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Biological and Physiological Changes in *Spodoptera frugiperda* Larvae Induced by Non-Consumptive Effects of the Predator *Harmonia axyridis*

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Abstract: The effects of predatory natural enemies on their prey or hosts involve both consumption and non-consumptive effects. This study investigated the non-consumptive effects of the predator, *Harmonia axyridis* (Coleoptera: Coccinellidae) on 1st, 2nd and 3rd instar larvae of *Spodoptera frugiperda*. We exposed larvae of different instars to the predator and assessed various parameters using a combination of biological and biochemical methods. Exposure to the predator significantly affected the growth and development of the *S. frugiperda* caterpillars. Firstly, the developmental duration of *S. frugiperda* larvae in the 1st–3rd instars and the pupal stage were notably prolonged. Moreover, we observed significant effects on pupal mass, pupal abnormality rate and emergence rate. These non-consumptive effects were gradually weakened with an increase in the larval stage exposed to the predator. Antioxidant enzyme activities including catalase (CAT) peroxidase (POD) and superoxide dismutase (SOD) activity increased significantly. Additionally, organismal triglyceride, trehalose and glycogen content were significantly altered by non-consumptive effects, while protein content showed no significant change. *Spodoptera frugiperda* larvae increased the activity of antioxidant enzymes in response to potential predators to mitigate oxidative stress and reduce cellular and tissue damage. This resources redistribution towards survival may inhibit growth and development of the species and further exacerbate these non-consumptive effects. These findings highlight the importance of considering non-consumptive effects in pest-management strategies to optimize control measures in agricultural systems.

Keywords: biological control; *Harmonia axyridis*; non-consumptive effect; *Spodoptera frugiperda*

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1. Introduction

Classical biological control is an effective method of pest control utilizing exotic natural enemies. By employing efficient, cost-effective pest population management strategies, pest populations can be consistently suppressed, reducing reliance on conventional chemical insecticides, mitigating resistance and ecological risks, and minimizing economic losses [1]. Since the 19th century, numerous classical biological control programs have been successful. For instance, the effective management of the California red scale (*Aonidiella aurantii*), a major citrus pest, is largely credited to specific parasitoids of the *Aphytis* genus [2]. Natural enemies are essential in regulating pest population dynamics and abundance [3]. The interactions encompass both the consumptive effect (CE) of direct feeding and non-consumptive effects (NCEs) induced by predation risk [4].

Although non-consumptive effects do not result in direct mortality, they increase the complexity of prey-predator interactions and can alter prey's ecological habits [5,6],

stress levels [7], feeding habits [8], spawning behaviors [9,10], physiology [11], host preferences [12] and dispersal patterns [13]. Such indirect effects may reduce prey fitness and abundance. For instance, *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) populations decrease under non-consumptive effects [14], and *Ischnura rufostigma* Selys (Odonata: Coenagrionidae) and *Manduca sexta* L. (Lepidoptera: Sphingidae) show altered growth and metabolic responses, respectively [15]. Non-consumptive effects could contribute to pest management with a comparatively modest number of individual predators, keeping pest populations below economically damaging thresholds [16,17]. Despite these insights, the impact of non-consumptive effects on prey development, survival and physiology remains underexplored. This study aims to address this gap by investigating the non-consumptive effects of multicolored Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). While previous research has examined non-consumptive effects on various prey species, our study is unique in its comprehensive approach to evaluating both biological and biochemical changes in *S. frugiperda* larvae. Unlike prior studies that often focus on a single aspect of prey response, our research integrates assessments of growth, development, antioxidant enzyme activities and nutrient changes.

Spodoptera frugiperda is an agriculturally significant pest native to the Americas, and this pest has a broad host range and causes severe economic losses worldwide [18]. The larvae are voracious feeders, with later larvae (4–6 instars) capable of consuming entire plant leaves or attacking the ears and cobs of maize. These later instars larvae (4–6 instars) can significantly impact maize yield and even lead to crop failure, meanwhile adults are characterized by rapid migration and invasive ability, and they have already invaded dozens of countries in Asia, Africa and Europe in a short period of time [19,20]. Effective management of *S. frugiperda* is challenging because it inhabits the whorl and ear of maize plants, which is difficult to reach with chemical insecticides [18]. However, natural enemies of *S. frugiperda* are abundant. The use of natural enemies such as *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae), *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), *Arma chinensis* (Fallou) (Hemiptera: Pentatomidae) and *Picromerus lewisi* Scott (Hemiptera: Pentatomidae) for control has been reported [21–24]. *Harmonia axyridis* is widely recognized as an effective predator of aphids, mites and scale insects [25]. Additionally, it has demonstrated potential as a natural enemy against Lepidoptera, including species such as *S. frugiperda* [26,27].

