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RESEARCH

Viral Infection Induces Changes to the Metabolome, Immune Response and Development of a Generalist Insect Herbivore

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Abstract

Host plant consumption and pathogen infection commonly influence insect traits related to development and immunity, which are ultimately reflected in the behavior and physiology of the insect. Herein, we explored changes in the metabolome of a generalist insect herbivore, *Vanessa cardui* (Lepidoptera: Nymphalidae), in response to both dietary variation and pathogen infection in order to gain insight into tritrophic interactions for insect metabolism and immunity. Caterpillars were reared on two different host plants, *Plantago lanceolata* (Plantaginaceae) and *Taraxacum officinale* (Asteraceae) and subjected to a viral infection by Junonia coenia densovirus (JcDV), along with assays to determine the insect immune response and development. Richness and diversity of plant and caterpillar metabolites were evaluated using a liquid chromatography-mass spectrometry approach and showed that viral infection induced changes to the chemical content of *V. cardui* hemolymph and frass dependent upon host plant consumption. Overall, the immune response as measured by phenoloxidase (PO) enzymatic activity was higher in individuals feeding on *P. lanceolata* compared with those feeding on *T. officinale*. Additionally, infection with JcDV caused suppression of PO activity, which was not host plant dependent. We conclude that viral infection combined with host plant consumption creates a unique chemical environment, particularly within the insect hemolymph. Whether and how these metabolites contribute to defense against viral infection is an open question in chemical ecology.

Keywords *Vanessa cardui* · *Plantago lanceolata* · *Taraxacum officinale* · Junonia coenia densovirus · Chemical diversity · LC-MS

Introduction

Insect herbivores are exposed to a variety of chemically-mediated interactions involving host plants and natural enemies, including predators, parasitoids and pathogens (Mooney et al. 2012; Dyer et al. 2018; Slinn et al. 2018). Research on these interactions has revealed that plant chemistry influences insect immunity, which consequently mediates how insects interact with and respond to their natural enemies (Ode 2006; Kaplan et al. 2016; Smilanich and Muchoney 2022; Ghosh et al. 2023). Whereas effects of parasitoids on insect development and immune responses have been increasingly investigated in a tritrophic context, relatively few studies have focused on how host plant chemistry influences insect physiology, behavior and immunity upon a viral infection (Cory and Hoover 2006; Ode 2019). Even less is known about metabolic and chemical changes in insect tissues due to pathogen infection and host plant consumption (Jousse et al. 2020).

Most of the research exploring the effects of viral infection on plant-insect interactions is focused primarily on changes in plant phenotypes and in phytohormone dynamics (i.e., jasmonate, salicylic acid and ethylene pathways) induced by phytopathogenic viruses (i.e., plant-infecting viruses) (Mauck et al. 2015; Peñaflor et al. 2016; Pan et al. 2021; Zhang et al. 2022). Nevertheless, a handful of studies have investigated the metabolic changes in insect hemolymph after entomopathogen infection (i.e., insect-infecting viruses and fungi) and demonstrated differential regulation of carbohydrate, amino acid and protein metabolism in infected individuals (Wang et al. 2019a, b; et al. 2019;

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Tian et al. 2023). However, little attention has been given to investigating changes in the secondary metabolite profile in insect tissue and hemolymph following viral infection and the subsequent consequences for development and immunity.

Diversity indices are commonly used to describe species richness and abundance of natural systems and to compare different taxa, niches, and trophic levels (Whittaker 1972). The ecological literature encompasses several metrics for measuring diversity, which include basic indices such as species richness and compound indices that combine measures of richness and abundance (e.g., Shannon's diversity and Simpson's diversity) (Putman and Wratten 1984; Morris et al. 2014). Traditionally, diversity measures have focused on species diversity (i.e., the variety and abundance of species in a given environment), yet novel forms of diversity such as chemical and genetic diversities have been proposed as informative and complementary measures (Richards et al. 2015; Marion et al. 2015; Wetzel and Whitehead 2020). In this context, chemical diversity has been used to investigate synergistic effects of plant metabolites on insects, as well as the interface between insect immunity, plant chemistry and parasitoids (Richards et al. 2015, 2016; Dyer et al. 2018; Slinn et al. 2018; Glassmire et al. 2020; Defossez et al. 2021).

Chemical diversity describes the richness and abundance of compounds in an organism (composition diversity) and may also describe the chemical complexity of an organism (structural complexity), which is measured as the number of chromatographic signals or spectroscopic peaks and their relative abundances (Philbin et al. 2021). Compositional diversity is obtained from complex extracts using separation methods such as liquid chromatography (LC) or gas chromatography (GC), which are usually coupled to mass spectroscopy (MS), while structural complexity is obtained from crude extracts or isolated compounds using proton nuclear magnetic resonance (^{1}H NMR) (Philbin et al. 2021). Herein, we employed a LC-MS approach to investigate the chemical diversity (i.e., composition diversity) of *Vanessa cardui* caterpillars subjected to a viral infection and reared on two host plants that vary in secondary metabolite content, *Plantago lanceolata* L. (Plantaginaceae) and *Taraxacum officinale* L. (Asteraceae).

In insect herbivores, immune responses are commonly activated against parasitoids and pathogen invaders (Beckage 2008; Rolff and Reynolds 2009), yet other types of defenses (e.g., sequestered secondary metabolites) may contribute to protecting insects against natural enemies (Ode et al. 2004; Muchoney et al. 2022). The immune response in insects has been increasingly measured via phenoloxidase (PO) activity, since this enzyme is involved in the activation of melanin production and therefore in the defense of insects against pathogens, including viruses (González-Santoyo and Córdoba-Aguilar 2012; Resnik and Smilanich 2020; Li et al. 2021). Nevertheless, hemocyte counts and abiotic implants have been used as complementary techniques for evaluating immune status in insects (Lavine and Strand 2002; Smilanich et al. 2009, 2018; Muchoney et al. 2022).

Vanessa cardui (Linnaeus, 1758), commonly known as the painted lady, belongs to the Nymphalidae family and is a generalist insect herbivore that feeds on numerous plant species from diverse families, but mostly on Asteraceae, Fabaceae and Malvaceae (Williams 1970; Poston et al. 1977; O'Neill et al. 2008; Stefanescu et al. 2012). Prior work with *V. cardui* showed that they are capable of sequestering low amounts of iridoid glycosides that occur in *P. lanceolata* (Lampert et al. 2014). Recent investigation by Gallon and Smilanich (2023) found that metabolites occurring in *P. lanceolata* (i.e., iridoid glycosides, phenylethanoids and flavonoids) and *T. officinale* (i.e., phenylpropanoids, flavonoids and sesquiterpene lactones – germacranolide-, eudesmanolide- and guaianolide- types) were not detrimental to *V. cardui* development. However, the immune response, measured as PO enzymatic activity, of larvae reared on *T. officinale* was lower compared with larvae reared on *P. lanceolata* (Gallon and Smilanich 2023). In addition, Resnik and Smilanich (2020) found that *V. cardui* larvae infected with an entomopathogenic virus had lower PO activity compared to uninfected larvae, and that the effect size was greatest when reared on *P. lanceolata* compared to another host plant (*Lupinus albifrons*, Fabaceae), suggesting host plant dependent effects of viral infection on immunity.

The entomopathogenic virus Junonia coenia densovirus (JcDV) (*Parvoviridae: Densovirinae*: *Protoambidensovirus lepidopteran1*) is a nonenveloped DNA virus that belongs to the *Parvoviridae* family (Bruemmer et al. 2005). This virus was first isolated from the common buckeye butterfly (*Junonia coenia* Hübner, Nymphalidae), but it is not exclusive to this host species and studies have demonstrated that JcDV is able to infect various lepidopteran species across several families, including Noctuidae, Erebidae and Lycaenidae (Rivers and Longworth 1972; McKeegan et al. 2024; Mutuel et al. 2010; Yoon 2021; Muchoney et al. 2022). JcDV infection impairs oxygen delivery to insect tissues and leads to caterpillar death through anoxia and diverse physiological disruptions, including molting arrest and oxidative stress (Mutuel et al. 2010). Notably, consumption of host plants differing in secondary metabolites has been shown to strongly influence insect survival and viral burdens following experimental infection with JcDV in multiple nymphalid species (Smilanich et al. 2018; Muchoney et al. 2022, 2023), including the dietary generalist *V. cardui* (Resnik and Smilanich 2020).

In this study, we explored whether a viral infection by JcDV influenced the metabolome, immunity, and development of *V. cardui* caterpillars reared on host plants that vary in secondary metabolite content, *P. lanceolata* and *T. officinale*. Using a LC-MS-based approach, we investigated the

chemical diversity of the consumed host plant leaves, as well as the chemical diversity of uninfected and JcDV-infected caterpillars and their corresponding frass and hemolymph, which were correlated to insect immune response and development. Since viral infections have been associated with differential expression of primary metabolites (e.g., carbohydrates, amino acids and lipids) in insect tissues (Wang et al. 2019a, b; et al. 2019; Tian et al. 2023), we predicted JcDV infection to cause changes in the secondary metabolite content of *V. cardui* tissues. More specifically, we predicted *V. cardui* caterpillars experiencing viral infection to present higher richness and abundance of metabolites, exhibiting greater chemical diversity indices. In addition, based upon prior data (Resnik and Smilanich 2020), we predicted JcDV infection to suppress the *V. cardui* immune response.

