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Nutrition of host plants influence the infectivity of nucleopolyhedrovirus to polyphagous caterpillar, *Hyphantria cunea*

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Abstract

Plants play an important role in interactions between insect herbivores and their pathogens. The ability of host plants to modify the infectivity of entomopathogens in herbivorous insects has been widely documented. However, the plants' nutrients have always been neglected as a factor contributing to variation in the susceptibility of insect herbivores to entomopathogens. The fall-webworm (FWW), *Hyphantria cunea* Drury, is a typical polyphagous caterpillar, and the *Hyphantria cunea* nucleopolyhedrovirus (HycuNPV) is a distinctly specialized baculovirus for the FWW, which is safe for other organisms and has been effectively used as a biological insecticide against *H. cunea* in China. In this study, we investigated the nutrient components of four host plant species, i.e., *Prunus serrulate*, *Cerasus serrulate*, *Camptotheca acuminata*, and *Populus deltoides*, and their effects on the susceptibility of *H. cunea* larvae to HycuNPV. The HycuNPV-infected larvae fed on *P. deltoides* leaves exhibited higher survival rates, longer survival times, more food intake, and gained larger body size. These biological parameters were positively correlated with the nitrogen components of host plant leaves. Moreover, the larval antioxidant enzymes exhibited different responses to HycuNPV. HycuNPV infection significantly triggered the catalase (CAT) and prophenoloxidase (PPO) enzyme activity levels of *H. cunea* larvae. The uninfected larvae fed on poplar leaves induced a robust increase in the POD activity, which could scavenge extra reactive oxygen species and provide a protective effect against the HycuNPV. In conclusion, the plant-mediated effects of HycuNPV on the FWW have been investigated in this study. The nitrogen content in dietary was an essential factor in determining the insect herbivore susceptibility to entomopathogenic viruses, and it helped explain variations in the susceptibility of pests to the entomopathogenic viruses and aid in developing more robust tolerance monitoring assays in the lab that reflect the performance of pests in the field.

Keywords Polyphagous caterpillar, Host plants, Nucleopolyhedrovirus, Tri-trophic interactions, *Hyphantria cunea*

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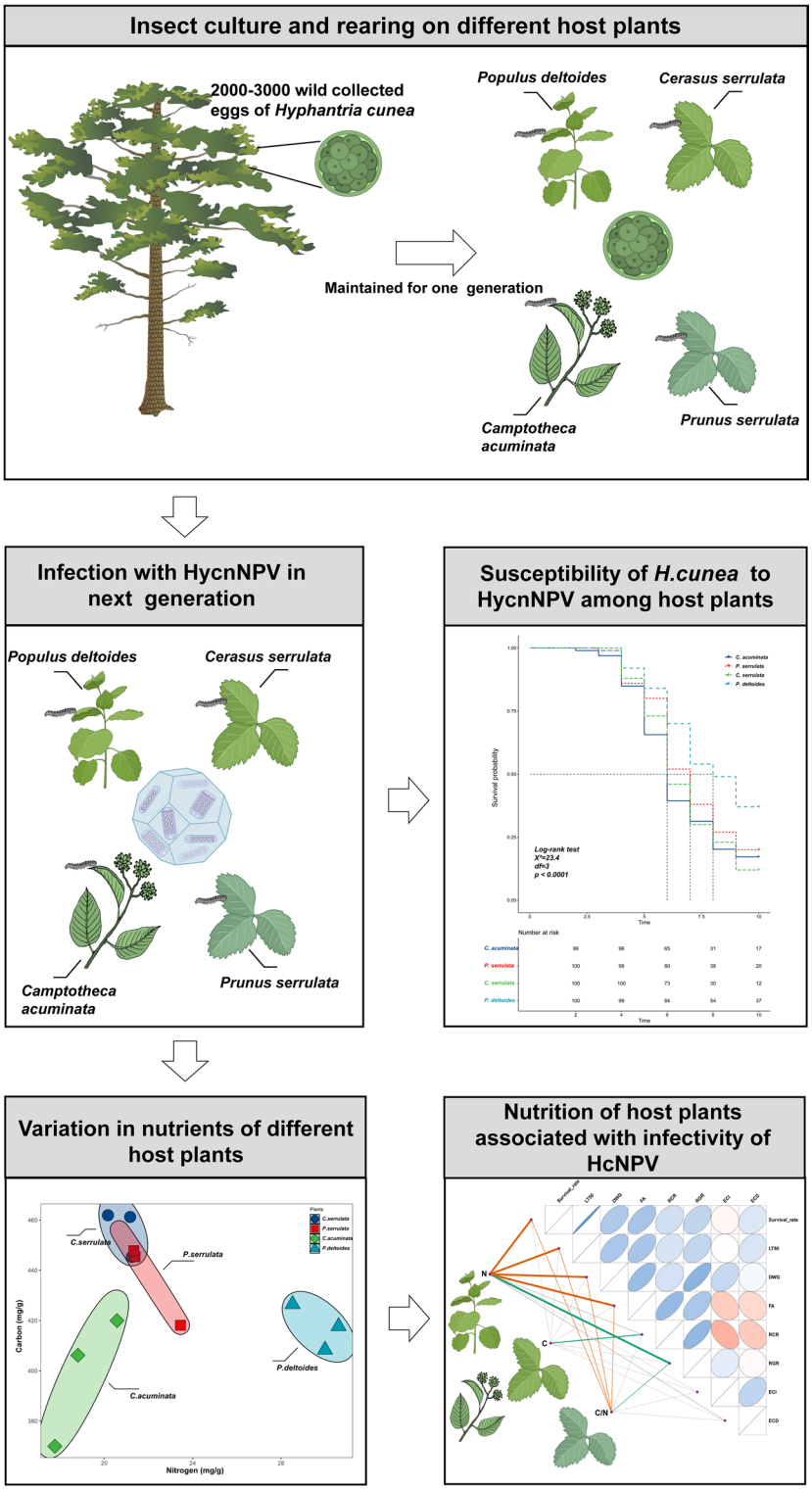
Graphical Abstract



Introduction

As an essential environmental factor and food resource, the host plants significantly influence the occurrence and distribution of herbivorous insects [1, 2]. Through long-term adaptation and co-evolution, a complex and intensive relationship has been formed between herbivorous insects and host plants [3]. The host plants play a significant role as food resources for phytophagous insects during their growth and development and provide them with the primary mating and oviposition sites [4, 5]. In addition to host plants, the pathogenic microorganisms (e.g., bacteria, fungi, and viruses) in nature can also affect the behavioral, morphological, and physiological performance of herbivorous insects [6, 7]. Microbial communities can influence the expression of life-history features in herbivorous insects via an extensive communication network, including the immune system [6, 8], affecting the expression of traits linked to not only the immune system but also metabolism and development [9, 10]. The host plants are the main drivers of the interactions between herbivorous insects and entomopathogenic viruses [11, 12]. Among such tritrophic interactions, host plants-mediated effects on the susceptibility of insect herbivores to entomopathogenic viruses have received particular attention. In recent decades, researchers have discovered that changes in phytochemicals, such as certain primary and secondary metabolites, might cause variances in viral susceptibility [13, 14]. In general, plants play an essential role in interacting with insect herbivores and their pathogens.

Intriguingly, some insects have demonstrated the ability to acquire particular phytochemicals and nutrients as a preventative or curative measure against entomopathogen threat or infection [14]. Insects' tolerance to entomopathogens is dependent on dietary protein. Whether increased dietary protein increases or decreases host resistance varies among insect-entomopathogen systems based on how protein influences insect immune function and entomopathogen growth. Either before or after the pathogen challenge, increased dietary protein consumption relative to carbohydrates can enhance the survival of larval lepidopterans against baculoviruses [15–17]. There can be substantial variation in the macronutrient and micronutrient content of different plants. These include differences among plant species, genotypes, plant segments, ontogenetic stages, seasons, and fertilization interventions [18].

