated using logistic regression, with viral dose (low or medium; expressed as an ordinal variable), host plant species (*Chelone* or *Plantago*), and their interaction as predictors and survival to the adult stage (Y/N) as the response. Individuals that received the highest viral dose were omitted from this analysis, as 100 % mortality was observed within this group. Due to the minimal impact of host plant and its interaction with viral dose on survival (see Results), a simplified model including only the effect of viral dose (treated as a continuous variable and expressed as log-transformed viral equivalent genomes) was used to estimate the dose required to kill 50 % of insects prior to the adult stage (LD_{50}) on both host plants. Using the 'survival' package (Therneau, 2022), a Cox proportional hazard model was fitted to assess the effects of viral dose (low or medium/high, expressed as an ordinal variable), host plant, and their interaction on survival following inoculation, and Kaplan-Meier survival analysis was used to illustrate daily survivorship across viral doses. Due to similarity in survival patterns between the medium- and high-dose groups (see Results), these treatments were pooled within the Cox proportional hazard model but are depicted separately within the Kaplan-Meier plot for illustrative purposes.

The probability of detecting JcDV (Y/N) in virus-challenged insects following death in the larval, pupal, or adult stage was compared across viral doses (low or medium; expressed as an ordinal variable) and host plants using logistic regression, and the relationship between viral detection and survival was examined using Pearson's chi-squared test. A separate logistic regression model was used to examine the effect of host plant species on the probability of viral detection in individuals that survived to the adult stage after receiving the lowest viral dose. To examine the outcomes of harboring a detectable infection for pupal mass and adult longevity (n days between eclosion and death), two linear regression models were used, which both included postmortem viral detection (Y/N) and sex as predictors. Sex was included as a predictor in these models to account for sexual dimorphism in body size (i.e., larger females) in this species. In addition, pupal mass and adult longevity were compared between virus-challenged individuals (low, medium, and high dose) and uninoculated controls using separate linear regression models, which both included viral exposure during the larval stage (Y/N) and sex as predictors. Within the subset of insects that harbored a detectable infection, multiple regression was used to evaluate the effects of viral dose (low, medium, or high; expressed as an ordinal variable), host plant species, the interaction between dose and host plant, and life stage at death (larva, pupa, or adult) on postmortem viral load. In addition, the correlation between survival time and postmortem viral load was separately evaluated among virus-infected individuals that died as larvae and those that died as pupae using Pearson's correlation coefficients.

The probability of detecting JcDV (Y/N) in the frass of virus-challenged larvae on days 1–5 following inoculation was evaluated using mixed-effects logistic regression, with viral dose (low, medium, or high; expressed as an ordinal variable) and collection day (expressed as continuous variable) as fixed effects and random intercepts for individual larvae. Viral quantities in frass were compared across host plant species and doses using a linear mixed-effects model, which included dose, host plant, day, and the interaction between dose and host plant as fixed effects, with random intercepts for individuals. Both mixed-effects models were fitted using the 'lme4' package (Bates et al., 2015), with p-values for the linear mixed-effects model generated using 'lmerTest' (Kuznetsova et al., 2017). Finally, the relationship between initial viral dose, postmortem viral load, and the quantity of virus excreted in the frass was investigated using a multiple regression model, with viral dose (treated as a continuous variable and expressed as log-transformed viral equivalent genomes), mean viral quantity in frass, and their interaction as predictors and postmortem load in each insect as the response. To probe this interaction, a Johnson–Neyman interval for significance of the conditional effect of JcDV quantity in frass on viral load in insects was calculated using the 'interactions' package (Long, 2019).

2.5.2. Experiment 2: Anartia jatrophae

The probability of detecting JcDV (Y/N) in *A. jatrophae* caterpillars following inoculation was compared across viral doses (low, medium, or high; expressed as an ordinal variable), host plant species (*Bacopa* or *Plantago*), and day sacrificed (day 2–6 post-inoculation) using logistic regression. For the subset of caterpillars that harbored a detectable infection, multiple regression was used to evaluate the effects of ordinal viral dose, host plant species, and day sacrificed on viral load. Viral loads were also compared between individuals that were sacrificed during the larval stage and those that were sacrificed as pupae using a multiple regression model, which evaluated the effects of viral dose, host plant, life stage (larva or pupa), and the interaction between host plant and life stage. The effects of host plant species and ordinal viral dose on the body mass of caterpillars and pupae at their time of sacrifice were investigated

using separate multiple regression models, which included viral dose, host plant, and day post-inoculation (for larvae only) as predictors and larval or pupal mass as the response.

3. Results

3.1. Experiment 1: Euphydryas phaeton

3.1.1. Survival and longevity

In *E. phaeton* challenged with JcDV, survival to the adult stage decreased with viral dose (Fig. 2a–b). Survival was reduced in individuals that received the medium dose, compared to the low dose [odds ratio (OR) = 0.042, 95 % confidence interval (CI) = (0.003–0.239), $z = -3.0$, $p = 0.002$], and 100 % mortality was observed in individuals that received the highest dose. In contrast, 100 % of uninfected controls successfully reached the adult stage (Appendix A: Fig. A.1). This negative relationship between viral dose and survival was consistent across individuals reared on the two host plant species (Fig. A.1) [host plant x dose interaction: OR=1.3, 95 % CI = (0.1–29.0), $z = 0.2$, $p = 0.9$]. Based on a simplified logistic regression model including solely the effect of dose on survival [OR=0.11, 95 % CI = (0.03–0.29), $z = -4.1$, $p < 0.0001$], the LD_{50} for sixth instar *E. phaeton* on both plants was estimated to be 1.0 x 10^8 veg.