Materials and Methods

Experiment Overview

To evaluate plant-insect-pathogen interactions, *V. cardui* caterpillars were reared on either *P. lanceolata* or *T. officinale* leaves and orally inoculated with JcDV. A metabolomic approach based on LC-MS was used to investigate the metabolome and chemical diversity of host plant leaves and caterpillar samples (caterpillar tissue, frass and hemolymph). Additionally, an immune assay for PO enzymatic activity was employed to measure the immunocompetence of uninfected and JcDV-infected caterpillars. Finally, caterpillar performance was evaluated by means of developmental rate and survivorship.

Insect and Host Plants

Vanessa cardui eggs were purchased from Carolina Biological (Carolina Biological Supply Company, Burlington, NC, USA). *Plantago lanceolata* and *T. officinale* were grown from seeds to adult plants (approximately three months) in a climatic chamber under controlled conditions (16 h photoperiod with day temperature of 25 °C and night temperature of 22 °C) using plastic nursery pots filled with soil (Miracle-Gro potting mix, The Scotts Company, LLC). *Vanessa cardui* eggs were placed with either *P. lanceolata* or *T. officinale* leaves and reared in a climatic chamber (16 h photoperiod with day temperature of 25 °C and night temperature of 20 °C) throughout the duration of development. Upon entering the 3rd instar, caterpillars were separated to be individually reared in 16 oz plastic cups and fed *ad libitum* on the corresponding host plant leaves ($n = 20$ for metabolomic analyses and $n = 50$ for developmental and immune assays, for each host plant). To keep leaves fresh, host plant leaves with petioles were placed in hydrophilic sponges soaked in water. At the beginning of the 4th instar, half of the caterpillars ($n = 10$ for metabolomic analyses and $n = 25$ for developmental and immune assays, for each host plant) were randomly assigned to be inoculated with JcDV and half ($n = 10$ for metabolomic analyses and $n = 25$ for developmental and immune assays, for each host plant) were kept in a virus-free environment.

Virus Assay

A dose of 1×10^6 viral equivalent genomes (v.e.g.) of JcDV suspended in 1 µL of phosphate-buffered saline (PBS) (VWR Life Science, VWR International, Radnor, PA, USA) was applied to an 8 mm leaf disk of each corresponding host plant. This dose is similar to average viral loads found in wild populations of the Baltimore checkerspot butterfly (*Euphydryas phaeton*, Nymphalidae) (N.D. Muchoney, unpublished data) and corresponds to an LD_{60} for *V. cardui* based upon prior data. Before inoculations, food (i.e., host plant leaves) was removed and caterpillars were starved for 24 h to ensure consumption of the inoculated leaf disk. One leaf disk was added to each plastic cup and caterpillars were allowed 24 h to consume the leaf disk, before returning to their assigned host plant. For the uninfected treatment (i.e., control group), caterpillars were provided with an 8 mm leaf disk of their corresponding host plant with 1 µL of deionized water added to the surface and the same procedure used for JcDV-infected caterpillars was employed.

Metabolomic Analyses

A total of 80 samples, comprising host plant leaves and different types of *V. cardui* samples (caterpillar tissue, frass and hemolymph), were collected across insect development. Leaves of *P. lanceolata* and *T. officinale*, growing in the climatic chambers, were sampled during insect development assays at different days ($n = 5$ for each host plant). Caterpillar tissue (whole body, containing hemolymph) and frass were sampled from a subset of 20 caterpillars reared on each corresponding host plant under the same conditions used for developmental and immune assays. Before JcDV infection, we sampled 4th instar caterpillars reared on *P. lanceolata* and *T. officinale* ($n = 5$ for each treatment). Additionally, we sampled 5th instar caterpillars (uninfected and JcDV-infected) reared on each host plant ($n = 5$ for each treatment) and the total frass produced during the 5th instar by a caterpillar (uninfected and JcDV-infected) reared on each corresponding host plant ($n = 5$ for each treatment). Caterpillar hemolymph ($n = 5$ for each treatment) was collected from the subset of 50 caterpillars used for developmental and immune assays (see below for hemolymph extraction procedure). For sampling, caterpillars were starved for 24 h to clear the gut of host plant material prior to chemical analysis, and then freeze-killed. All other samples were immediately frozen

and kept at -10°C. Host plants and insect samples (except hemolymph samples) were freeze-dried for 48 h (FreeZone 2.5 L − 84 C Benchtop, Lanconco Corporation, Kansas City, MO, USA) and pulverized. Extracts for the LC-MS analyses were prepared by adding 1 mL of a solution of MeOH:H_2O (7:3, v:v) with hydrocortisone (10 µg.mL^{-1}) to 10 mg of each plant or insect sample. After vortex agitation (30 s, K-550-G, Scientific Industries, Bohemia, NY, USA), extractions were performed in an ultrasonic bath (10 min, 75T Aquasonic, VWR Scientific Products, Radnor, PA, USA). Extracts were centrifuged (21,130 g, 5 min, Centrifuge 5425, Eppendorf™, Fisher Scientific, Hampton, NH, USA) and the supernatant was filtered through a 0.22 µm PTFE syringe filter into glass vials. For preparing the extracts of the hemolymph samples, we used a proportion of hemolymph:extraction solution of 1:9 (v:v) and the same protocol described above. A quality control (QC) sample was prepared by combining aliquots (20 µL) of each extract.

LC-MS analyses were performed in a high-performance liquid chromatography (HPLC) system coupled to a time-of-fly (TOF) mass spectrometer (Agilent 6230, Agilent Technologies, Santa Clara, CA, USA), using a C18 column (5 µm, XB-C18 Kinetex, 100Å, 150 × 3 mm, Phenomenex, Torrance, CA, USA). The mobile phase was composed of water and acetonitrile (CH_3CN), both with 0.1% formic acid at a flow rate of 0.75 mL.min $^{-1}$, and the following gradient was employed: 0–2 min, 5% CH_3CN; 2–30 min, 5–100% CH_3CN; 30–35 min, 100% CH_3CN; 35–38 min, 100–5% CH_3CN and 38–40 min, 5% CH_3CN. For each sample, an injection volume of 20 µL was used and samples were kept at 4 °C. The column oven was set at 45 °C. Chromatograms were acquired in the negative ionization mode, and the following parameters were employed for the mass spectrometer: dry temperature, 325 °C; dry gas flow, 10 L.min^{-1}; nebulizer pressure, 20 psig; capillary voltage, 3200 V; fragmentor, 100 V; skimmer, 65 V; Oct 1RF Vpp, 750 V; acquisition range, *m/z* 100 to 1500; 2 spectra/sec; 500 ms/spectrum. Blank (i.e., extraction solution) and QC samples were injected five times throughout the LC-MS analyses and used to certify the reliability and accuracy of the data acquisition and processing.

Raw LC-MS data were converted to .mzXML format using the software ProteoWizard-MSconvert (version 3 for Windows, Proteowizard Software Foundation, Palo Alto, CA, USA) and processed with the software MzMine (version 2.53 for Windows, BMC Bioinformatics, United Kingdom). The following parameters were used for data processing: raw data methods - feature detection, mass detection, filter - retention time (0.04–30.00 min), mass detector – centroid (noise level, 1.0×10^4); ADAP chromatogram builder, filter – retention time (0.04–30.00 min), min group size in # of scans, 5; group intensity threshold, 1.0×10^4; min highest intensity, 3.0×10^4; *m/z* tolerance, 0.02 *m/z*; feature list methods – feature detection, chromatogram deconvolution, algorithm – wavelets (ADAP) (S/N threshold, 10; S/N estimator, intensity window SN; min feature height, 1.0×10^4; coefficient/area threshold, 100; peak duration range, 0.02–2.0; RT wavelet range, 0.01–0.20), *m/z* center calculation – median; isotopes – isotopic peak grouper (*m/z* tolerance, 0.02 *m/z*; retention time tolerance, 0.2 min (absolute); maximum charge, 2; representative isotope, most intense) and alignment – join aligner (*m/z* tolerance, 0.05 *m/z*; weight for *m/z*, 50; retention time tolerance, 0.2 min (absolute) and weight for retention time, 50). After processing, data were exported as a .csv spreadsheet, which was further used in multivariate statistical analysis and for calculating phytochemical diversity indices.

Principal Component Analysis (PCA) was performed in the software SIMCA (version 13.0.3.0 for Windows, Umetrics, Umeå, Sweden) using the .cvs spreadsheet data, which were log-transformed. To compare the chemical profiles of uninfected and JcDV-infected *V. cardui* individuals reared on *P. lanceolata* or *T. officinale* leaves, we performed PCA using insect samples collected across the *V. cardui* life cycle (caterpillar tissue, frass and hemolymph samples of uninfected and JcDV-infected individuals reared on each host plant) and host plant samples (*P. lanceolata* and *T. officinale* leaves). Thereafter, we performed individual PCA for each set of samples (host plant, caterpillar tissue, frass or hemolymph) to verify the effects of JcDV infection and host plant consumption on the chemical profile of *V. cardui* caterpillar tissue, frass and hemolymph, and also to investigate the varying chemical profiles between the host plants.