Non-consumptive effects of natural enemies on prey may play an important role in the ecological regulation of pest populations [28]. This study aims to evaluate non-consumptive effects of *H. axyridis* on 1st–3rd instar larvae of *S. frugiperda*. Specifically, we will investigate its impact on the growth, development, antioxidant enzyme activities and nutrient changes in *S. frugiperda* larvae. We propose the following hypotheses: (1) *Harmonia axyridis* exerts non-consumptive effects on early instar larvae (1st–3rd instar) of *S. frugiperda*, influencing their growth and development; (2) exposure to *H. axyridis* induces changes in antioxidant enzyme activities in *S. frugiperda* larvae, potentially affecting their physiological stress responses; (3) non-consumptive effects of *H. axyridis* alter nutrient availability or utilization in *S. frugiperda* larvae, impacting their fitness and development. By examining these interactions, we aim to enhance our understanding of natural enemies in pest management and improve integrated pest management strategies.

2. Materials and Methods

2.1. Plants and Insects

Spodoptera frugiperda were reared on maize plants in our study. We planted maize seeds (Zhongnong Sweet Maize 488) in plastic pots (28 cm in diameter, 20 cm in height) filled with a soil-sand mixture (10% sand, 5% clay and 85% peat). The plants were cultivated in a greenhouse free from arthropod infestations and maintained under natural temperature and light conditions. They were regularly watered and used for the experiment once they developed 6–8 fully expanded leaves.

Spodoptera frugiperda adult individuals were collected in May 2022 from a maize field in Huadu District (113.16° E, 23.35° N), Guangzhou City, Guangdong Province, China. The specimens were then reared on maize seedlings for over three generations in the laboratory of the Engineering Research Center of Biological Control, Ministry of Education, South China Agricultural University (SCAU), Guangzhou, China. The insect rearing laboratory was maintained with a temperature of 25 ± 3 °C, relative humidity between 60% and 90% and a photoperiod of 14 h:10 h (L:D).

The predator, *H. Axyridis* adult individuals, were collected in 2018 from lettuce in the farms of South China Agricultural University (SCAU). They were reared under laboratory conditions (temperature of 25 ± 3 °C, relative humidity between 60% and 90% and a photoperiod of 14 h:10 h (L:D)) using pea aphids *Acyrthosiphon pisum* as food to maintain the colony. *Harmonia axyridis* were fed with *S. frugiperda* larvae for three generations prior to the beginning of the experiment.

2.2. Experimental Setup

In this study, a double-layer cage (35 mm diameter, 30 mm height and the two parts of the cage had the same height (i.e., 15 mm)) modified from a single-layer leaf cage was used (Figure 1). A thin nylon net divided the leaf cage into two parts (upper and lower). This nylon layer was designed to avoid direct contact between natural enemies and prey but allowing chemical and visual signals to pass through. *Spodoptera frugiperda* larvae were introduced into the lower layers covering young maize leaves (the second and third leaves) on which they could feed (fresh maize leaves were cut into small pieces in the leaf cage). Young (3–5 days old) adult *H. axyridis* were introduced into the upper layer of the leaf cage.

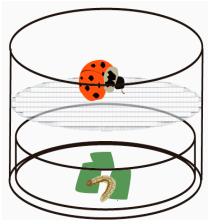

Figure 1. Experimental apparatus for testing the non-consumptive effects of *Harmonia axyridis* on *Spodoptera frugiperda*.

2.3. Non-Consumptive Effects of Predator Presence on the Biology of S. frugiperda

The presence of *H. axyridis* may influence the growth and development of *S. frugiperda*. In addition, the age at the time of exposure may also influence the interaction. Therefore, we tested different *S. frugiperda* early instars as treatments. Initially, adult *H. axyridis* within 3 days of eclosion were selected and starved for 24 h. Subsequently, one 1st instar (1 day old), one 2nd instar (4 days old) and one 3rd instar (6 days old) of *S. frugiperda* larvae were selected and each was individually transferred to lower leaf cages containing young and tender maize leaf. Then, one *H. axyridis* individual was introduced to the upper layer of the leaf cage for the threat treatment. Each larva was exposed to a single predator (to avoid cannibalism). On average, larvae were exposed to the predation stress for 2–3 days, or until progressing to the next larval stage (confirmed using the cephalic capsule width [29]. And each larva was individually transferred to a new petri dish (35 mm diameter) (to avoid cannibalism) and reared on young maize leaves until adulthood. During the experiment, pieces of maize leaves were added every 3 h to prevent any shortage of food. Development and survival of *S. frugiperda* larvae were observed every 24 h. Larvae without the predator (*H. axyridis*) threat were used as the control, and 20 fall armyworms constituted a replicate, with three replicates per experiment.