Survival time following inoculation (i.e., time to death) was also reduced at medium and high viral doses, compared to the lowest dose (Fig. 2a) [Cox proportional hazard ratio (HR) = 9.3, 95 % CI = (2.2–39.1), $z = 3.0$, $p = 0.002$], and this pattern was consistent among individuals reared on the two host plant species [host plant x dose interaction: HR=1.0, 95 % CI = (0.1–7.4), $z = -0.02$, $p > 0.9$]. Individuals that received the lowest dose of JcDV survived for an average of 38 days, successfully reaching the adult stage in 90 % of cases (Fig. 2b), compared to 15 and 13 days in the medium- and high-dose groups, respectively. Though survival time was similar following medium and high doses, individuals that received the highest dose were more likely to die during the larval stage, while a greater proportion of those that received the medium dose died as pupae (Fig. 2b) ($X^2 = 9.2$, $df = 1$, $p = 0.002$).

3.1.2. Viral detection and loads in insects

The likelihood of detecting JcDV in virus-challenged individuals following death in the larval, pupal, or adult stage increased with dose (Fig. 3a). All individuals that received the highest dose harbored a detectable infection at their time of death, compared to 95 % of the medium-dose group and 60 % of the low-dose group [OR=5.9, 95 % CI = (1.6–49.4), $z = 2.2$, $p = 0.03$]. This pattern was mediated by the higher frequency of survival in these groups (see Fig. 2b), as JcDV was detected in only 55 % of individuals that reached the adult stage, compared to 100 % of individuals that died during the larval or pupal stages ($X^2 = 17.0$, $df = 1$, $p < 0.0001$).

Though patterns of viral detection were similar in individuals reared on the two host plant species [Fig. 3a; OR=2.2, 95 % CI = (0.4–13.6), $z = 0.9$, $p = 0.4$], a greater proportion of butterflies that had been reared on *Plantago* maintained a detectable infection after receiving the lowest dose (75 %), compared to butterflies that had been reared on *Chelone* (40 %) [OR=4.5, 95 % CI = (0.6–43.4), $z = 1.5$, $p = 0.1$]. Virus-challenged butterflies that maintained detectable infections exhibited lower pupal masses than those that did not ($\beta = -42 \pm 17$, $t = -2.5$, $df = 15$, $p = 0.02$), with pupal mass reduced by an average of 21 % in females (undetected mean: 428 ± 15 mg; detected mean: 340 ± 30 mg) and 5 % in males (undetected mean: 274 ± 27 mg; detected mean: 261 ± 29 mg). However, pupal masses were higher in virus-challenged individuals than uninoculated controls ($\beta = 76 \pm 15$, $t = 5.1$, $df = 25$, $p < 0.0001$). Adult longevity (mean: 11 ± 6 d) was similar between virus-challenged butterflies that maintained detectable infections and those that did not ($\beta = 0.9 \pm 3.0$, $t = 0.3$, $df = 15$, $p = 0.8$) and did not differ substantially between virus-challenged individuals and uninoculated controls ($\beta = -1.2 \pm 2.4$, $t = -0.5$, $df = 25$, $p = 0.6$).

Within the subset of individuals that harbored detectable infections at their time of death (including those that died as larvae, pupae, and adults), postmortem viral loads also varied according to dose (Fig. 3b). There was a positive relationship between initial dose and postmortem load ($\beta = 2.5 \pm 1.0$, $t = 2.6$, $df = 41$, $p = 0.01$), which did not differ based on host plant species (host plant x dose interaction: $\beta = -0.6 \pm 1.0$, $t = -0.6$, $df = 41$, $p = 0.5$). In addition, individuals that died as larvae and pupae harbored substantially higher viral burdens than those that reached the adult stage (Fig. A.2) (deceased larvae: $\beta = 6.1 \pm 1.1$, $t = 5.3$, $df = 41$, $p < 0.0001$; deceased pupae: $\beta = 7.6 \pm 1.3$, $t = 5.9$, $df = 41$, $p < 0.0001$). Viral loads of individuals that died during the larval and pupal stages were similar ($\beta = 1.51 \pm 0.76$, $t = 2.0$, $df = 41$, $p = 0.1$), and there was not a strong correlation between survival time and postmortem viral load within either group (larvae: Pearson's R=-0.03, $df = 23$, $p = 0.9$; pupae: Pearson's R=-0.47, $df = 11$, $p = 0.1$).