Chemical Diversity

We measured phytochemical diversity as richness (i.e., number of metabolites) and Simpson's index [1 - D; where $D = \Sigma(n/N)^2$, *n* is the peak area at a specific retention time and *N* is the total usable peak area] using the software Past (version 4.03 for Windows, Oyvind Hammer, Oslo, Norway). We used the .csv spreadsheet obtained after data processing (see above) with no data transformation (i.e., relative peak abundances were estimated as peak areas). To correct for metabolites being represented multiple times (as fragments, adducts and dimers, for example) and reduce data redundancy, we removed from the spreadsheet redundant MS features exhibiting equivalent retention times for each set of samples (host plant, caterpillar tissue, frass and hemolymph) considering a retention time threshold of 0.1 min. Therefore, we kept only a single MS feature for each potential metabolite (i.e., the most intense peak area corresponding to a certain retention time). For each set of samples, we averaged richness and Simpson's index according to the treatment (*P. lanceolata* or *T. officinale* consumption, uninfected or JcDV-infected). Statistical significances between

treatments were evaluated in the software GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, CA, USA) using *t* tests for host plant and 4th instar caterpillar tissue samples, and two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test for 5th instar caterpillar tissue, frass and hemolymph samples. To minimize the potential of a type II error, an alpha level of 0.1 was considered for statistical significance.

Hemolymph Extraction and Immune Assay

Hemolymph was extracted by piercing the cuticle of the 5th abdominal segment with a sterilized insect pinning needle. For JcDV-infected individuals, hemolymph was extracted from caterpillars five days post-infection (dpi) ($n = 20$ for *P. lanceolata* and $n = 8$ for *T. officinale*). For uninfected individuals, hemolymph was extracted five days after the beginning of the 4th instar ($n = 20$ for *P. lanceolata* and $n = 14$ for *T. officinale*). A total of 10 µL of hemolymph was collected from each individual, comprising 5 µL for metabolomics analyses which was collected in tubes and immediately frozen, and 5 µL for immune assay which was added to 250 µL of ice-cold PBS solution and vortexed.

PO activity was measured to evaluate the immune response of uninfected and JcDV-infected caterpillars reared on each host plant. For the immune assay, once all hemolymph-bound PBS samples were prepared, 100 µL of each sample was loaded in a 96-well microplate and 200 µL of 4 mM dopamine solution was added as the substrate (Alfa Aesar, Thermo Fisher Scientific, Haverhill, MA, USA). Using an iMark Microplate Absorbance Reader (Bio-Rad), a spectrophotometric assay was immediately performed to determine the PO enzymatic activity by measuring the absorbance at 490 nm every 30 s for 45 min. PO activity was measured as the slope over the time period during which the reaction was linear (from 5 to 30 min). Negative measurements of PO activity were removed from analyses (*P. lanceolata*, $n = 5$ for JcDV-infected caterpillars and $n = 6$ for uninfected caterpillars; *T. officinale*, $n = 2$ for JcDV-infected caterpillars and $n = 8$ for uninfected caterpillars). Statistical significance was evaluated in the software GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, CA, USA) using two-way ANOVA with Tukey's multiple comparisons test.

Survivorship

Larval and pupal survival were measured as the percentage of caterpillars and pupae that thoroughly completed each corresponding developmental stage. Survival to adulthood was measured as the percentage of adults that survived the larval and pupal stages and eclosed in good health (i.e., no observable physical damage or signs of disease). Larval and pupal survival curves were created in the software GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, CA, USA) using the Kaplan-Meier method and compared using the overall Logrank test. For each host plant, we analyzed whether time to death of caterpillars and pupae was dependent upon JcDV infection. Larval survival was measured as the elapsed time from hatch to caterpillar's death or pupae formation (censored observation). Pupal survival was measured as the elapsed time from pupa formation to pupa's death or eclosion as adults (censored observation).

Developmental Rate

The development time of uninfected and JcDV-infected caterpillars reared on each host plant was measured during the 4th and 5th instars. Additionally, caterpillars and pupae were weighed at specific time points and further compared. Larval masses were recorded at the beginning of the 4th instar (corresponding to the day of inoculation with JcDV), three dpi, five dpi (corresponding to the day of hemolymph extraction), seven dpi and nine dpi. Uninfected caterpillars were weighed at time points equivalent to the time points employed for JcDV-infected caterpillars and used as control group. Pupal masses were recorded five days after pupation, for both uninfected and JcDV-infected individuals. All statistical analyses were performed in the software GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, CA, USA) using t-test for comparing development times, mixed effects model with Bonferroni's multiple comparisons test to evaluate statistical differences in mean larval masses across insect development, and two-way ANOVA with Tukey's multiple comparison test for verifying the effects of JcDV infection and host plant consumption on pupal masses.

Results

Metabolomic Analyses

The score plot for the PCA that included both host plant- and insect-derived samples showed a cluster tendency according to the sample type (host plant, caterpillar tissue, frass, or hemolymph samples) (Fig. 1). Interestingly, host plant and frass samples were clustered close to each other, suggesting that these samples presented some metabolites in common. Conversely, caterpillar tissue and hemolymph samples were clustered apart from each other, and from host plant and frass samples, which indicated chemical differences among the sample types.

Further PCA analyses focused on the effects of JcDV infection and host plant identity within each individual set of samples (host plant, caterpillar tissue, frass or hemolymph samples) (Fig. 2).

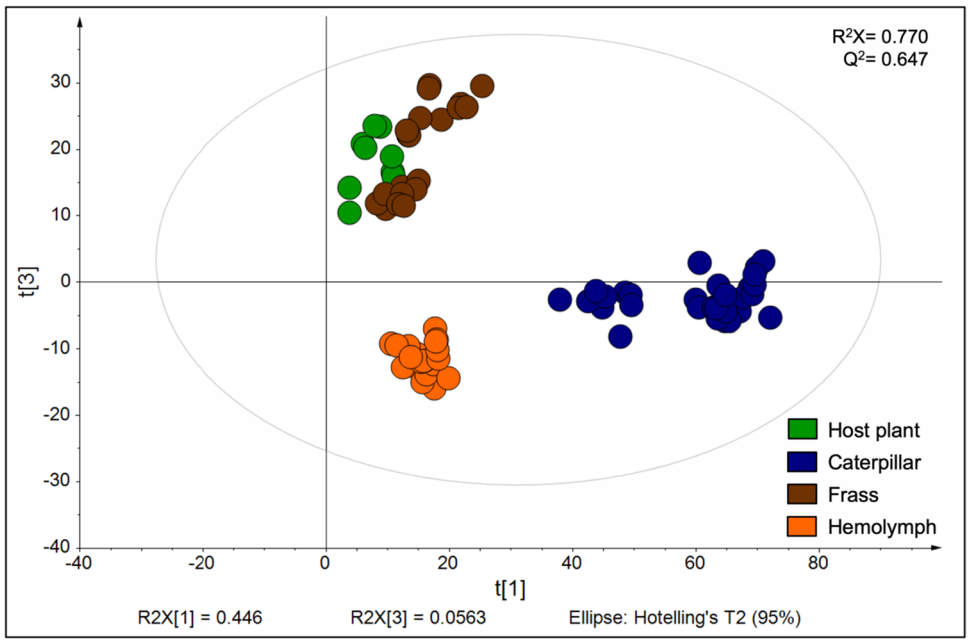

Fig. 1 PCA score plot of LC-MS data in the negative ionization mode for host plant and insect samples obtained from uninfected and JcDV-infected individuals. Green, host plants (*P. lanceolata* and *T. officinale* leaves); blue, caterpillars (uninfected 4th instar caterpillars, uninfected 5th instar caterpillars and JcDV-infected 5th instar caterpillars reared on *P. lanceolat*a or *T. officinale*); brown, frass (from uninfected and JcDV-infected caterpillars reared on *P. lanceolata* or *T. officinale*); orange, hemolymph (from uninfected and JcDV-infected individuals reared on *P. lanceolata* or *T. officinale*). R^2X, goodness of fit; Q^2, goodness of prediction

The PCA score plot for host plant samples confirmed the expected differences in terms of secondary metabolites between *P. lanceolata* and *T. officinale* leaves (PC = 2; $R^2X = 0.677$, $Q^2 = 0.490$, Fig. 2A), which in turn validated the assumption that caterpillars reared on each host plant are in contact with distinctive chemical environments and ingesting different secondary metabolites.

For caterpillar tissue samples, the PCA score plot of the second versus the fourth PC showed a separation between 4th instar caterpillars (upper left and lower left quadrants) and 5th instar caterpillars (upper right and lower right quadrants) (PC = 5, $R^2X = 0.846$, $Q^2 = 0.751$, Fig. 2B). A cluster tendency according to host plant consumption was observed for 4th instar caterpillars, in which caterpillars reared on *P. lanceolata* were clustered in the upper left quadrant and most of the caterpillars reared on *T. officinale* were clustered in the lower left quadrant. However, 5th instar caterpillars exhibited no clustering tendency regardless of host plant consumption or JcDV infection.