Insects are also known to defend themselves against pathogens through various enzyme systems [19, 20]. Several insect enzymes, such as catalase (CAT), prophenoloxidase (PPO), superoxide dismutase (SOD), and peroxidase (POD), contribute to defenses against infections and pesticides [21]. Under the stress of pathogens, insects accumulate superoxide anion free radicals (O_2^-), hydroxyl radicals (-OH), hydrogen peroxide (H_2O_2), and other reactive oxygen species (ROS), which can cause damage to the organism [21]. However, insects possess a protective enzyme system consisting of SOD, POD, and CAT, which can scavenge excess free radicals, among which SOD can catalyze O_2^- disproportionation to generate H_2O_2, while CAT and POD can degrade H_2O_2. The three enzymes coordinate with each other to maintain the dynamic balance of free radical metabolism in the body, so that the insects can control the damage levels [22]. Due to its function in cellular and humoral defense, insect prophenoloxidase (PPO) is an essential innate immunity protein [23]. The cascade of polyphenol oxidases protects insects from microbial infections by participating in the melanization of hemocytes attached to pathogen surfaces [24], and increased PPO activity can increase tolerance to pathogens and promote wound healing [25].

The fall webworm (FWW), *Hyphantria cunea* Drury (Lepidoptera: Erebidae), is a typical polyphagous pest [26]. It is native to North America, ranging from Canada to Mexico, and was first reported in Dandong City, Liaoning Province in China in 1979, and spread to Shandong, Shaanxi, Hebei, Tianjin, and Shanghai, causing significant damage to forests, fruit trees, and even field crops [27–29]. Its population is below the threshold level in its native habitats due to the presence of numerous natural enemies. Consequently, it does not cause severe damage. However, the pest has caused a significant loss of forest trees in newly distributed areas. Generally, the first step in its introduction was the application of insecticides [30], but using indiscriminate insecticides has detrimental effects on beneficial organisms and the environment, particularly when sprayed in forests, which are the primary source of beneficial organisms. Baculoviruses are species-specific, double-stranded DNA viruses with limited host ranges and high virulence, rendering them ecologically and environmentally safe biopesticides [31]. The baculovirus *Hyphantria cunea* nucleopolyhedrovirus (HycuNPV) has been isolated from the larvae of the fall webworm [32]. At present, several HycuNPV strains have been isolated in China and successfully used as biological insecticides against *H. cunea* [33, 34].

Due to its polyphagous traits, the larvae of the *H. cunea* could feed on nearly all deciduous tree species. Currently, more than 400 plant species have been recorded to serve as host plants for *H. cunea* worldwide [26]. Previous studies have revealed that different host plant species/food resources could significantly affect the biological performance of *H. cunea* larvae [28, 35]. However, it remains unknown whether host plant species and variations in nutrient components among host plants affect

the susceptibility of *H. cunea* to HycuNPV. In this study, strains of *H. cunea* raised on four common host plant species were established in the lab. The tolerance of *H. cunea* larvae fed on different host plants to HycuNPV and critical enzyme response was investigated. Furthermore, we determined the carbon, nitrogen contents, and ratios of C/N in different host plants and analyzed the correlation between the biological parameters of HycuNPV-infected *H. cunea* larvae and the nutrient components of host plants. Variations in virulence of insect viruses and host susceptibility by plant-mediated can significantly impact the timing, duration, and severity of epidemics in herbivorous insects. Determining plant quality influences this variation is essential for enhancing the efficacy of microbial pesticides, comprehending disease-mediated insect population dynamics, and predicting and controlling pest populations, which could have implications for forest ecosystem health and biodiversity.

Materials and methods

Insect culture and rearing on different host plants

A total of 2000–3000 *H. cunea* eggs were collected from mulberry leaves in Huaian, Jiangsu Province, in China (33.62°N, 119.02°E) in early May 2020. Before we collected the healthy eggs of *H. cunea* from mulberry leaves, we ensured that pesticides were not used before this collection. After being sterilized with 5% formaldehyde, the newly-hatched larvae were reared on artificial diets for one generation to eliminate host plant memory at 25 ± 1 °C, 60 ± 5% RH, and a 16:8 h light: dark period and supplied fresh diets every 2 days until pupation. We determined the gender of each pupa via the presence (female) or absence (male) of a line intersecting the first abdominal sternite [36, 37]. The pupae were individually placed in glass jars (4.5 cm high and 2.5 cm diameter) according to sex to facilitate the collection of adults. After eclosion, at least 30 pairs of adults were randomly selected and placed in screen cages (25 × 25 × 15 cm) with the bottom covered with cotton wool soaked in 10% honey solution, and the corrugated paper replaced daily for mating and oviposition. In the following generation, neonates were reared on different host plant leaves at the rearing box (13.5 × 8 × 6.5 cm). The Japanese flowering cherry (*Prunus serrulata* var. lannesiana (Carri.) Makino), Chinese flowering cherry (*Cerasus serrulata* (Lindl.) G. Don ex London), happy tree (*Camptotheca acuminata* Decne), and necklace poplar (*Populus deltoides* Marshall) were common host plants of *H. cunea* in the Jiangsu area in a previous survey. The host plant leaves were collected at Nanjing Forestry University (32.08°N, 118.82°E) at 8:00 pm, and 75% ethanol was utilized to clean the leaves' surface to remove impurities and microorganisms.

Efficacy of HycuNPV against *H cunea* larvae reared on different host plants

The viral stock of *H. cunea* nucleopolyhedrovirus (HycuNPV) was obtained from the Research Institute of Forest Ecology, Environment, and Protection, Chinese Academy of Forestry (Beijing, China). It was resuspended in distilled water at a concentration of 3×10^8 mL^{-1} polyhedra (sublethal concentrations of HycuNPV) for this experiment to avoid excessive larval mortality [38, 39].

The newly molted 3rd instar larvae [38, 39] of similar sizes reared on different host plants were randomly selected, starved for 12 h, and then moved to a new Petri dish (diameter 35 mm, depth 12 mm) individually. Once the caterpillars were placed into cells and covered with ventilated clear plastic covers. Each caterpillar was fed a leaf disk (diameter 5 mm) treated with HycuNPV or distilled water [14, 40]. Either 2 μL of HycuNPV suspension or sterilized water was applied onto each leaf disk and then air-dried. After that, we covered the lid and isolated the HycuNPV-infected and non-infected (control) caterpillars in climate chambers in different rooms to avoid cross infection. After feeding for 24 h, larvae that had consumed the entire disk (6×10^5 polyhedra per leaf disk) were transferred to a clean rearing box with fresh leaves without HycuNPV. Those larvae that failed to consume the entire disk were discarded, at least 100 larvae for each treatment.