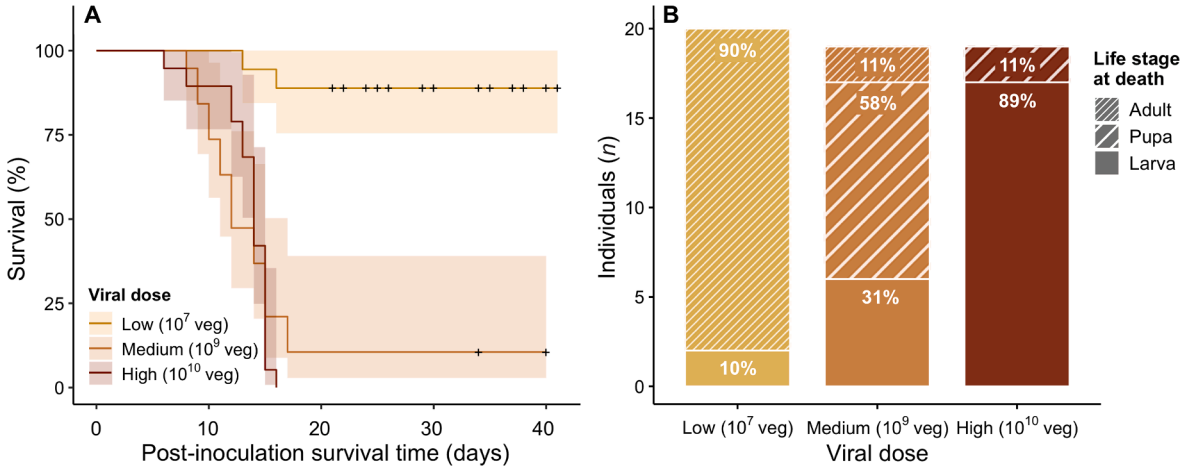

Fig. 2. Effects of Junonia coenia densovirus dose on survival time and development in *Euphydryas phaeton* (Experiment 1). Results include individuals reared on both the native host plant, *Chelone glabra,* and the exotic host plant, *Plantago lanceolata.* (A) Kaplan–Meier survival plot of individuals inoculated with either a low (n = 18), medium (n = 19), or high (n = 19) dose of JcDV. Time to death (n days between inoculation and death in the larval, pupal, or adult stage) was faster in insects inoculated with medium and high viral doses (means: 15 d and 13 d, respectively), compared to the lowest viral dose (mean: 38 d). The medium- and high-dose groups were pooled for Cox proportional hazard analysis due to overlap in survival patterns, but are depicted separately for illustrative purposes. (B) Life stage at death following inoculation with a low, medium, or high dose of JcDV. A relatively high proportion of individuals that received the lowest dose survived to reach the adult stage (90 %), while mortality during the larval and pupal stages increased at higher viral doses.

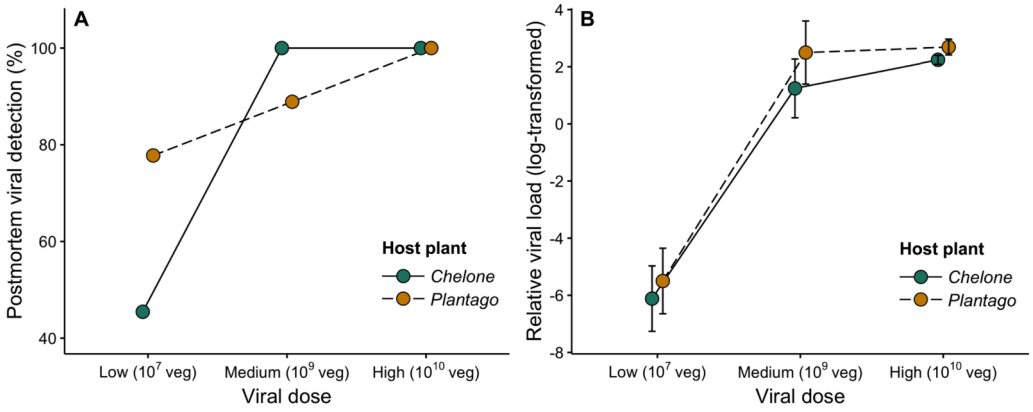

Fig. 3. Effects of host plant species on postmortem viral detection frequencies and viral burdens of *Euphydryas phaeton* inoculated with a low, medium, or high dose of Junonia coenia densovirus (Experiment 1). (A) Frequency of viral detection in JcDV-challenged insects following death in the larval, pupal, or adult stage. Points represent % inoculated individuals reared on the native host plant, *Chelone glabra,* or the exotic host plant, *Plantago lanceolata,* in which the virus was detected following death. (B) Postmortem viral loads of JcDV-challenged insects following death in the larval, pupal, or adult stage. Points represent mean load (log-transformed, relative to an internal control gene) ± SE of individuals reared on the native or exotic host plant. Patterns of viral detection and loads were similar on the two plant species, though infection was detected in a higher proportion of individuals reared on *Plantago* following inoculation with the lowest dose.

3.1.3. Viral detection and loads in frass

JcDV was detected in frass samples from 100 % of virus-challenged caterpillars and was detected on all five sampling days in 76 % of individuals. Overall, the likelihood of detecting JcDV in frass increased with viral dose [OR=7.5, 95 % CI = (1.3–42.5), $z = 2.3$, $p = 0.02$] and decreased slightly across days following inoculation [OR=0.68, 95 % CI = (0.46–1.00), $z = -2.0$, $p = 0.05$]. The amount of JcDV in each frass sample mirrored this pattern: viral quantity was strongly influenced by the dose that was ingested (Fig. 4a) ($\beta = 1.84 \pm 0.29$, $t = 6.4$, $df = 47$, $p < 0.0001$) and decreased over time following inoculation ($\beta = -0.649 \pm 0.042$, $t = -15.6$, $df = 192$, $p < 0.0001$). The relationship between viral dose and the amount of virus excreted in frass did not differ based on host plant use (Fig. A.3) (host plant x dose interaction: $\beta = 0.18 \pm 0.40$, $t = 0.4$, $df = 48$, $p = 0.7$).