The PCA score plot for frass samples showed a cluster tendency based on host plant consumption (PC = 4, $R^2X = 0.686$, $Q^2 = 0.488$, Fig. 2C). In the PCA score plot of the first versus the third PC, frass of caterpillars reared on *P. lanceolata* was clustered in the lower quadrants, while frass of caterpillars reared on *T. officinale* was clustered in the upper quadrants. For frass of caterpillars reared on *P. lanceolata*, we observed a cluster tendency according to JcDV infection, however no clear cluster tendency was found for frass of uninfected and JcDV-infected caterpillars reared on *T. officinale*.

Hemolymph samples exhibited a clustering tendency based on host plant consumption and on JcDV infection (PC = 4; $R^2X = 0.803$, $Q^2 = 0.655$, Fig. 2D). In the PCA score plot of the second versus the third principal component, hemolymph samples of caterpillars reared on *P. lanceolata* were clustered in the left quadrants. More specifically, samples from uninfected caterpillars reared on *P. lanceolata* were clustered in the upper left quadrant, while most of the hemolymph samples from JcDV-infected caterpillars reared on *P. lanceolata* were clustered on the lower left quadrant. The same pattern was observed for hemolymph samples of caterpillars reared on *T. officinale*: all hemolymph samples of caterpillars reared on *T. officinale* were clustered in the right quadrants, in which hemolymph samples from uninfected caterpillars were clustered in the upper right quadrant and most of the hemolymph samples of JcDV-infected individuals were clustered in the lower right quadrant.

Chemical Diversity

Subsequently, diversity indices were measured to evaluate metabolite richness and evenness across host plant and insect samples. No significant differences were found in chemical diversity between host plants ($t = 0.1917$; df = 8; $P = 0.8528$ for richness, and $t = 0.2138$; df = 8; $P = 0.8360$ for Simpson's index) (i.e., richness and Simpson's index for *P. lanceolata* and *T. officinale* leaves were not significantly different) (Figs. 3A and 4A).

Significant differences in richness of metabolites were found between 4th instar caterpillars (before JcDV infection) reared on each host plant ($t = 2.1580$; df = 8; $P = 0.0630$), where caterpillars reared on *T. officinale* had more detected metabolites (mean = 136.20 metabolites, SD = 6.91, $n = 5$) than caterpillars reared on *P. lanceolata* (mean = 128.60

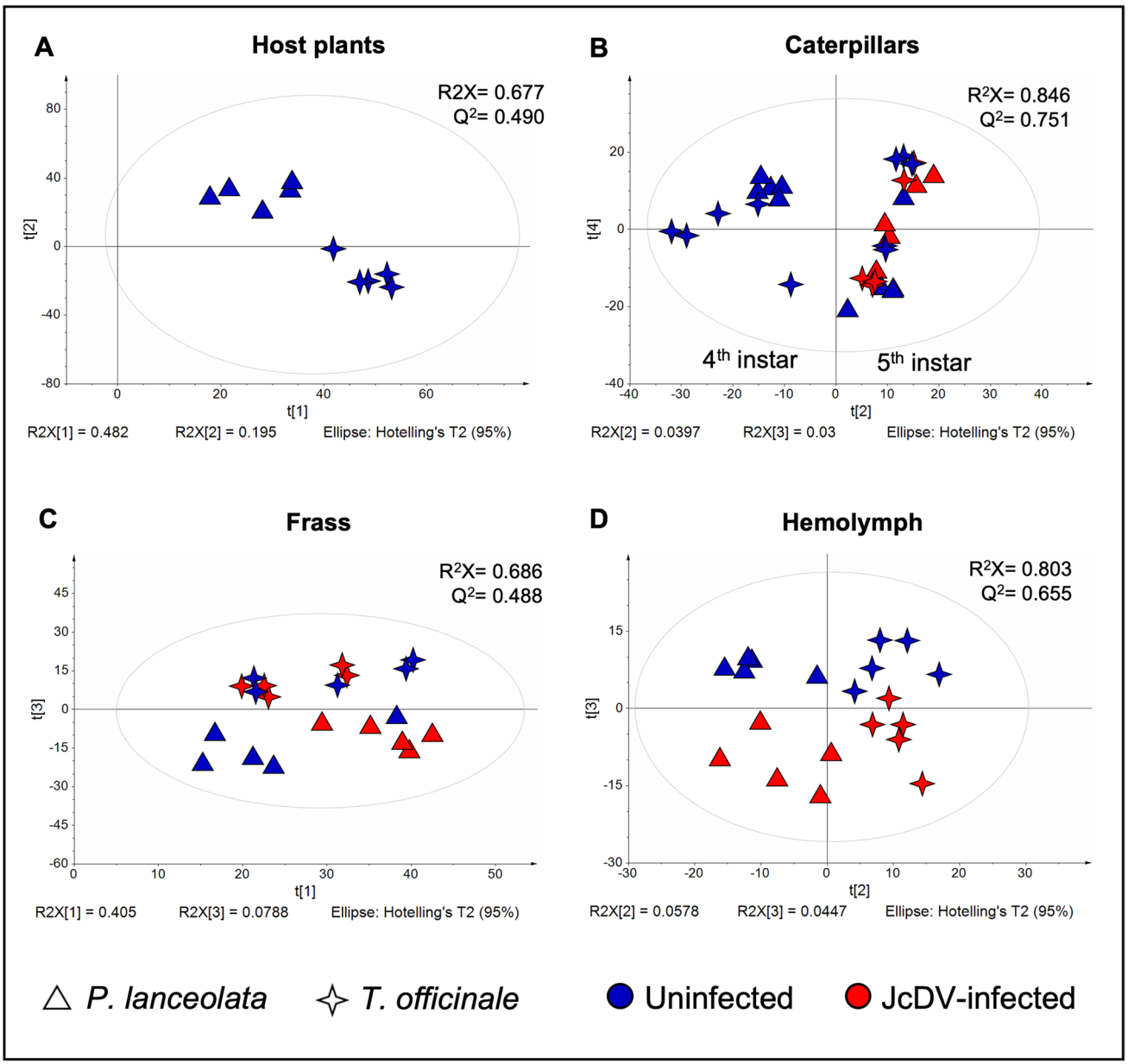

Fig. 2 PCA score plot of LC-MS data in the negative ionization mode for each type of samples, including host plant (**A**), caterpillar tissue (**B**), frass (**C**) and hemolymph (**D**) samples. Samples of *P. lanceolata* leaves are represented as blue triangles; samples of *T. officinale* leaves are represented as blue 4-point stars; samples obtained from caterpillars reared on *P. lanceolata* are represented as triangles; samples obtained from caterpillars reared on *T. officinale* are represented as 4-point stars; uninfected samples are colored in blue; JcDV-infected samples are colored in red. R^2X, goodness of fit; Q^2, goodness of prediction

metabolites, SD = 3.78, $n = 5$) (Fig. 3B). Additionally, richness in 4th instar caterpillars was different from richness in 5th instar caterpillars for both uninfected and JcDV-infected individuals reared on either host plant, with fewer metabolites detected in 5th instar caterpillars. However, for 5th instar caterpillars, no statistical differences in richness of metabolites were found between uninfected and JcDV-infected caterpillars reared on either *P. lanceolata* (mean = 107.00 metabolites; SD = 23.82; $n = 5$ and mean = 87.80 metabolites; SD = 24.39; $n = 5$ respectively) or *T. officinale* (mean = 84.20 metabolites; SD = 27.93; $n = 5$ and mean = 79.40 metabolites; SD = 22.88; $n = 5$ respectively). Therefore, richness of metabolites in 5th instar caterpillars was not influenced by host plant consumption ($F_{(1,16)} = 0.9510$; $P = 0.3440$) or JcDV infection ($F_{(1,16)} = 2.7570$; $P = 0.1163$).