Feeding inhibition

After virus treatment, half of the larval mortality occurred in roughly 5 to 8 days for the different treatments. Therefore, we chose 3 days post virus inoculation for the feeding inhibition experiment. After inoculating viral suspensions 3 days, larvae from each host plant were individually reared in a new Petri dish (diameter 9 cm, depth 1.8 cm) with sufficient clearance between the lip and the bottom to ensure air circulation in the microenvironment. Before the feeding experiment started, the larvae were starved for 12 h to allow them to excrete feces. Subsequently, the larvae were weighted and supplied with pre-weighed host plant leaves. At least 15 larvae from each host plant colony were used for the test. A Petri dish with a leaf of each plant (approximately 1 g) was provided for each larva. After 48 h, fed larvae were starved again for 12 h to allow them to excrete feces. After excreting feces, the remaining diet, feces, and larvae were dried in an oven (at 60 ℃ for 48 h) and weighed by a balance (ME204T, Switzerland, $d = 0.0001$ g). The dry weight gained (DWG), food amount (FA), relative growth rate (RGR), relative consumption rate (RCR), the efficiency of converting ingested food into body matter (ECI), and the efficiency of conversion of digested food

into body matter (ECD) to analyze the consumption and feeding efficiency. The formulas used to calculate these nutritional indices are as follows:

$$\text{The dry weight gained } (\text{DWG/mg}) = \text{B - A};$$

$$\text{The food amount } (\text{FA/mg}) = \text{D - C};$$

$$\text{The relative growth rate (RGR) } (\text{mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}) = (D - C) / \left[\left((A + B) / 2 \right) \times T \right]$$

$$\text{The relative consumption rate (RCR) } \left(mg \cdot mg^{-1} \cdot d^{-1} \right) = (D - C) / \left[\left((A + B) / 2 \right) \times T \right]$$

$$\text{The efficiency of conversion of ingested food (ECI) \%} = (\text{B - A}) / \text{E};$$

$$\text{The relative efficiency of conversion of digested food } (ECD)\% = (B - A) / \left[\left((D - C) / -E \right) \times T \right]$$

A is the dry mass of the larva before feeding; B is the dry mass of the larva after feeding; *C* is the dry mass of food before larvae feeding; *D* is the dry mass of food after larvae feeding; E is the dry mass of feces, and *T* is the duration of the feeding period.

Determination of carbon and nitrogen content of host plants

The leaves of different host plants were collected at Nanjing Forestry University (32.08°N, 118.82°E) at 8:00 pm (75% ethanol was utilized to clean the leaves' surface to remove impurities and microorganisms) in July and August according to the feeding habits and periods of the second generation of *H. cunea* larvae in the wild. The prominent veins of the leaves were removed according to the forage characteristics of the *H. cunea* larvae. The leaf flesh was used to determine the chemical content. At least 10 different leaves from different host plants were used for one treatment. The wet weight of leaf flesh obtained from each treatment was at least 15 g, and the dry weight was at least 5 g. Three biological replicates were obtained for each treatment.

Total nitrogen content was determined by H_2SO_4-H_2O_2 digestion method of Kjeldahl [41] and was used to measure the total nitrogen concentration of different host plant leaves. Nitrogen content was expressed as the product of concentration and dry weight. Leaf carbon concentration of different host plants was assessed following potassium dichromate oxidation outside the heating method [42].

Enzyme analysis

The 3 days larvae post virus inoculation were chosen for the enzyme analysis following the methods of Sun [38]. At least 10 replicates from each treatment were stored at − 80 °C after being rapidly frozen in liquid nitrogen. The frozen larvae were randomly selected from each treatment for enzyme analysis.

The peroxidase (POD) activity assay was performed by following Shannon et al. [43]. In brief, 2.1 ml of H_2O, 0.32 ml of 14 mM potassium phosphate buffer, 0.16 ml of 0.027% (*v*/*v*) hydrogen peroxide, and 0.32 ml of 0.5% (*w*/*v*) pyrogallol were mixed with 3.00 ml of water and incubated at 20 °C for 10 min. After adding 0.1 ml of 14 mM potassium phosphate buffer and 0.1 ml of the sample, the mixture was mixed by inversion. Spectrophotometry was used to measure the change in absorbance of 420 nm. The activity of an enzyme was shown as *U* per mg of protein.

Nitroblue tetrazolium (NBT) reduction was used to evaluate the superoxide dismutase (SOD) activity in cell-free extracts [44]. The assay mixture included 0.2 ml of 0.1 M Ethylenediaminetetraacetic acid (EDTA) containing 0.3 mM sodium cyanide (0.2 ml), 0.1 ml of 1.5 mM Nitroblue tetrazolium chloride (NBT), 3 ml of 0.067 M potassium phosphate buffer, pH 7.8, and a series of samples ranging from 0.1 to 10 mg of protein in separate tubes. The tubes were put in a lightbox, which provided consistent illumination. The tubes were incubated for 5 to 8 minutes to reach a standard temperature. At zero time, 0.05 ml 0.12 mM riboflavin was introduced, all tubes were incubated for 12 min, and the absorbance was measured every minute at 560 nm. One unit of SOD activity was defined as the quantity of SOD necessary to prevent the 50% decrease of NBT (A_{560}) and was represented as units per mg of protein (U/mg protein).

CAT activity was assayed by the method described by Beers and Sizer [45], in which the decomposition of H_2O_2 was analyzed spectrophotometrically at 240 nm. Reagent-grade water (1.9 ml) and 0.059 M hydrogen peroxide (1.0 ml) were pipetted into the cuvette. The cuvette was incubated in a spectrophotometer for 4–5 min to achieve temperature equilibration and to establish a blank rate if any. After 5 min, 0.1 ml of sample was added, and a change in absorbance at 240 nm was observed for 2–3 min. Change in absorbance per minute was calculated from the initial (45 s) linear portion of the curve. One unit of CAT activity was defined as the amount of enzyme that decomposed 1 mmol H_2O_2/min at an initial H_2O_2 concentration of 30 mM at pH 7.0 and 25 °C and was expressed as U/mg protein.

Following the methods of Thipyapong et al. [46], the prophenoloxidase (PPO) activity was evaluated spectrophotometrically using 2 mM 4-methylcatechol as a substrate. To eliminate peroxidase-mediated phenolic oxidation, 280 U of catalase was administered to each sample for 15 min before testing. Specific activity was reported regarding micromoles of quinone produced per min per mg of protein at 525 nm.

Statistical analysis

The general linear models (GLM) were used to analyze the effects of HycuNPV infection and different host plants on the feeding inhibition and enzyme activities of *H. cunea*. The age-specific survival rates of larvae reared on different host plants after HycuNPV infection were compared with the log-rank test. The LT50 (median lethal time) was estimated by probit analysis, and the survival time values were transformed using log transformation at the base of 10. The χ^2 tests are used to test the goodness-of-fit of the $\log_{10}$ (survival time)-probit regression model [47, 48]. The difference in carbon components, nitrogen components, and carbon-to-nitrogen ratios among different host plants was compared by one-way variance analysis (ANOVA), followed by Tukey's honest significant difference (HSD) test. Pearson's correlation analysis analyzes the correlations among the biological parameters of *H. cunea* after HycuNPV infection. The correlations between the biological parameters of *H. cunea* after HycuNPV infection and the nutrients of the host plants were correlated by the Mantel test [49].

All statistical tests were conducted in R (version 4.2.0) and the statistical software package SPSS 22.0 (IBM Inc., New York, USA). Plots were generated using R 4.2.0 within Rstudio (RStudio Team 2022), with some modifications to the graphic layout being made using Illustrator CC 2018 (Adobe Systems, San Jose, California).

Results

Effects of different host plants on the virulence of HycuNPV-infected *H. cunea* larvae

The virulence of HycuNPV-infected *H. cunea* larvae was significantly affected by feeding on different host plants. The survival rate of infected larvae feeding on poplar leaves was significantly higher than those feeding on other host plant leaves (Additional file 1: Table S1, Fig. 1, log-rank test, $\chi^2 = 23.4$, $df = 3$, $P<0.0001$). The highest mortality rate of infected larvae was detected when feeding on the leaves of *C. acuminata*. Feeding on poplar leaves significantly enhanced the survival time of larvae (Table1). The infected larvae survived longer (measured as LT50) on *P. deltoides* followed by *P. serrulate* then *C. serrulate*, and the lowest LT50 was detected for larvae fed *C.acuminata*. In conclusion, when feeding *C. serrulata* and *C. acuminata*, the infected larvae had higher mortalities and shorter mean time to death than when feeding *P. deltoides*.