The postmortem viral load of each insect (including those that died as larvae, pupae, and adults) was positively associated with both the initial dose that was ingested ($\beta = 11.3 \pm 1.6$, $t = 6.9$, $df = 41$, $p < 0.0001$) and the average amount of JcDV that was excreted in frass ($\beta = 15.5 \pm 2.6$, $t = 5.9$, $df = 41$, $p < 0.0001$). Notably, there was also a negative interaction between viral dose and viral content in frass ($\beta = -1.47 \pm 0.25$, $t = -5.8$, $df = 41$, $p < 0.0001$). This interaction indicates that when larvae consumed lower doses of JcDV, viral quantity in frass was a strong predictor of the postmortem viral burden of the insect; however, this relationship attenuated at higher doses (Fig. 4b). The threshold dose at which the viral content of frass was no longer positively associated with viral load was calculated as 1.1 x 10^{10} veg (Johnson-Neyman interval); above this value, the quantity of virus excreted in the frass was not a reliable predictor of the postmortem infection burden of the insect.

3.2. Experiment 2: Anartia jatrophae

3.2.1. Viral detection and loads in insects

In virus-challenged *A. jatrophae*, JcDV was detected in 95 % of individuals that were sacrificed as larvae (2–6 days post-inoculation) and 100 % of individuals that were sacrificed as pupae. The likelihood of viral detection in larvae did not differ based on viral dose [OR=1.3, 95 % CI = (0.6–3.3), $z = 0.6$, $p = 0.5$] and decreased slightly across days following inoculation [OR=0.61, 95 % CI = (0.40–0.89), $z = -2.4$, $p = 0.02$], with the highest probability of viral detection (99 %) occurring on days 2 and 3 post-inoculation, and the lowest probability of detection (91 %) occurring on day 5 post-inoculation. The probability of detecting JcDV was also greater in caterpillars that were reared on the exotic host plant, *Plantago*, compared to those reared on the native *Bacopa* (Fig. 5a)

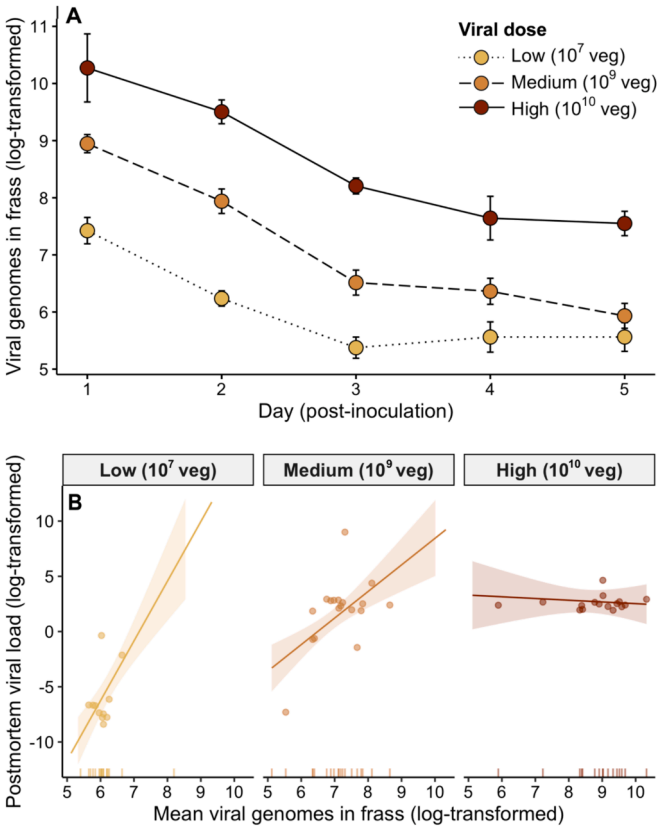

Fig. 4. Detection of Junonia coenia densovirus in *Euphydryas phaeton* frass following inoculation with a low, medium, or high viral dose (Experiment 1). Results include individuals reared on the native host plant, *Chelone glabra,* and the exotic host plant, *Plantago lanceolata.* (A) Daily amount of JcDV excreted in caterpillar frass. Points represent mean quantity of JcDV [log-transformed number of viral equivalent genomes (veg)] ± SE on days 1–5 following inoculation. Viral content of frass increased with dose and decreased slightly across days following inoculation. (B) Relationship between the quantity of JcDV excreted in frass (averaged across days 1–5 post-inoculation) and the postmortem viral load of each insect. There was a positive association between these two parameters in individuals inoculated with low and medium doses of the virus, but not in those inoculated with the highest dose of the virus.