For Simpson's diversity, 4th instar caterpillars reared on *P. lanceolata* (mean = 0.976, SD = 0.002, $n = 5$) were similar to 4th instar caterpillars reared on *T. officinale*

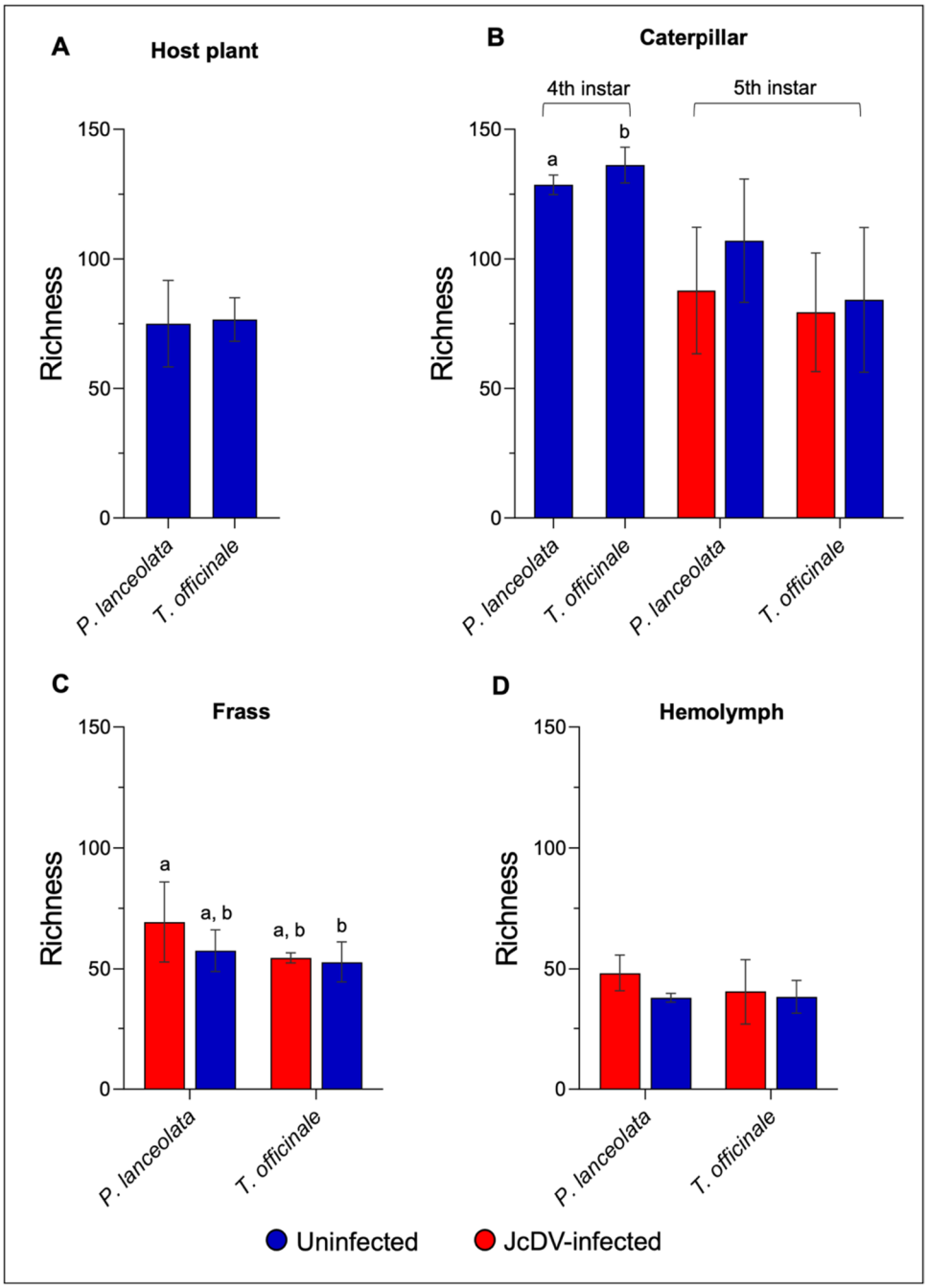

Fig. 3 Chemical diversity measured as richness in host plant leaves (**A**) and uninfected and JcDV-infected *Vanessa cardui* caterpillar tissue (**B**), frass (**C**) and hemolymph (**D**) samples. Uninfected samples are colored in blue; JcDV-infected samples are colored in red. Different letters represent statistical differences between samples ($P < 0.1$; *t* test for host plant samples and 4th instar caterpillar samples; two-way ANOVA with Tukey's multiple comparisons test for 5th instar caterpillar, frass and hemolymph samples)

(mean = 0.968, SD = 0.011, $n = 5$) (i.e., no significant differences in Simpson's index were found between 4th instar caterpillars) ($t = 1.4190$; df = 8; $P = 0.1938$) (Fig. 4B). Caterpillars reared on *P. lanceolata* showed significant differences in the Simpson's index between 4th instar caterpillars and 5th instar caterpillars, however caterpillars reared on *T. officinale* exhibited no significant differences in the Simpson's index between 4th and 5th instar caterpillars. Regarding 5th instar caterpillars, host plant consumption had no significant effect on metabolite diversity ($F_{(1,16)} = 0.0051$; $P = 0.9437$), however JcDV had a significant effect on metabolite diversity ($F_{(1,16)} = 4.2130$; $P = 0.0568$) resulting in a significant interaction between JcDV infection and host plant consumption ($F_{(1,16)} = 3.5710$; $P = 0.0771$). Therefore, the Simpson's index of uninfected and JcDV-infected caterpillars reared on *P. lanceolata* did not differ significantly (mean = 0.967; SD = 0.005; $n = 5$ and mean = 0.966; SD = 0.010; $n = 5$), yet caterpillars reared on *T. officinale* exhibited significantly different Simpson's index between uninfected and JcDV-infected caterpillars (mean = 0.972; SD = 0.005; $n = 5$ and mean = 0.960; SD = 0.007; $n = 5$).

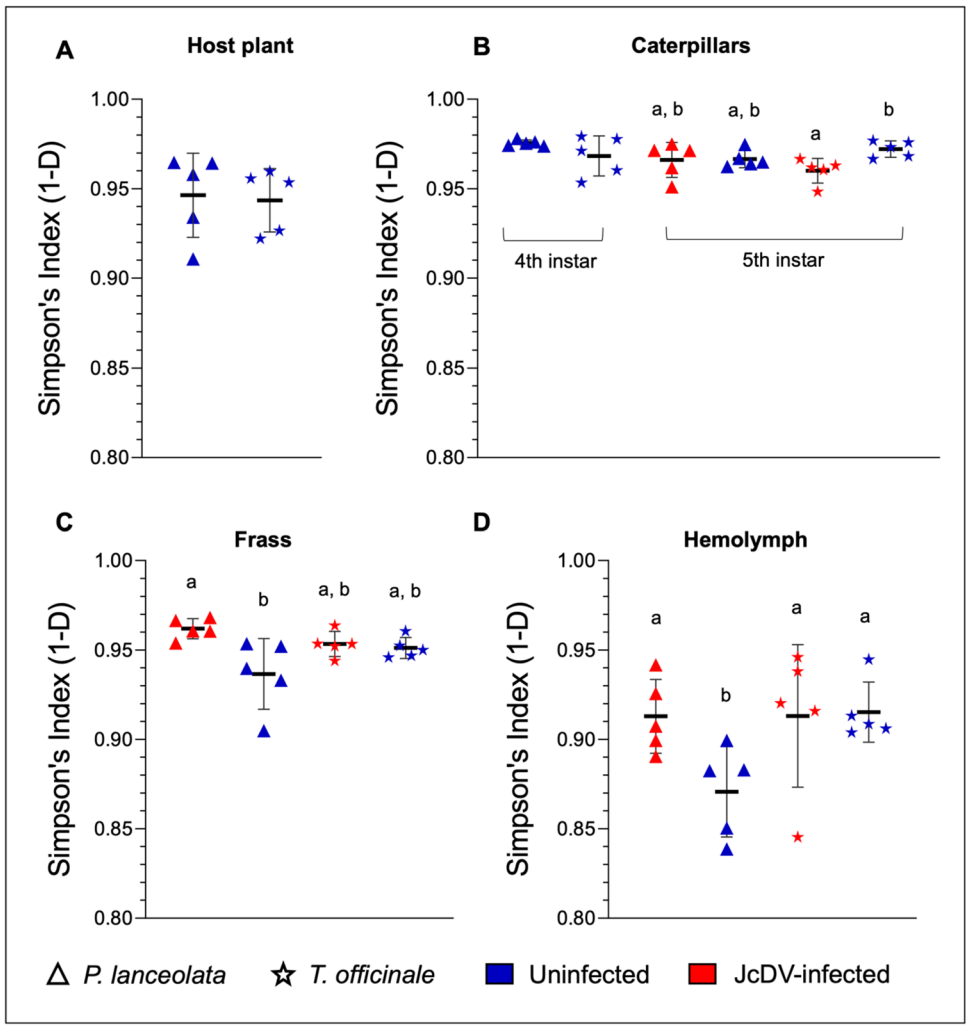

Fig. 4 Chemical diversity measured as Simpson's index in host plant leaves (**A**) and uninfected and JcDV-infected *Vanessa cardui* caterpillar tissue (**B**), frass (**C**) and hemolymph (**D**) samples. Samples of *P. lanceolata* leaves are represented as blue triangles; samples of *T. officinale* leaves are represented as blue stars; samples obtained from caterpillars reared on *P. lanceolata* are represented as triangles; samples obtained from caterpillars reared on *T. officinale* are represented as stars; uninfected samples are colored in blue; JcDV-infected samples are colored in red. Bold lines in black represent the mean Simpson's index. Different letters represent statistical differences between samples ($P < 0.1$; *t* test for host plant samples and 4th instar caterpillar samples; two-way ANOVA with Tukey's multiple comparisons test for 5th instar caterpillar, frass and hemolymph samples)