Effects of different host plants on the feeding inhibition of HycuNPV-infected and uninfected *H. cunea* larvae

The dry mass of each host plant leaves before larva feeding was regressed against the wet mass, and the wet-dry regression formula for the linear regression was calculated (*P. deltoides* leaves, $n=5$, dry mass $=-0.0489+0.33444\times$wet mass, $R^2=0.99$; *P. serrulata* leaves, $n=5$, dry mass $=-0.00752+0.38266\times$ wet mass, $R^2=0.99$; *C. serrulata* leaves, $n=5$, dry mass $=0.01492+0.27501\times$wet mass, $R^2=0.99$; *C. acuminata* leaves, $n=5$, dry mass $=-0.0085+0.30757\times$wet mass, $R^2=0.99$). The dry mass of larvae before feeding is also converted according to this formula (*H. cunea* larvae, $n=32$, dry mass $=-0.00371+0.18715\times$wet mass), and the HycuNPV infection significantly affected the feeding behaviors of *H. cunea.* The DWG, FA, RGR, ECI, and ECD of larvae significantly decreased after HycuNPV infection (Table 2; DWG: $\chi^2=126.6$, $P<0.001$; FA: $\chi^2=17.3$, $P<0.001$; RGR: $\chi^2=43.9$, $P<0.001$; ECI: $\chi^2=44.5$, $P<0.001$; ECD: $\chi^2=20.9$, $P<0.001$). On the contrary, the RCR of larvae increased significantly after infection ($\chi^2=11.6$, $P<0.01$). In the case of the uninfected larvae, there was no significant effect of host plant species on the amount of food intake by the healthy *H. cunea* larvae (FA and RCR, Fig. 2B, C). However, the larvae feeding on poplar leaves had higher ECI and ECD (Fig. 2E, F), and therefore also possessed greater DWG (Fig. 2A) and higher RGR (Fig. 2D). After HycuNPV infection, there were no significant differences in ECI, ECD, and DWG among larvae feeding on different host plants (Fig. 2A, E, and F), while the *C. acuminata* leaves-fed larvae showed the lower FA, RCR and RGR.

Effects of different host plants on the enzyme activities of HycuNPV-infected and uninfected *H. cunea* larvae

Host plant species and HycuNPV infection both significantly affected the activity of antioxidant enzymes as well as PPO in *H. cunea* larvae (Table 3). On average, the CAT and PPO enzyme activities were significantly higher in HycuNPV-infected larvae (CAT activity: χ^2=6.6, P<0.01; PPO activity: χ^2=79.9, P<0.001). In contrast, the POD and SOD enzyme activities were significantly lower in the HycuNPV-infected larvae (POD activity: χ^2=53.9, P<0.001; SOD activity: χ^2=37.1, P<0.001). The larvae fed on poplar leaves showed the lowest CAT enzyme activity, while the larvae fed on *C. acuminata* leaves exhibited the highest CAT enzyme activity (Fig. 3A, P<0.001). The effect of host plant species on larval PPO enzyme activity was similar to that of CAT, with the lowest level of

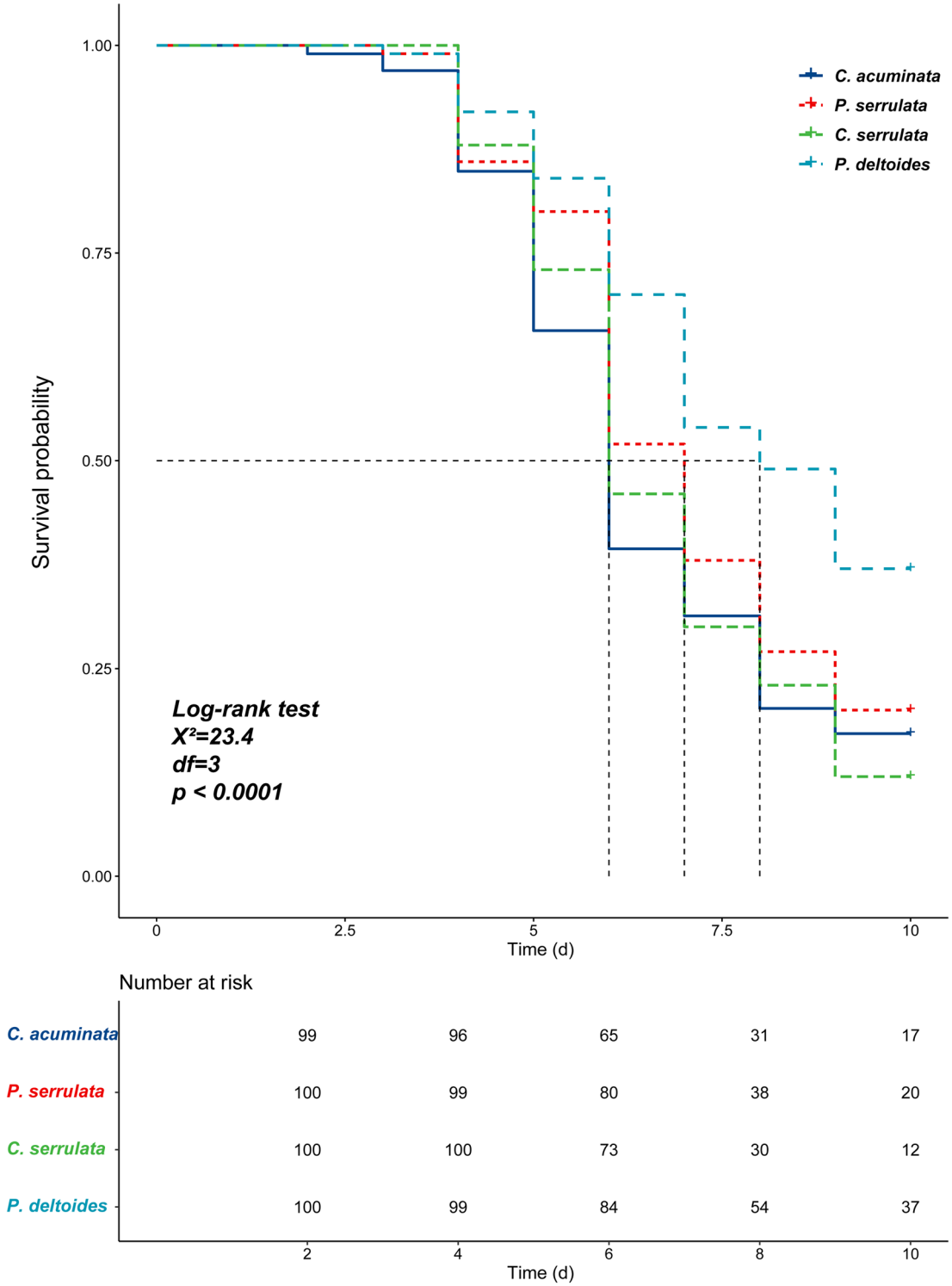


Fig. 1 Survival analysis of *H. cunea* larvae infected with HycuNPV feeding on different host plants

PPO enzyme activity in larvae feeding on poplar leaves (Fig. 3B, $P<0.05$). Furthermore, the larvae feeding on poplar leaves exhibited the highest POD enzyme activity among the uninfected larvae, significantly higher than those feeding on other host plants. Meanwhile, there was no significant difference in POD enzyme activity in larvae