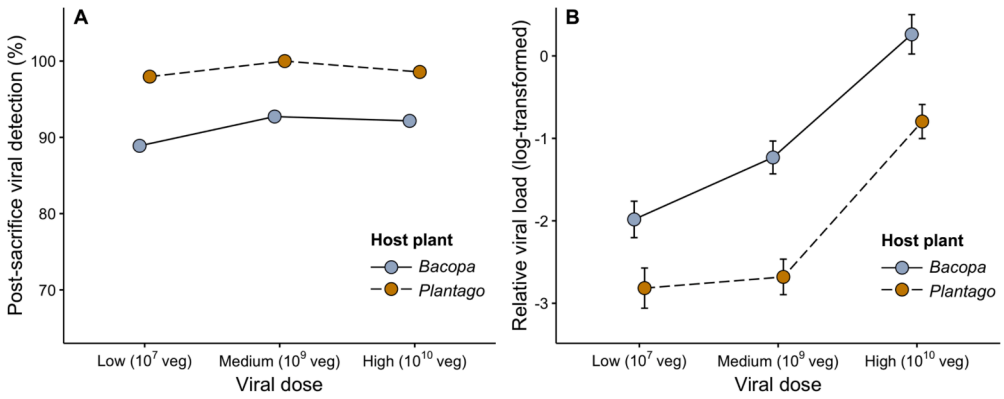

Fig. 5. Effects of host plant species on viral detection frequencies and viral burdens of *Anartia jatrophae* caterpillars inoculated with a low, medium, or high dose of Junonia coenia densovirus (Experiment 2). Results include individuals sacrificed on days 2–6 following inoculation. (A) Frequency of viral detection in JcDV-challenged individuals following sacrifice. Points represent % inoculated individuals reared on the native host plant, *Bacopa monnieri,* or the exotic plant, *Plantago lanceolata,* in which the virus was detected following sacrifice. (B) Viral loads of JcDV-challenged caterpillars following sacrifice. Points represent mean JcDV load (log-transformed, relative to an internal control gene) ± SE of individuals reared on the native or exotic host plants. JcDV was detected at a higher frequency in larvae reared on *Plantago,* while viral burdens were substantially higher in individuals reared on *Bacopa* across all three doses.

[OR=9.7, 95 % CI = (2.6–63.1), $z = 2.9$, $p = 0.003$].

In caterpillars that harbored detectable infections, viral loads increased according to dose (Fig. 5b) ($\beta = 1.53 \pm 0.16$, $t = 9.8$, $df = 306$, $p < 0.0001$) and were higher in individuals reared on *Bacopa* than those reared on *Plantago* (β = -1.07 ± 0.18, t = -5.8, df = 306, $p < 0.0001$) across all doses (Fig. 5b) and sampling days (Fig. A.4). Altogether, larvae that consumed the native *Bacopa* exhibited 12-fold higher loads than those that consumed the exotic *Plantago.* In addition, infection loads decreased slightly over time following inoculation (Fig. 6a) (β = -0.118 ± 0.064, t = -1.8, df = 306, $p = 0.07$). However, individuals that were sacrificed as pupae harbored lower viral burdens than those sacrificed as larvae (Fig. 6b). This decrease in JcDV load following pupation was more pronounced in insects reared on *Bacopa* (β = -2.90 ± 0.42, t = -6.8, df = 345, $p < 0.0001$) than those reared on *Plantago* (β = -0.75 ± 0.37, t = -2.0, df = 345, $p = 0.04$) (Fig. A.4).

3.2.2. *Caterpillar growth and pupal mass*

Caterpillar body mass was higher in individuals that developed for a longer period of time before sacrifice ($\beta = 27.6 \pm 3.9$, $t = 7.2$, $df = 320$, $p < 0.0001$), increasing by an average of 38 % between day 2 and day 6 post-inoculation, but did not vary depending on the viral dose received (β = -8.8 ± 9.3, t = -1.0, df = 320, $p = 0.3$). However, larvae reared on *Plantago* were larger across all sampling days ($\beta = 37 \pm 11$, $t = 3.3$, df = 320, $p = 0.001$), with 15 % higher overall mass. A similar pattern was evident in individuals that were sacrificed as pupae: pupal mass was 63 % higher in *Plantago*-fed individuals than *Bacopa*-fed individuals (β = 139 ± 33, $t = 4.2$, $df = 34$, $p = 0.0002$) but was not impacted by viral dose (β = -7 ± 27, t = -0.3, $df = 34$, $p = 0.8$).

4. Discussion

4.1. *Experiment 1: Euphydryas phaeton*

In *E. phaeton,* the outcomes of JcDV infection were highly dose-dependent, with higher viral doses resulting in faster time to death (Fig. 2a), greater mortality during the larval and pupal stages (Fig. 2b), and higher postmortem viral burdens (Fig. 3b). However, patterns of survival (Fig. A.1) and viral loads (Fig. 3b) were largely similar in herbivores reared on the native and exotic host plant species, suggesting that use of these two host plant species did not strongly mediate