In frass samples, host plant consumption exhibited a significant effect on metabolite richness ($F_{(1,16)} = 4.5290$; $P = 0.0492$) while JcDV infection exhibited no significant effect ($F_{(1,16)} = 2.1810$; $P = 0.1592$) which resulted in a non-significant interaction between host plant consumption and JcDV infection ($F_{(1,16)} = 1.790$; $P = 0.2937$) (Fig. 3C). Therefore, frass of caterpillars reared on *P. lanceolata* exhibited no significant differences in richness of metabolites between uninfected and JcDV-infected treatments, and a similar pattern was observed for frass of uninfected and JcDV-infected caterpillars reared on *T. officinale*. However, richness was significantly different between frass of JcDV-infected caterpillars reared on *P. lanceolata* (mean = 69.40 metabolites; SD = 16.56; $n = 5$) and frass of uninfected caterpillars reared on *T. officinale* (mean = 52.80 metabolites; SD = 8.44; $n = 5$). For Simpson's index, we observed a similar pattern where metabolite diversity was dependent upon host plant consumption ($F_{(1,16)} = 7.5080$; $P = 0.0145$) yet it was not dependent upon JcDV infection ($F_{(1,16)} = 0.3521$; $P = 0.5612$) (Fig. 4C). Nevertheless, the interaction between host plant consumption and JcDV infection exhibited a significant effect on metabolite diversity in frass samples ($F_{(1,16)} = 5.2810$; $P = 0.0354$). Thus, significant differences were found between frass of uninfected and JcDV-infected caterpillars reared on *P. lanceolata* (mean = 0.937; SD = 0.020; $n = 5$ and mean = 0.962; SD = 0.006; $n = 5$ respectively), yet no significant differences were found between frass of uninfected and JcDV-infected caterpillars reared on *T. officinale* (mean = 0.951; SD = 0.006; $n = 5$ and mean = 0.953; SD = 0.007; $n = 5$).

In general, hemolymph samples exhibited the lowest richness and Simpson's diversity, which may reflect a limitation of our LC-MS method for detecting lipophilic metabolites potentially present in this type of sample. Even though JcDV infection had greater (yet non-significant) influence on metabolite richness ($F_{(1,16)} = 2.7570$; $P = 0.1163$), no statistical significance was found for host plant consumption ($F_{(1,16)} = 0.9510$; $P = 0.3340$) or the interaction between JcDV infection and host plant consumption ($F_{(1,16)} = 1.1680$; $P = 0.2959$) (Fig. 3D). For Simpson's index, we observed a

significant effect of host plant consumption ($F_{(1,16)}=3.2240$; $P=0.0915$) and JcDV infection ($F_{(1,16)}=3.0580$; $P=0.0995$), resulting in a significant effect of the interaction between host plant consumption and JcDV infection on metabolite diversity ($F_{(1,16)}=3.7370$; $P=0.0711$) (Fig. 4D). Therefore, hemolymph of uninfected and JcDV-infected caterpillars reared on *P. lanceolata* significantly differed in terms of Simpson's diversity (mean = 0.871; SD = 0.025; $n=5$ and mean = 0.913; SD = 0.021; $n=5$ respectively), yet hemolymph of uninfected and JcDV-infected caterpillars reared on *T. officinale* were not significantly different (mean = 0.915; SD = 0.017; $n=5$ and mean = 0.913; SD = 0.040; $n=5$, respectively).

Immune Assays

Host plant consumption had a significant effect on PO activity ($F_{(1,37)}=92.61$; $P<0.0001$) with individuals reared on *P. lanceolata* exhibiting higher PO activity (mean = 41.98 OD_{490}/min, SD = 4.22 and mean = 30.43 OD_{490}/min, SD = 14.28 for uninfected and JcDV-infected caterpillars, respectively) than individuals reared on *T. officinale* (mean = 6.14 OD_{490}/min, SD = 7.51 and mean = 2.63 OD_{490}/min, SD = 3.62 for uninfected and JcDV-infected caterpillars, respectively) (Fig. 5). Infection with JcDV significantly reduced PO activity, although the effect was weaker than the effect of host plant consumption ($F_{(1,37)}=5.19$; $P=0.0286$). Consequently, the interaction between PO activity, host plant consumption and viral infection was not significant ($F_{(1,37)}=1.48$; $P=0.2318$).

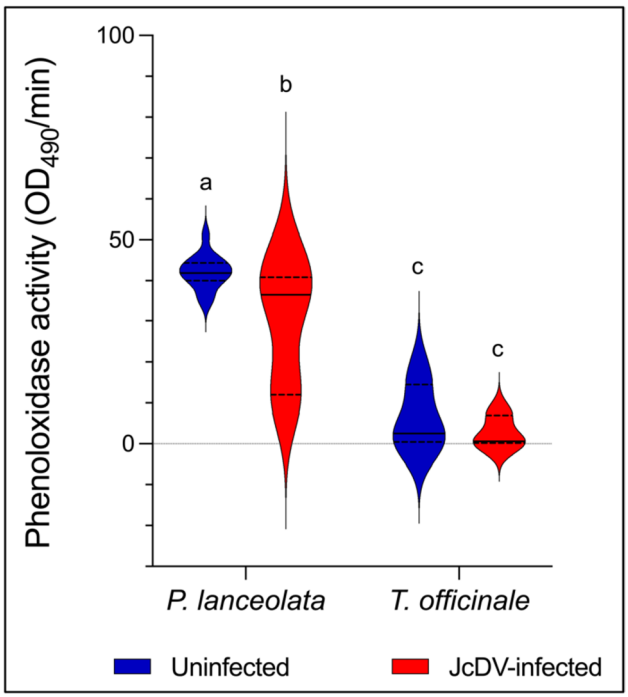

Fig. 5 Phenoloxidase activity of uninfected and JcDV-infected *Vanessa cardui* caterpillars reared on *Plantago lanceolata* or *Taraxacum officinale* leaves. Bold lines in black represent the median. Dotted lines in black represent the interquartile range. Different letters indicate significant differences ($P<0.05$, two-way ANOVA with Tukey's multiple comparisons test)

Survivorship

Comparing the survival curves of uninfected and JcDV-infected caterpillars reared on each host plant, we observed a tendency for caterpillars that did not experience JcDV infection to exhibit slightly higher probabilities of larval survival. Caterpillars reared on *P. lanceolata* leaves exhibited larval survival probability of 64% for uninfected individuals and 52% for JcDV-infected individuals. For caterpillars reared on *T. officinale*, uninfected individuals exhibited larval survival probability of 36%, while JcDV-infected individuals exhibited larval survival probability of 20%. However, no statistically significant differences were found between the larval survival curves of uninfected and JcDVinfected individuals (Logrank test – *P. lanceolata*, $P=0.2503$; *T. officinale,* $P=0.4986$) (Fig. 6).

Pupal survival curves of uninfected and JcDV-infected individuals reared on each host plant showed that uninfected individuals presented higher probabilities to survive throughout the pupal stage than JcDV-infected individuals. When caterpillars were reared on *P. lanceolata* leaves, the pupal survival was 75% for uninfected individuals, yet pupae that experienced JcDV infection were not able to complete the pupal stage and exhibited 100% mortality. Individuals reared on *T. officinale* leaves exhibited approximately 89% of pupal survival for uninfected individuals, while caterpillars that were inoculated with JcDV exhibited pupal survival probability of 20%. Additionally, survival to adulthood was greater for individuals that did not experience viral infection (48% and 32% for individuals reared on *P. lanceolata* and *T. officinale*, respectively) and lower for individuals that were inoculated with JcDV (0 and 4% for individuals reared on *P. lanceolata* and *T. officinale*, respectively). However, statistical analyses showed no significant differences between the pupal survival curves of uninfected and JcDV-infected individuals (Logrank test –*P. lanceolata*, $P=0.5949$; *T. officinale*, $P=0.1777$) (Fig. 7).

Developmental Rate

Vanessa cardui caterpillars reared on *P. lanceolata* leaves exhibited significant differences in the development time of uninfected and JcDV-infected caterpillars only for the 5th larval instar ($P=0.0001$) (Fig. 8A). Uninfected caterpillars remained approximately six days in the 5th instar

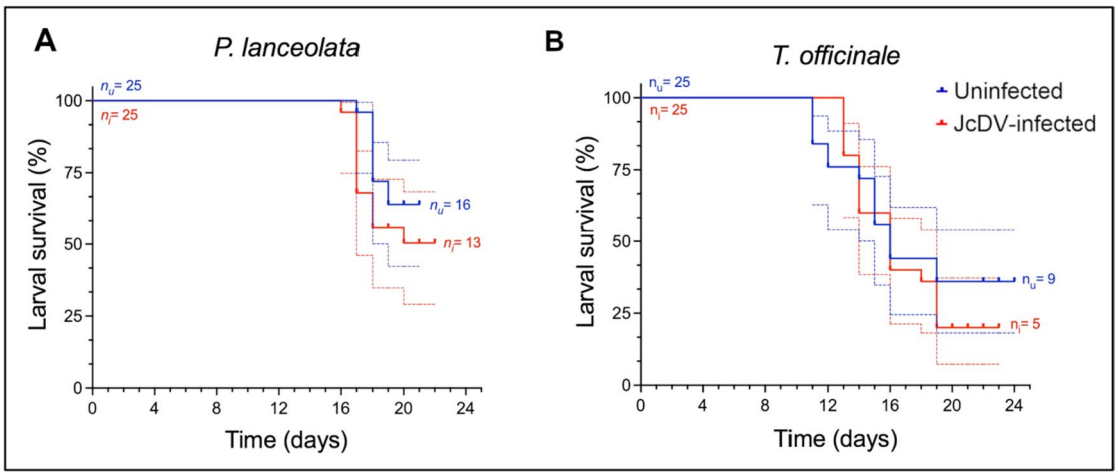

Fig. 6 Probability of larval survival of uninfected and JcDV-infected *Vanessa cardui* caterpillars reared on *Plantago lanceolata* (**A**) or *Taraxacum officinale* (**B**) leaves. Dotted lines represent 95% confidence intervals. Sample sizes for uninfected (n_u) and JcDV-infected (n_i) treatments at the beginning and end of the larval stage are indicated in the figure