Table 1 Mean time to death (logit-transformed) for the infectivity of HycuNPV to *H. cunea* larvae feeding on the foliage of different host plants

Host plants	*Probit regression*			*Pearson goodness-of-fit test*		
	Probit-log(time) regressions	LT_{50}(d)	95%CI	χ^2	*df*	*P*
C. acuminata	$Y = -4.41 + 5.75^* X$	5.84	5.61–6.11	5.67	7	0.57
P. serrulata	$Y = -4.98 + 6.20^* X$	6.37	6.12–6.64	5.05	7	0.65
C. serrulata	$Y = -5.51 + 7.06^* X$	6.03	5.81–6.25	4.13	7	0.76
P. deltoides	$Y = -4.36 + 4.77^* X$	8.17	7.69–8.82	4.78	7	0.73

Table 2 Effects of plant species and HycuNPV-infection on feeding behavior in *H. cunea*

Traits	Source	*df*	$\chi 2$	*P*
DWG	Host plants	3	23.19	< 0.001
	HycuNPV-infection	1	126.59	< 0.001
	Host plants × HycuNPV-infection	3	6.86	0.07
FA	Host plants	3	11.24	0.01
	HycuNPV-infection	1	17.27	< 0.001
	Host plants × HycuNPV-infection	3	6.78	0.07
RCR	Host plants	3	6.40	0.09
	HycuNPV-infection	1	11.59	< 0.01
	Host plants × HycuNPV-infection	3	11.21	< 0.01
RGR	Host plants	3	13.87	< 0.01
	HycuNPV-infection	1	43.86	< 0.001
	Host plants × HycuNPV-infection	3	2.10	0.55
ECI	Host plants	3	9.81	0.02
	HycuNPV-infection	1	44.50	< 0.001
	Host plants × HycuNPV-infection	3	10.88	0.01
ECD	Host plants	3	9.03	0.03
	HycuNPV-infection	1	20.93	< 0.001
	Host plants × HycuNPV-infection	3	5.24	0.15

feeding on different host plants after HycuNPV infection (Fig. 3C). The impact of host plant species on SOD enzyme activity was also observed in HycuNPV-infected larvae and feeding on *C. acuminata* leaves showed higher SOD enzyme activity (Fig. 3D).

Nitrogen and carbon concentration of different host plants

One-way ANOVA indicated that carbon content, nitrogen content, and carbon to nitrogen ratio of different host plant species varied significantly (Additional file 1: Table S2, *C* content, $F_{3,11}$= 6.64, P<0.05; N content, $F_{3,11}$= 53.09, P<0.01; C/N, $F_{3,11}$= 29.35, P<0.01). The *P. deltoides* leaves had the highest nitrogen content compared to the other host plant leaves, and the lowest nitrogen content was observed in the leaves of *C. acuminata* (Fig. 4). The carbon content was higher in the leaves of *C. serrulata*, and there was no significant difference in the carbon content of the leaves of the other three host plants (Additional file 1: Table S2). Furthermore, *P. deltoides* leaves had the lowest carbon-to-nitrogen ratio, significantly lower than other host plants (Additional file 1: Table S2).

Correlation analysis between biological parameters of HycuNPV infected larvae and nutrient components of host plants

Subsequently, we established a correlation analysis between the biological characteristics of HycuNPV-infected larvae and host plant nutrient components. The results showed that the nitrogen content of host plant leaves was significantly and positively correlated with the survival rate (mantel'r=0.99, mantel'p< 0.01), survival time (mantel'r=0.99, mantel'p< 0.01), DWG (mantel'r=0.53, mantel'p< 0.01), FA (mantel'r=0.57, mantel'p< 0.01), and RGR (mantel'r=0.41, mantel'p< 0.05) of HycuNPV-infected larvae (Additional file 1: Table S3, Fig. 5). Carbon content in plant leaves was positively correlated with RCR of HycuNPV-infected larvae (Additional file 1: Table S3, Fig. 5, mantel'r=0.38, mantel'p< 0.05). In contrast, the carbon-to-nitrogen ratio in plants was negatively correlated with partial biological parameters of infected larvae. The higher the carbon to nitrogen ratio content in plants, the lower the larval survival rate (mantel'r=− 0.97, mantel'p< 0.01), survival time (mantel'r=− 0.98, mantel'p< 0.01), DWG (mantel'r=− 0.58, mantel'p< 0.01), FA (mantel'r=− 0.57, mantel'p< 0.01), and RGR (mantel'r=− 0.36, mantel'p< 0.05) of larvae (Additional file 1: Table S3, Fig. 5).

Discussion

Tri-trophic interactions among plants, insect herbivores, and entomopathogens have received increasing attention in entomology and ecology over the last two decades. Among such tritrophic interactions, host plant-mediated effects on the susceptibility of insect herbivores to entomopathogenic viruses have been widely reported [50]. Plants, as important ecological factors and food resources, significantly affect the occurrence and distribution of insects. Numerous insect species require plant resources at a specific life stage [51, 52].

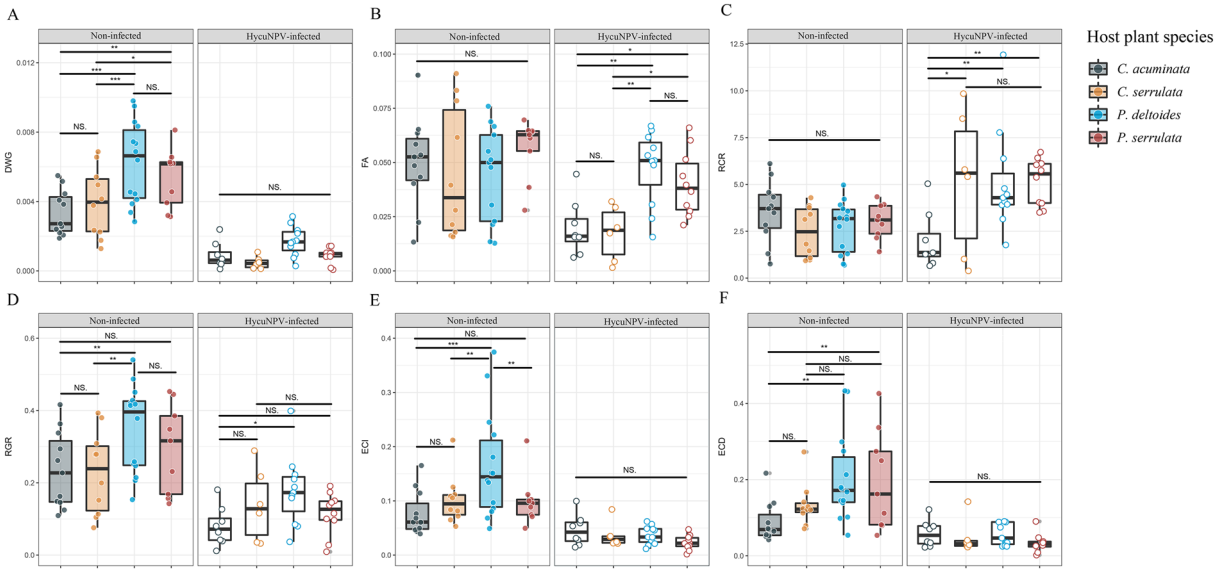


Fig. 2 Food utilization of *H. cunea* larvae infected with HycuNPV and uninfected larvae on different host plants. **A** the dry weight gain (DWG); **B** feed amount (FA); **C** the relative consumption rate (RCR); **D** the relative growth rate (RGR); **E** the conversion efficiency of ingested food (ECI); **F** the relative efficiency of conversion of digested food (ECD). The asterisks represent significant differences between HycuNPV-challenged larvae and non-challenged larvae by GLM. **P* < 0.05, ***P* < 0.001, NS, not significant