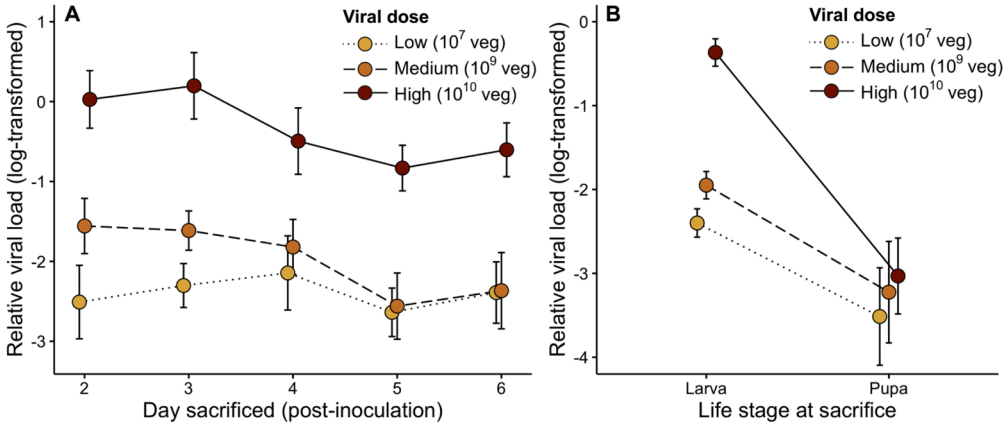

Fig. 6. Variation in viral loads across time and development in *Anartia jatrophae* following inoculation with a low, medium, or high dose of Junonia coenia densovirus (Experiment 2). Results include individuals reared on the native host plant, *Bacopa monnieri,* and the exotic host plant, *Plantago lanceolata.* (A) Viral loads of JcDV-challenged larvae following sacrifice on days 2–6 following inoculation. Points represent mean load (log-transformed, relative to an internal control gene) ± SE of individuals inoculated with either a low, medium, or high viral dose. (B) Viral loads of JcDV-challenged individuals following sacrifice in either the larval stage (averaged over days 2–6 following inoculation) or pupal stage (day 1 following pupation). Points represent mean load (log-transformed, relative to an internal control gene) ± SE of individuals inoculated with a low, medium, or high viral dose. Infection loads decreased slightly over time following inoculation in larvae, and were lower in pupae, relative to larvae, across all doses.

resistance to infection in this species within a controlled environment. Our previous research documented higher JcDV burdens in late-instar *E. phaeton* caterpillars utilizing the exotic plant, *Plantago,* compared to the native *Chelone,* in a field setting (Muchoney et al., 2022), raising the question of whether consuming *Plantago* increases *E. phaeton*'s vulnerability to this pathogen. However, the same study found that the survival rates of individuals consuming the two host plants when naturally infected with JcDV in the wild were similar (Muchoney et al., 2022). The findings of the present study are consistent with this result, indicating that both the severity of infection (Fig. 3b) and its outcomes for survival and development (Fig. 2a–b) were primarily determined by the viral dose that was ingested, rather than the host plant species that was consumed.

An important consideration in interpreting these results is the phytochemical similarity between *Chelone* and *Plantago,* which both contain iridoid glycosides, although the composition of their IG profiles differs (Bowers et al., 1992; Bowers and Stamp, 1992). In other nymphalid butterflies (*Junonia coenia* and *A. jatrophae*), the outcomes of JcDV infection have been found to differ when herbivores were reared on a high-IG plant species, compared to a low-IG plant species (Smilanich et al., 2018), or a plant species containing IGs, compared to a plant species lacking IGs (Muchoney et al., 2023). As such, it is possible that the magnitude of host plant effects on viral infection in *E. phaeton* would be greater when comparing the two primary host plants (*Chelone* and *Plantago*) to alternative host plant species containing lower concentrations of IGs (e.g., *Plantago major*; Smilanich et al., 2018) or comparing genotypes of the primary host plant species that vary in IG content (e.g., Laurentz et al., 2012). As IG concentrations and composition in *Chelone* and *Plantago,* along with *E. phaeton* sequestration of these compounds, may vary across local populations and seasons (Bowers and Stamp, 1993; Carper et al., 2021; Muchoney et al., 2022), it remains possible that intraspecific and/or interpopulation variation in host plant chemistry could mediate interactions between *E. phaeton* and JcDV in wild settings.

Notably, one of the only patterns associated with host plant use in *E. phaeton* involved the probability of detecting JcDV following death, which was higher in *Plantago*-fed individuals that survived to the adult stage after receiving the lowest viral dose, compared to *Chelone*-fed individuals (Fig. 3a). Assuming that viral infection was initially established after inoculation, this pattern may indicate a reduced capacity to suppress or clear infection before reaching the adult stage. Alternatively, it is possible that a greater proportion of *Plantago*-fed individuals initially contracted infection, while *Chelone*-fed individuals were better able to avoid or limit its establishment (i.e., qualitative resistance; de Roode et al., 2011; Gandon and Michalakis, 2000). Though this pattern was only evident at the lowest viral dose, we found that post-mortem viral loads of individuals that received this low dose [median (interquartile range): −6.7 (−7.5, −5.1)] were similar to those of JcDV-infected individuals collected from the wild in two different studies [median (IQR): −6.5 (−7.2, −5.7); Muchoney et al., 2022] [median (IQR): −6.7 (−7.1, −5.7); Muchoney et al., unpublished results]. Thus, the "low" dose employed in this study (1.0×10^7 veg) likely represents a field-relevant dose for *E. phaeton.* Higher incidence of infection in adults could provide increased opportunity for vertical transmission of the virus in populations consuming *Plantago.* Such transmission of JcDV from female to offspring has recently been documented in *E. phaeton* (Christensen et al., 2024). In addition, as maintaining a detectable infection into the adult stage following viral challenge entailed a cost for performance (lower pupal weight), we may expect this sublethal effect to be more prevalent in populations using *Plantago* as a primary host plant, compared to *Chelone*. However, given that virus-challenged individuals exhibited higher pupal weights than uninoculated controls, together with a limited sample size ($n = 28$ surviving butterflies), additional research will be necessary to elucidate the impacts of viral exposure and infection on *E. phaeton* body mass and adult performance.