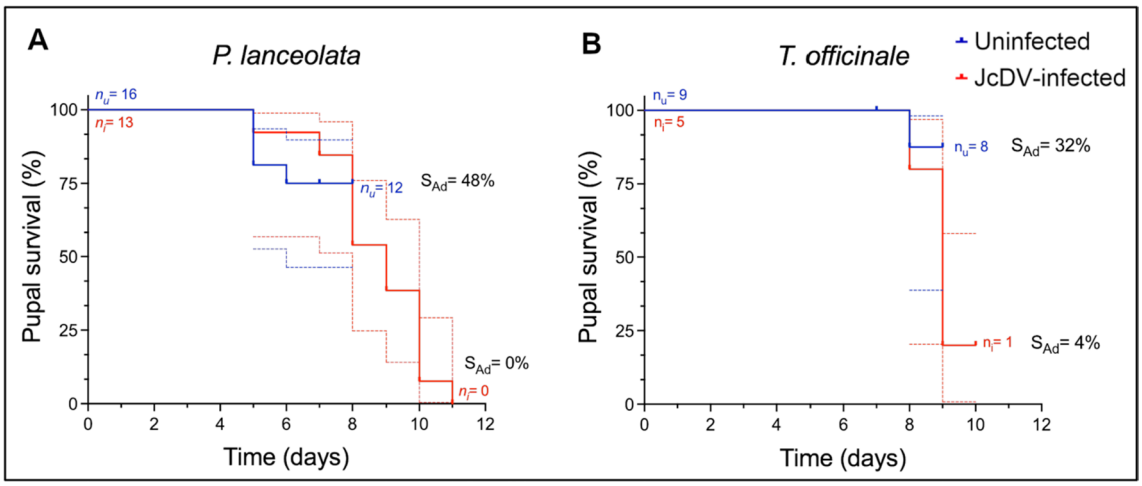

Fig. 7 Probability of pupal survival of uninfected and JcDV-infected *Vanessa cardui* individuals reared on *Plantago lanceolata* (**A**) or *Taraxacum officinale* (**B**) leaves. Dotted lines represent 95% confidence intervals. Sample sizes for uninfected (n_u) and JcDV-infected (n_i) treatments at the beginning and end of the pupal stage are indicated in the figure, as well as the survival to adulthood (S_{Ad})

(mean = 6.42 days; SD = 0.62; n = 17), while JcDV-infected caterpillars remained approximately seven days in this instar (mean = 7.57 days; SD = 0.85; n = 14). The 4th larval instar of both uninfected and JcDV-infected caterpillars lasted approximately four days (mean = 3.96 days; SD = 0.20; n = 25 and mean = 4.00 days; SD = 0.71; n = 25, respectively) (i.e., no significant differences were observed, P = 0.7867).

For caterpillars reared on *T. officinale*, significant differences were found between uninfected and JcDV-infected caterpillars for both the 4th and the 5th larval instars (P = 0.0002 for the 4th instar and P = 0.0088 for the 5th instar) (Fig. 8B). The 4th instar was shorter for JcDV-infected caterpillars (mean = 3.57 days; SD = 0.79; n = 7) than it was for uninfected caterpillars (mean = 5.09 days; SD = 0.53; n = 11). In contrast, the 5th instar was longer for JcDV-infected caterpillars (mean = 7.00 days; SD = 0.71; n = 5) than it was for uninfected caterpillars (mean = 5.89 days; SD = 0.60; n = 9).

Uninfected and JcDV-infected caterpillars reared on *P. lanceolata* exhibited similar mean weights across larval development at corresponding time points (i.e., no significant differences were found in the mean larval mass based on infection with JcDV) (Fig. 9A). Similarly, the mean mass of caterpillars reared on *T. officinale* leaves was not dependent upon JcDV infection (i.e., uninfected and JcDV-infected caterpillars exhibited similar mean mass across insect development) (Fig. 9B).

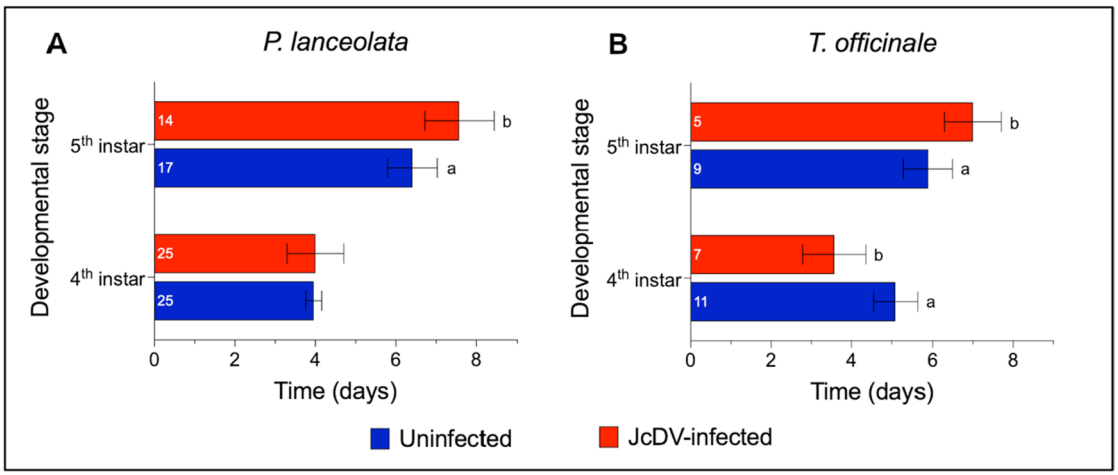

Fig. 8 Development time for uninfected and JcDV-infected *Vanessa cardui* caterpillars reared on *Plantago lanceolata* (**A**) or *Taraxacum officinale* (**B**) leaves throughout the 4th and 5th larval instars. Sample sizes (*n*) are indicated within bars. Different letters indicate significant differences between uninfected and JcDV-infected caterpillars within a developmental stage ($P < 0.01$, *t*–test)

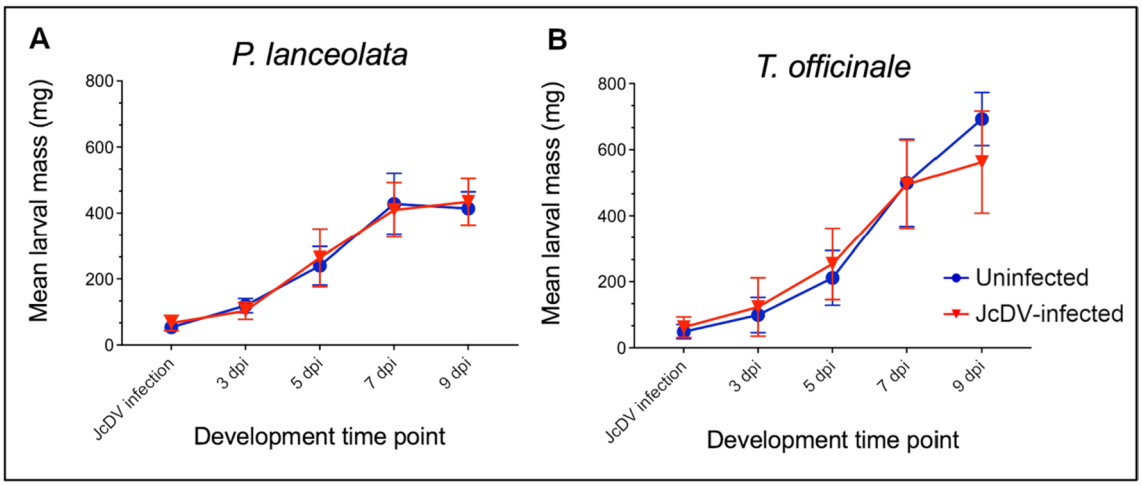

Fig. 9 Mean larval mass of uninfected and JcDV-infected *Vanessa cardui* caterpillars reared on *Plantago lanceolata* (**A**) or *Taraxacum officinale* (**B**) leaves at specific development time points. Error bars represent standard deviations; dpi, days post infection

The mean pupal mass of *V. cardui* individuals reared on each host plant did not differ significantly between uninfected and JcDV-infected individuals ($P = 0.1133$ for *P. lanceolata* and $P = 0.3546$ for *T. officinale*) (Fig. 10). However, significant differences were observed in the pupal masses based on the host plant provided as diet for both uninfected and JcDV-infected caterpillars (i.e., pupal masses of individuals reared on *P. lanceolata* significantly differed from the pupal masses of individuals reared on *T. officinale*) ($P < 0.0001$).