Table 3 Effects of plant species and HycuNPV-infection on activities of enzymes in *H. cunea* larvae

traits	source	*df*	$\chi 2$	*P*
CAT activity	Host plants	3	72.15	0.004
	HycuNPV-infection	1	6.56	< 0.01
	Host plants × HycuNPV-infection	3	1.06	0.8
SOD activity	Host plants	3	13.1	0.004
	HycuNPV-infection	1	37.1	< 0.001
	Host plants × HycuNPV-infection	3	8.2	0.042
PPO activity	Host plants	3	20.85	< 0.001
	HycuNPV-infection	1	79.85	< 0.001
	Host plants × HycuNPV-infection	3	8.30	0.04
POD activity	Host plants	3	65.2	< 0.001
	HycuNPV-infection	1	53.9	< 0.001
	Host plants × HycuNPV-infection	3	73.8	< 0.001

In the wild, a mutually beneficial symbiotic relationship exists between plants and entomopathogenic microorganisms, necessitating plants' ability to influence entomopathogens to compensate for the fitness loss caused by phytophagous insect foraging. Plants could increase pathogen exposure to phytophagous insects by modulating the behavior of phytophagous insects or altering their chemical compound components [53, 54]. Insect pathogens (entomopathogens) have been the subject of much investigation, emphasizing their potential as biological control agents [55]. On the global market, the use of microbiological pesticides has increased significantly over the past decade. It is primarily attributable to the European Union, as a decline in traditional broad-spectrum chemical insecticides has coincided with an increase in the organic sector and a more accommodating legislative environment for businesses to commercialize microbial pesticides [55]. Thus, it has become increasingly vital to ascertain the degree to which the efficacy of microbial pesticides varies across crop species, cultivars, and varieties [56].

The quality of host plants can directly affect the growth, development, and performance of insect herbivores [13, 27]. Due to the various primary and secondary metabolites present in host plants, they not only affect the growth, development, and reproductive capacity of insect herbivores but also have essential effects on immunity and tolerance to disease [7, 57]. In this study, host plant species significantly affected the susceptibility of *H. cunea* larvae to HycuNPV. Different host plants influenced the survival rate and time of HycuNPV-infected caterpillars. Entomopathogenic microorganisms are usually attached to the surface of host plants and enter the insects through foraging by phytophagous insects [58], while the morphology and structure of host plants directly affect the number of entomopathogenic microorganisms on their surfaces [59–61]. Differences in the types and amounts of pathogenic microorganisms within different host plants can significantly affect the survival of insects. In *Spodoptera exigua*, 14 host plant species

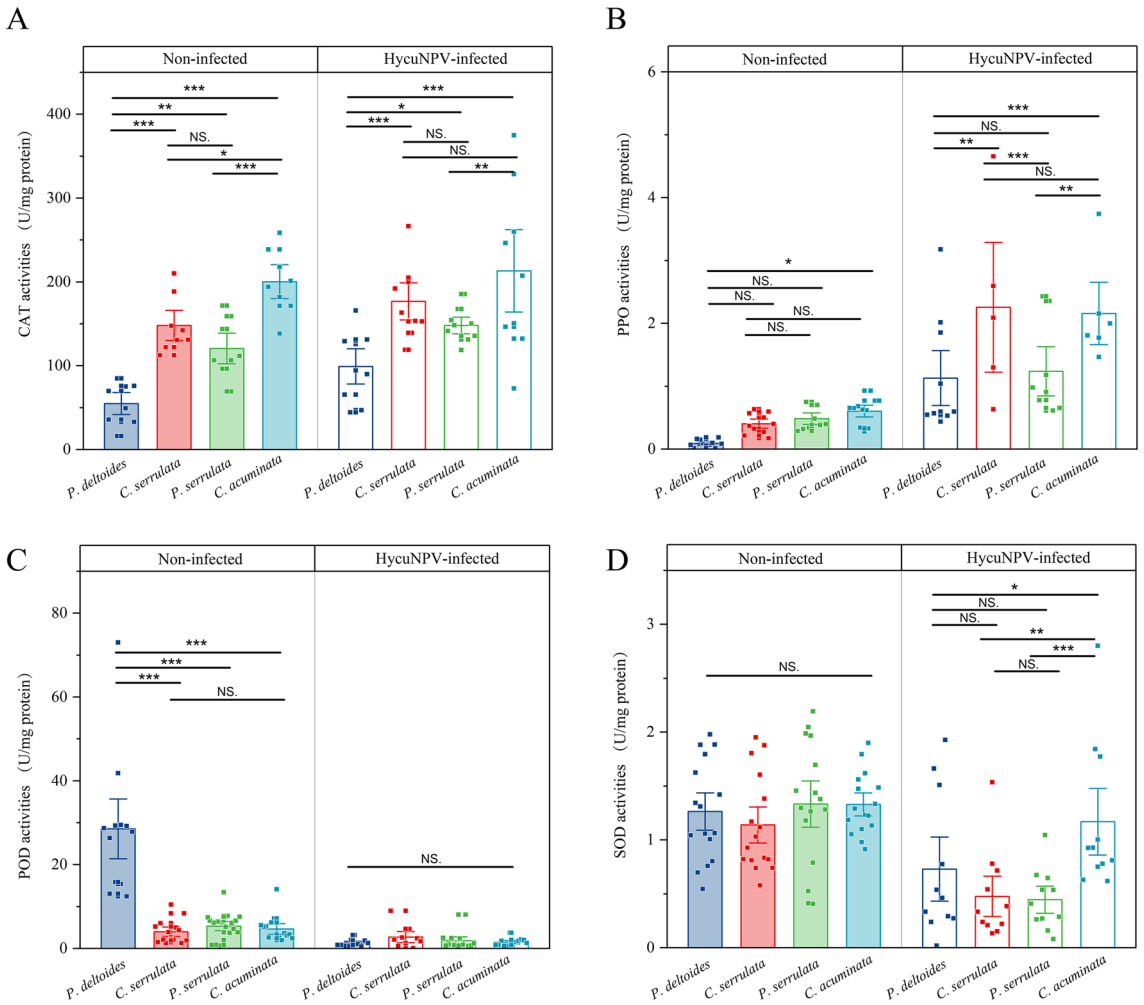


Fig. 3 Enzyme responses of *H. cunea* larvae infected with HycuNPV on different host plants. **A** CAT activity; **B** PPO activity; **C** POD activity; **D** SOD activity. The asterisks represent significant differences between HycuNPV-challenged larvae and non-challenged larvae by GLM. *$P < 0.05$, **$P < 0.001$, NS, not significant

on the infectivity of *S. exigua* nucleopolyhedrovirus (SeNPV) to larvae were measured, and the viral infectivity to beet armyworm larvae was the highest on *Glycine max* and lowest on *Ipomoea aquatica* compared to that on any other tested plant species [62]. The *Anagrapha falcifera* nucleopolyhedrovirus (AfNPV) was studied by Farrar and Ridgway [63] to determine how plant species affected the infectivity of beet armyworm on three different plant species, which found that tomato had significantly higher larval morality. In contrast, larvae fed on collard and cotton exhibited significantly shorter mean times to death. In practice, this difference in infectivity can guide agricultural operations and virus doses can be used rationally based on differences in virus infectivity on different crops [64].