Together, these results suggest that higher viral burdens observed in the wild in caterpillars consuming *Plantago* may be the product of additional abiotic or biotic factors that differ between populations utilizing the two host plants, rather than intrinsic differences in the herbivore's ability to resist infection. One possibility is that JcDV occurrence in the environment (i.e., on host plant surfaces) is higher in *Plantago*-utilizing populations than *Chelone*-utilizing populations, thereby influencing the amount of virus that is ingested throughout development. As host plant surface chemistry (Young et al., 1977), architecture (Duffey et al., 1995), and habitat (Raymond et al., 2005) have all been shown to influence entomopathogen persistence on the phylloplane, this possibility warrants investigation. In addition, spatial clustering and contact rates among caterpillars have been shown to vary across *E. phaeton* populations in the region (Carson et al., 2024), which may contribute to variation in viral accumulation and transmission. Though our results indicate a potential for increased rates of vertical transmission of JcDV in populations using *Plantago,* another study found that horizontal transmission rates of JcDV were slightly lower in *E. phaeton* reared on *Plantago,* compared to *Chelone,* in a laboratory setting (Christensen et al., 2024). Thus, the factors mediating variation in viral burdens across *E. phaeton* populations utilizing distinct dietary resources are likely complex and multifaceted.

4.2. Experiment 2: Anartia jatrophae

In contrast to the patterns documented in *E. phaeton*, JcDV infection was strongly modulated by host plant use in *A. jatrophae.* While viral burdens varied according to the dose that was ingested (Fig. 6a), they were also consistently higher in caterpillars reared on *Bacopa,* compared to the exotic plant, *Plantago,* across three infectious doses (Fig. 5b) and across time following inoculation (Fig. A.4). This indicates that viral infection was limited or suppressed in larvae consuming this exotic plant almost immediately following its establishment (2–6 d post-inoculation). These findings corroborate the results of our previous study within this system, which found that postmortem viral loads were reduced, and survival was dramatically enhanced, in individuals using *Plantago* following inoculation with the lowest dose of JcDV used in the present study (Muchoney et al., 2023). Together, these results support the hypothesis that survival of viral infection is improved when *A. jatrophae* is reared on *Plantago,* relative to *Bacopa,* due to an enhanced ability to limit pathogen burden during the early stages of infection.

The mechanisms underlying this improved resistance remain unknown. Our previous research documented similar patterns of immunocompetence in *A. jatrophae* reared on *Bacopa* and *Plantago* (Muchoney et al., 2023; see also Lampert et al., 2014), though the role of specific immune parameters in contributing to defense against JcDV appear to be complex and require further study (Muchoney et al., 2022; Resnik and Smilanich, 2020; Smilanich et al., 2018). It is therefore possible that immune responses that have not yet been quantified in *A. jatrophae* contribute to defense against JcDV and are enhanced in larvae consuming *Plantago.* However, given that host plant-mediated differences in viral burden were evident as early as day 2 post-inoculation, it appears more likely that consuming *Plantago* reduces the effective dose of viral particles that initially establishes infection (de Roode et al., 2011), rather than suppressing viral replication or enhancing viral clearance following infection (i.e., quantitative resistance; Gandon and Michalakis, 2000). Given these findings, investigating how barriers to the establishment of JcDV infection, including the physiology of the peritrophic matrix and/or midgut (Pigeyre et al., 2019), vary across *A. jatrophae* caterpillars consuming different host plant species (Plymale et al., 2008) may yield insight into the mechanisms underlying enhanced resistance on *Plantago.*

Additionally, differences in the chemistry of the two host plants may play a role in mediating resistance (Cory and Hoover, 2006). As previously noted, *Plantago* contains iridoid glycosides, a class of secondary metabolites that are highly consequential for multitrophic interactions (Bowers, 1991; Dyer and Bowers, 1996; Smilanich et al., 2009), whereas

the native plant *Bacopa* does not contain these compounds. Previous research indicates that sequestration of IGs may contribute to defense against JcDV in *E. phaeton,* as a negative relationship between the amount of IGs sequestered from host plants and JcDV loads was documented in wild-collected caterpillars (Muchoney et al., 2022; see also Christensen et al., 2024). Survival following JcDV infection in another nymphalid butterfly, *Junonia coenia*, was higher in individuals reared on *Plantago lanceolata,* compared to a congeneric species containing lower IG concentrations, *P. major* (Smilanich et al., 2018). As *A. jatrophae* is capable of sequestering IGs when reared on *Plantago* (Knerl and Bowers, 2013), the possibility that IG sequestration, or IG presence in the midgut, is directly or indirectly linked to suppression of JcDV infection is a compelling one.