Discussion

Overall, we found that *V. cardui* development and immunity were more strongly affected by host plant consumption than by JcDV infection, which corroborated the assumption that insect development is greatly influenced by the nutritional value and chemical content of the host plant (Knolhoff and Heckel 2014; Lampert et al. 2014; Simpson et al. 2015). Even so, JcDV infection induced

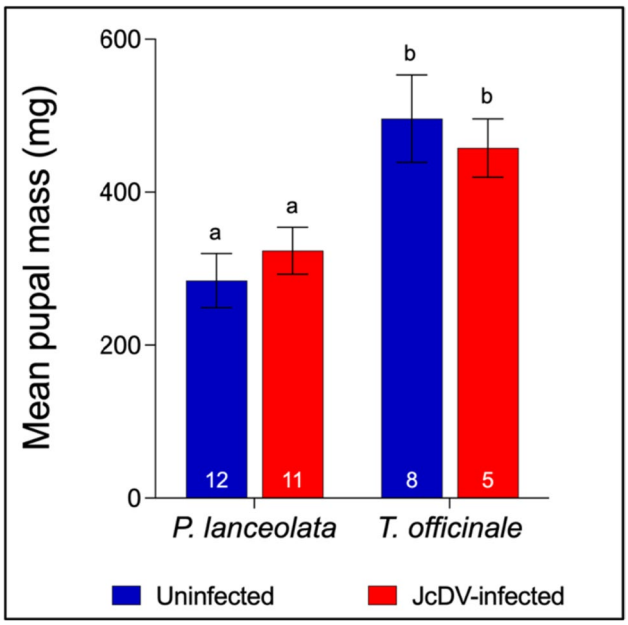

Fig. 10 Mean pupal mass of uninfected and JcDV-infected *Vanessa cardui* individuals reared on *Plantago lanceolata* or *Taraxacum officinale* leaves. Sample sizes (*n*) are indicated within bars. Different letters indicate significant differences ($P < 0.0001$, two-way ANOVA with Tukey's multiple comparisons test)

changes to the *V. cardui* metabolome, especially related to the chemical diversity in frass and hemolymph, which were dependent upon both viral infection and host plant consumption.

Even though *P. lanceolata* and *T. officinale* leaves were characterized by similar richness (Fig. 3A; mean = 75.00 metabolites, SD = 16.67, $n = 5$ and mean = 76.60 metabolites, SD = 8.38, $n = 5$, respectively) and Simpson's diversity (Fig. 4A; mean = 0.946, SD = 0.024, $n = 5$ and mean = 0.943, SD = 0.018, $n = 5$, respectively) of metabolites, PCA showed that the chemical content was different between the two host plants (Fig. 2A). This finding was reported in our previously study, where we demonstrated the occurrence of iridoid glycosides, phenylethanoids and flavonoids in *P. lanceolata* leaves, and phenylpropanoids, sesquiterpene lactones and flavonoids in *T. officinale* leaves (Gallon and Smilanich 2023). Therefore, *V. cardui* caterpillars experienced a similar intake in terms of richness and diversity of secondary metabolites within each diet, however they were exposed to different types of secondary metabolites, which may have influenced the immune response to JcDV infection. Many prior studies have demonstrated that secondary metabolites can influence the immune response in both positive and negative directions (reviewed in Smilanich and Muchoney 2022). In particular, Slinn et al. (2018) showed that caterpillars feeding on plants with higher chemical diversity had lower immunity as measured by PO activity. Since the two plants compared here had similar chemical diversity, we were unable to test whether this result was supported in our study. Nonetheless, caterpillars feeding on *P. lanceolata* had higher PO activity compared to those feeding on *T. officinale* (Fig. 5), which is further discussed below.

Phenoloxidase is an important enzyme that activates the production of melanin (i.e., a brownish pigment with antimicrobial activity) and it is involved in insect defense against foreign invaders (e.g., pathogens and parasitoids) and wound healing of mechanical injuries (Marmaras et al. 1996; Eleftherianos and Revenis 2011). Since melanin is a nitrogen-rich compound, considerable supplies of proteins and nitrogen are required for its biosynthesis (González-Santoyo and Córdoba-Aguilar 2012). Consequently, the production and maintenance of PO is diet-depend, which suggest that healthy individuals are likely to exhibit high PO activity (Povey et al. 2009; González-Santoyo and Córdoba-Aguilar 2012). Our results confirmed this assumption, since uninfected caterpillars reared on *P. lanceolata* exhibited higher PO activity (Fig. 5), as well as higher larval and pupal survivals (Figs. 6 and 7), than JcDV-infected caterpillars reared on this host plant. Additionally, PO activity was higher in caterpillars reared on *P. lanceolata* for both uninfected and JcDV-infected treatments, than it was in caterpillars reared on *T. officinale* also for both uninfected and JcDV-infected treatments. Therefore, caterpillars reared on *P. lanceolata* exhibited greater immunocompetence, in terms of PO activity, than caterpillars reared on *T. officinale* regardless of JcDV infection, indicating that host plant consumption affected the *V. cardui* immune response.

A prior study with the common buckeye caterpillar (*J. coenia*) showed that JcDVinfected individuals exhibited faster larval development than uninfected individuals, suggesting that quickening development time may be advantageous to resist viral infections (Smilanich et al. 2018; Muchoney et al. 2022). Herein, the differences in the development time between uninfected and JcDV-infected 4th instar caterpillars reared on *T. officinale* (Fig. 8B) may be associated with caterpillars attempting to escape the viral infection by shortening the 4th larval instar and, therefore, expediting molt to the 5th instar. Additionally, a study with *Spodoptera frugiperda* demonstrated that JcDV amplification was exponential at four days post infection (Mutuel et al. 2010), supporting the assumption that during the 4th larval instar *V. cardui* caterpillars reared on *T. officinale* developed faster to avoid high viral loads and consequently succumbing to JcDV. In contrast, JcDV-infected caterpillars exhibited longer duration for the 5th instar regardless of host plant consumption, which showed that different strategies for modulating development during a viral infection may be involved across the insect life cycle. Developmental defects, including prolonged larval development and pupation failure, have also been associated with viral infection in lepidopteran insects

(Nakai et al. 2002, 2016). Therefore, extended duration of the 5th instar in *V. cardui* caterpillars may indicate that even after molting, viral loads were high enough to maintain JcDV infection in progress. Consequently, JcDV-infected caterpillars may have been in very poor health by this point or may have invested more in defending themselves against the virus rather than fitness or performance, which explains a prolonged larval instar.

Our findings suggest that host plant consumption influences how *V. cardui* caterpillars chemically respond to JcDV infection, since for caterpillars reared on *P. lanceolata* significant differences were found in the chemical diversity of frass and hemolymph samples between uninfected and JcDV-infected individuals while no differences were found in the chemical diversity of frass and hemolymph of uninfected and JcDV-infected individuals when caterpillars were reared on *T. officinale* (Fig. 4C and D). Therefore, changes in the insect metabolome and consequently, in the metabolites being mobilized into insect hemolymph and eliminated in insect frass, were dependent upon viral infection along with host plant consumption. A recent study by Tian et al. (2023) also found that viral infection caused changes to the metabolome of cotton bollworm, *Helicoverpa armigera* (Noctuidae), infected with *Helicoverpa armigera* NPV (HearNPV). In this case, infection by HearNPV caused a dysregulation of glucose metabolism, which in turn caused suppression of the immune response. In our study, it appears that both infection and host plant played a role in altering the metabolome of the infected caterpillar.

Differences in richness and Simpson's index for hemolymph of uninfected and JcDV-infected caterpillars reared on *P. lanceolata* (Figs. 3D and 4D) may be associated with the differential PO activity found between uninfected and JcDV-infected caterpillars reared on this host plant (Fig. 5). While uninfected caterpillars exhibited higher PO activity and lower chemical diversity (i.e., lower richness of metabolites and Simpson's index), JcDV-infected caterpillars exhibited lower PO activity and higher chemical diversity. This finding suggested that the insect immune response was suppressed upon JcDV infection, while novel and more diverse metabolites were biosynthesized or mobilized into the insect hemolymph to aid in defending *V. cardui* caterpillars against JcDV. Thus, these additional metabolites may be a byproduct of the caterpillar responding metabolically to the infection and even though the PO activity was suppressed, there may still be metabolites that were synthesized by other parts of the insect immune system. In any case, it is clear that the hemolymph is a primary location where the JcDV infection is manifesting large changes to the physiology of the insect, and the outcomes of these changes for pathogen suppression warrant further investigation.

Taken together, our results provided insights into the chemical environment within a virus-infected insect and presented novel perspectives for multitrophic interactions in a plant-insect-pathogen context. Therefore, we demonstrated that changes to the chemical content, immunity and development of a generalist insect were influenced by a viral infection. Going forward, studies considering the roles of specific metabolites in defending insects against viruses may benefit from the background gained herein.

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Author Contributions MEG and AMS contributed to the study conception and design. Data collection and analyses were performed by MEG. NDM contributed to the statistical analyses.The first draft of the manuscript was written by MEG. All authors commented on previous versions of the manuscript, as well as read and approved the final manuscript.

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Data Availability The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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