Variations in insect herbivores food ingestion caused by differences in host plant quality can also affect the susceptibility of phytophagous insects to entomopathogens [65]. In this study, the caterpillars could also improve their tolerance to the entomopathogens by increasing the amount of foraging. The survival rate and time, the larvae dry wet gain, and the feeding amount of *H. cunea* were significantly higher when the larvae was fed on poplar leaves compared to other host plants. Pearson correlation analysis showed that dry weight gain (DWG) was significantly and positively correlated with survival rate and time. Larvae gained more body size through increased feeding, which suggested a higher tolerance to pathogens [66]. Similarly, later instars of *S. exigua* were more tolerant to NPV than early instars when fed on an artificial diet [67]. In *Daphnia magna*, larger Daphnia were less likely to be infected, and there was a tendency for fast-feeding Daphnia was less likely to become infected [66].

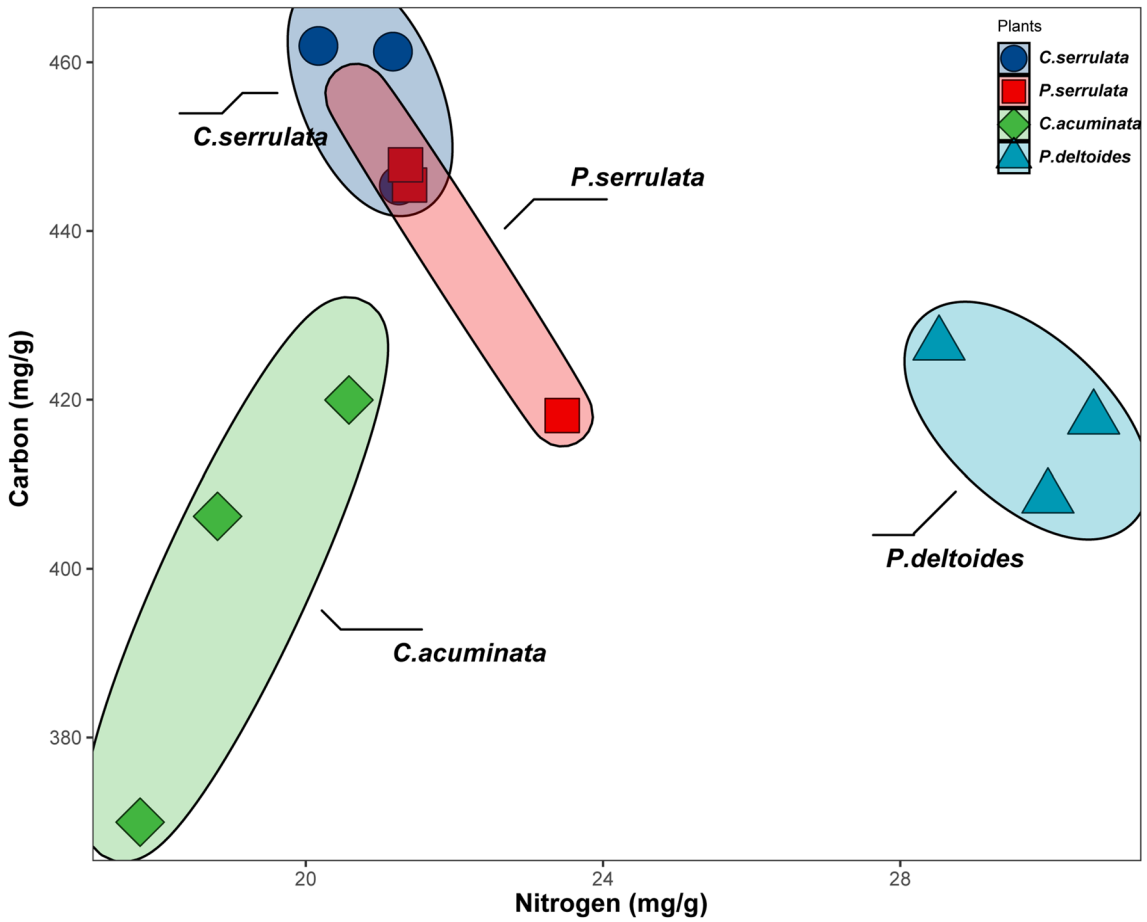


Fig. 4 Carbon and nitrogen content of different host plants

Oxidative stress is a state of imbalance between ROS production and the biological system's capacity to detoxify ROS or restore the damage they directly cause. When the normal redox state of a physical system is disrupted (e.g., by a pathogen), peroxides and free radicals can damage proteins, lipids, and DNA, impairing the function of various intercellular mechanisms/organelles [68]. The antioxidant enzymes (CAT, SOD, and POD) are known as cellular protective systems. SOD is recognized to increase the production of H_2O_2 from O_2 through dismutation, while CAT and POD could catalyze the production of H_2O from H_2O_2. Through reactive oxygen species, these reactions can reduce the damage to the biomembranes [69]. In general, CAT enzymes offer a fundamental protective mechanism against H_2O_2 toxicity in herbivores. In this study, HycuNPV infection significantly triggers the CAT enzyme level of *H. cunea* larvae. Similarly, in *Spodoptera litura*, the pathogens infection prominently enhanced the CAT enzyme level of larvae [70]. Antioxidant activities can significantly raise mortality by reducing oxidative stress, damaging midgut cells, and leading to more frequent sloughing of infected gut cells [14]. In addition, host plant species could also influence the antioxidant enzymes of caterpillars. In this study, uninfected larvae fed on poplar leaves showed the highest POD and the lowest CAT activities. Meanwhile, the HycuNPV-infected larvae dramatically decreased the POD activity. The POD inhibits CAT activity because POD uses H_2O_2 as a co-enzyme, so breaking down H_2O_2 with POD may reduce the activity of CAT. The higher POD activity in uninfected larvae that have fed on poplar leaves may be a result of the POD in *H. cunea* reacting to the phytochemicals in the poplar leaves to flush out excessive reactive oxygen species, and the raised POD activity in caterpillars could have provided a shield against the entomopathogenic viruses. Consequently, infected larvae feeding on poplar leaves exhibited higher survival rates and longer survival times. Furthermore, recent research has documented that activating melanogenesis with PO is an important tool against several pathogens [24].