Surprisingly, the frequency with which JcDV was detected in *A. jatrophae* on days 2–6 following inoculation was higher in caterpillars reared on *Plantago* (98 %) than *Bacopa* (91 %) (Fig. 5a), while no difference in postmortem detection was found between individuals reared on the same two host plants in our previous study (Muchoney et al., 2023). Overall, the virus was detected in a higher proportion of caterpillars in the days immediately following inoculation (95 % across both host plants in the present study) than following death in the larval, pupal, or adult stage (57 %; Muchoney et al., 2023), indicating that JcDV is highly infective in this species at the experimental doses, but that some insects are able to effectively clear infection by the time they die. In addition, JcDV was detected in 100 % of individuals sacrificed during the pupal stage on both plants. Thus, although initial establishment of infection was higher in larvae reared on *Plantago,* these larvae maintained lower viral burdens than those using *Bacopa* (Fig. 5b), which is highly consequential for survival in this species (Muchoney et al., 2023; see also Fig. 2).

4.3. Viral infection dynamics in wild hosts

Beyond providing insight into the role of host plants in mediating viral infection, these experiments afforded opportunities to compare the dynamics of JcDV infection in two wild hosts of this pathogen (*E. phaeton* and *A. jatrophae*) to its effects in the model host in which it has primarily been studied, *Spodoptera frugiperda* (Mutuel et al., 2010; Wang et al., 2013). Overall mortality following viral inoculation appears to be greater in final-instar *E. phaeton,* compared to fifth-instar *S. frugiperda* (Fig. 2a) (e.g., 100 % mortality at the highest dose, compared to 80 % in *S. frugiperda*; Mutuel et al., 2010). However, it is important to note that research focusing on *S. frugiperda* utilized semi-artificial diet for caterpillar feeding and oral inoculation of the virus, which may differ significantly from the entirely plant-based diets utilized in this study in its effects on infection (Hoover et al., 2000; Plymale et al., 2008). In addition, mortality following inoculation with a lower, and likely field-relevant, dose of the virus was slightly lower in *E. phaeton* than *A. jatrophae* (e.g., 10 % mortality at the lowest dose, compared to 25 % in *A. jatrophae*; Muchoney et al., 2023). Our results are consistent with previous findings that mortality following viral inoculation primarily occurs before or during pupation at high doses (Fig. 2b) (Mutuel et al., 2010); however, JcDV infection did not appear to persist into the adult stage in *S. frugiperda*, whereas it was detected in 55 % of *E. phaeton* butterflies (Fig. 3a).

Viral quantities excreted in *E. phaeton* frass closely mirrored the doses that were ingested by their hosts, particularly on the first day following inoculation (Fig. 4a). This result is consistent with the research of Mutuel et al. (2010), which demonstrated that a low proportion of JcDV (0.1 % of viral particles) crosses the midgut epithelium to establish in host tissues. In addition, postmortem viral loads of insects were positively associated with the amount of JcDV that was excreted in the frass at low to medium viral doses (Fig. 4b), suggesting that viral content in frass may be a reliable indicator of the severity of infection that is established in the insect host. At the highest dose, however, herbivores experienced uniformly high postmortem burdens that were not correlated with frass content, which may be the product of the relatively low proportion of particles required to establish infection at this high viral concentration. Altogether, characterizing the dynamics of viral excretion in herbivore frass represents an important component of understanding horizontal transmission via the fecal-to-oral route in these systems.

5. Conclusions

These results provide insight into the relative roles of host plant use and pathogen dose in mediating the outcomes of infection in herbivorous insects. Together, the patterns documented in *E. phaeton* and *A. jatrophae* provide an interesting contrast, illustrating that host plant identity may exert a stronger influence on resistance to infection in certain scenarios, relative to others. Use of the exotic host plant, *Plantago,* did not impact JcDV yield or mortality in *E. phaeton,* though the potential for sublethal effects in infected adults and/or vertical transmission to offspring may differ between the two host plants. In contrast, host plant use had a substantial effect on viral burdens in *A. jatrophae,* with consumption of *Plantago* appearing to enhance resistance to infection. One notable difference between the two experiments relates to the specific host plants compared: in *E. phaeton*, which specializes on host plants containing iridoid glycosides, both the native and exotic host plant contained IGs and were therefore relatively similar in their secondary chemistry. In contrast, *A. jatrophae* is known to consume host plants with varying secondary chemistry, and the native and exotic host plants used for this herbivore differed in the presence of IGs (the native *Bacopa* lacks these compounds, while the exotic *Plantago* contains them). Given that the effect of host plant use was stronger in *A. jatrophae* than *E. phaeton*, these results suggest that dietary effects on infection severity may be greatest in magnitude when herbivores consume chemically dissimilar host plants that produce distinct chemical environments within the gut and/or hemocoel (Gallon et al., 2024).

These experiments contribute examples from a relatively understudied group of pathogens, the densoviruses (François et al., 2016), to a rich literature demonstrating that host plant use can, but does not always, influence interactions between herbivores and their natural enemies (Cory and Hoover, 2006; Kaplan et al., 2016; Ode, 2006). These experiments, conducted within controlled settings at field-relevant doses, provide critical context for field-based studies documenting patterns of infection across wild populations (MacDonald et al., 2023). In addition, characterizing the role of host plants in mediating vulnerability to infection provides insight in the tritrophic costs and benefits of utilizing different plant species, with implications for understanding the influence of pathogens on herbivore host range.

CRediT authorship contribution statement

Nadya D. Muchoney: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amy M. Watanabe:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Mike B. Teglas:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Angela M. Smilanich:** Writing – review & editing, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2024.108176.

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