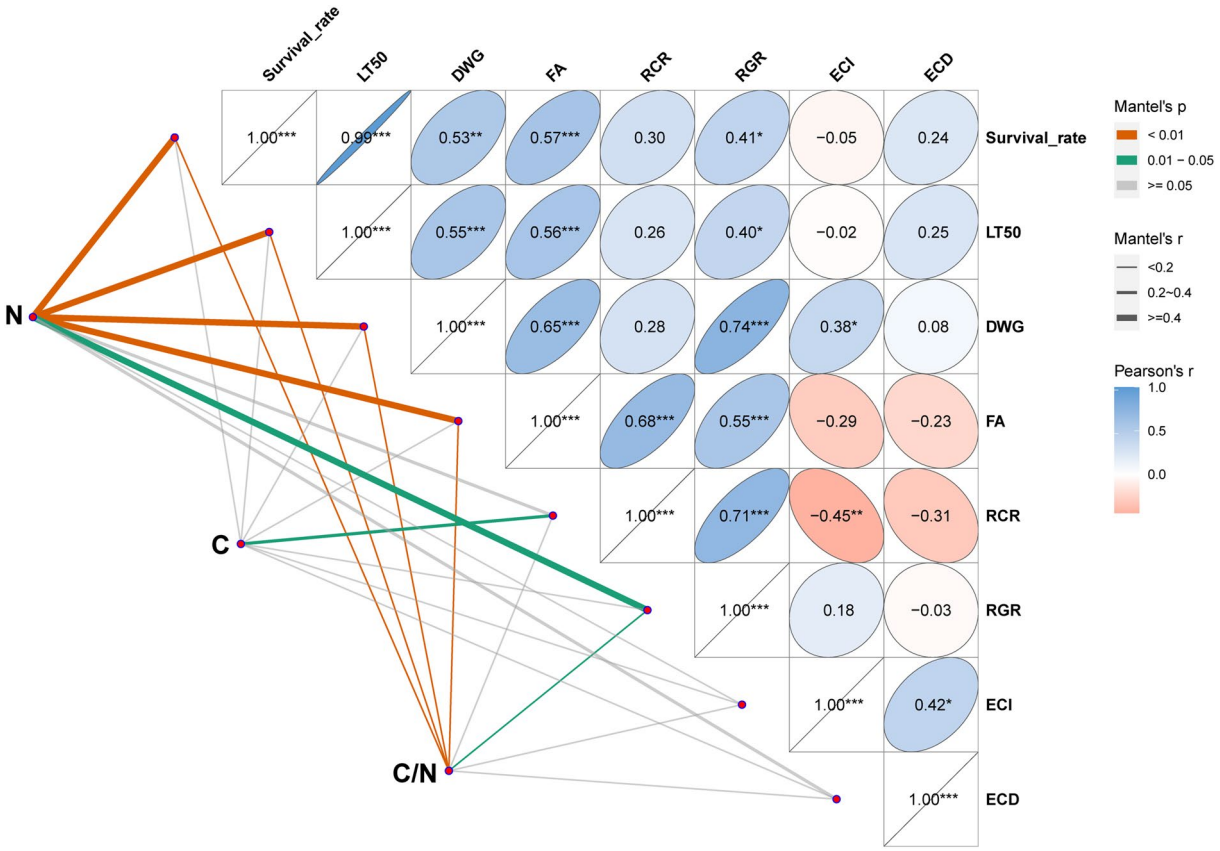


Fig. 5 Correlation analysis of host plant nutrient components with biological parameters of HycuNPV-infected larvae. In the matrix of biological parameters of *H. cunea* larvae, blue ovals represent positive correlations, red ovals represent negative correlations, and asterisks represent significant correlations between different biological parameters by Pearson's correlation analysis ($P < 0.05$). The thicker the line represents the correlation between the plant nutrient components and biological parameters of HycuNPV-infected larvae, and the thicker the line the higher the correlation. Where the green (P: 0.01–0.05) and red ($P < 0.01$) line represent significant correlation, while the gray line indicates that the correlation is not significant ($P > 0.05$) by Mantel test

Enzymes protect cells from pathogens such as bacteria, fungi, and even viruses by encapsulating, repairing, and destroying them [71]. The PPO enzyme activity of the infected *H. cunea* significantly increased in the other three host plants, except for feeding on poplar leaves. Fanny Vogelweith et al. [72] reported that larvae of an inbred strain of the European grape berry moth *Eupoecilia ambiguella* were reared on five artificial diets, each based on a different grape variety changed in activities of the PPO. Similarly, Wang et al. [10] considered that host plant quality could affect immune defenses and potentially tolerance to disease. Relating the results to the host quality of this study, there was a significant difference in the carbon-to-nitrogen ratio in poplar leaves from the other host plants. Whether nitrogen is the fundamental substance that enhances host immunity and defense against entomopathogenic viruses infection or not, it deserves further study.

Nutrition is of fundamental importance to all animals, including insect herbivores [73], but has been neglected as a factor contributing to variation in insect herbivores susceptibility to pathogens [74]. Plants are a major food resource for phytophagous insects, where the plants nutrients have been demonstrated to be temporally and spatially variable, indicating that insect herbivores forage in a highly heterogeneous nutritional landscape [75, 76]. Carbon (C) and nitrogen (N) are required for plants to carry out normal and essential cellular processes [77]. C compounds contain various carbohydrates, including sucrose (Suc) and glucose (Glc). These photosynthetic products supply the energy and C-skeletons required for plant ammonium uptake during amino acid biosynthesis. Moreover, nitrogen is an essential component of plant proteins and nucleic acids [77]. C and N nutrients are essential for various cellular functions. Therefore, an adequate supply of these two nutrients is critical for plant

growth and development [78]. Insect herbivores forage in a highly varied nutritional landscape due to plant nutrient content's geographical and temporal variability, especially protein and carbohydrates [50, 79]. There is also substantial evidence that carbon-to-nitrogen ratios in insect herbivore diets significantly impact their performance, including growth rate, reproductive capabilities, tolerance to toxins [80, 81], and immunity [15, 82]. In this study, the carbon content, nitrogen content, and carbon-to-nitrogen ratio varied significantly among the host plants, and higher nitrogen content in host plants was significantly and positively correlated with larval survival rate, LT50, and DWG of *H. cunea* larvae. On the contrary, the C/N ratio was negatively correlated with the survival rate, LT50, and DWG of larvae. It is assumed that insects that consume different dietary sources might have different nitrogen sinks, impacting growth, maturation, and tolerance to pathogens[65]. Caterpillars are generally less mobile and contain significantly fewer contact and taste receptors [73, 83]. Previous studies have revealed that caterpillars tend to self-select so that protein ingestion is equal to or greater than carbohydrate ingestion, and more protein intake facilitates tolerance to pathogens [73]. In *Spodoptera littoralis*, the post-ingestive utilization of nitrogen was increased for larvae on the high-quality protein diet, and larvae given a high-quality protein diet had higher survival and faster growth rates than larvae on the low-quality protein diet [65]. During experiments on caterpillars with SlNPV, it was observed that infected caterpillars with protein-rich diets had a greater survival rate. Similarly, the most significant levels of antibacterial activity, encapsulation response, and a lesser degree of phenoloxidase activity were observed in caterpillars fed on diets with high protein-carbohydrate ratio. In addition, when the caterpillars were allowed to choose their diets, those that survived the NPV challenge were seen to have a higher relative protein consumption than the control group and those larvae that died from infection [15]. Similarly to NPV infection, both C and N in diet have a predictably significant impact on the survival and performance of caterpillars challenged with Bt toxins and facilitate plasticity in sensitivity to Cry1Ac, which could account for substantial changes in LC_{50} values [74]. In *Helicoverpa zea*, larvae were more resistant to Cry1Ac when reared on a diet with a higher protein: carbohydrate ratio, as indicated by a markedly higher survival rate and LC_{50} values when exposed to lethal dosages of Cry1Ac [84]. In *T. ni*, the LC_{50} of resistant strains, but not susceptible strains, decreased when fed diets with excess protein [85].

In conclusion, this study has demonstrated that the host plant species impacted the infectivity of the entomopathogen towards polyphagous caterpillars. Furthermore, the plants nutrients were an essential factor in determining the caterpillar's susceptibility to nucleopolyhedrovirus. Moreover, the plants could mediate the tolerance of caterpillars against entomopathogenic viruses by shaping the feeding behaviors and antioxidant enzyme responses. Plants nutrients mediated effects could contribute to the theoretical understanding of the regulatory mechanisms of host plants interactions between phytophagous insects and entomopathogens. It provides a scientific foundation for underscoring the importance of considering dietary influences on pathogen susceptibility in pest management strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00535-7.

Additional file 1: Table S1. Pairwise comparison of survival rate of *H. cunea* after HycuNPV-infection on different host plants. **Table S2.** Carbon, nitrogen content and carbon to nitrogen ratio of different host plants. **Table S3.** Correlation analysis between biological parameters of *H. cunea* after HycuNPV-infection and nutrient components of host plants.

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Author contributions
TH: data curation. XZ and YG: formal analysis. YL and WX: investigation. HQ and YG: project administration. XZ: writing—original draft. DH: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors consent for publication.

Competing interests
The authors declare that the research has no competing interests.

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