2.4. Antioxidant Enzyme Activities of S. frugiperda

To perform the enzyme assays, we randomly collected and weighed 1st, 2nd and 3rd instar larvae individually after exposure to stress (1st instar: 30 larvae, 2nd instar: 25 larvae,

and 3rd instar: 20 larvae). Larvae without the predator (*H. axyridis*) threat were used as the control. Each treatment or control trial was conducted with three replicates. We then froze them in liquid nitrogen, ground them into a fine powder and homogenized in PBS (pH 7.4). They were centrifuged at 4 °C at 10,000 rpm/min for 10 min and stored at −80 °C [30]. The concentration of hydrogen peroxide (H_2O_2), the activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) in *S. frugiperda* larvae in response to the threat of *H. axyridis* were determined according to the manufacturer's instructions on the assay kits provided by Grace Biotechnology (Suzhou, China). Absorbance was read on a microplate spectrophotometer (XMarkTM, BIO-RAD, Hercules, CA, USA).

H_2O_2 concentration was determined by reacting H_2O_2 with titanium salts to produce a yellow precipitate of a peroxide-titanium complex, which can be dissolved by concentrated sulfuric acid. The maximum absorption peak occurs at a wavelength of 415 nm. The depth of color is linearly related to the concentration of H_2O_2.

CAT catalyzes the decomposition of hydrogen peroxide into water and oxygen. The remaining hydrogen peroxide reacts with a novel chromogenic probe that has a maximum absorption peak at 510 nm [31]. The activity of CAT in the sample is calculated based on the decrease in hydrogen peroxide.

POD activity was determined by catalyzing the oxidation of substrate in the presence of H_2O_2 [32]. In the presence of peroxidase, H_2O_2 oxidizes a specific substrate. The resulting reddish-brown product exhibits maximum light absorption at 470 nm, allowing the determination of peroxidase activity by measuring changes in absorbance at 470 nm.

SOD activity assessed using the WST-8 method, where WST-8 reacts with the superoxide anion (O^{2-}) catalyzed by Xanthine oxidase (XO) to produce water-soluble Formazan dye. This dye exhibits maximum absorption at 450 nm.

2.5. Determination of Nutrients in Larvae after Stress

For nutrient measurements, we first collected and weighed *S. frugiperda* 1st, 2nd and 3rd instar larvae from all treatments and the control (larvae not exposed to predators) (1st: 30 larvae, 2nd: 25 larvae and 3rd: 20 larvae). Larvae without the predator (*H. axyridis*) threat were used as the control. Each treatment or control trial was conducted with three replicates. We then froze them in liquid nitrogen, ground them into a fine powder and homogenized in PBS (PH 7.4). They were centrifuged at 4 °C at 10,000 rpm/min for 10 min and stored at −80 °C. Protein, triglyceride, trehalose and glycogen extraction and measurement followed kits from Grace Biotechnology (Suzhou, China).

Glycogen content was determined by Anthrone-H_2SO_4 colorimetry. Glycogen undergoes dehydration in concentrated sulfuric acid to form glycoaldehyde derivatives. These derivatives subsequently react with anthrone to produce blue-colored compounds. The colorimetric intensity of these compounds are quantitatively compared with a standard glucose solution treated under the same conditions. Glyceraldehyde interacts with anthrone and has a maximum absorption peak at 620 nm.

Protein can reduce Cu^{2+} to Cu^{+} and the Cu^{+} ion can chelate with BCA reagent to form a violet-blue complex. There is a maximum light absorbance value at 562 nm, and the intensity of color is proportional to the protein content, so the concentration of protein can be determined based on the absorbance value.

Trehalose content is determined by using the anthrone-sulfuric acid method. In this method, carbohydrates are dehydrated by concentrated sulfuric acid at elevated temperatures to form furfural or hydroxymethylfurfural. These compounds then react with anthrone to produce a blue-green derivative, which exhibits maximum absorption at 620 nm. The depth of its color is proportional to the sugar content.

Triglyceride is hydrolyzed by lipoprotein lipase to glycerol and free fatty acids, which ultimately oxidize to form red quinone compounds. Triglyceride has a characteristic absorption peak at 510 nm, and the absorbance value at 510 nm can be used to determine the triglyceride content.

2.6. Data Analysis

Statistical analyses were conducted using SAS software (v.8.01). Prior to performing parametric tests, the Shapiro–Wilks test was employed to assess the normality of data distributions, which is a prerequisite for many parametric tests. Levene's test was used to check for homogeneity of variances across groups, which is crucial for the reliability of ANOVA results. Data on pupation rate and pupal abnormality were square-root transformed to stabilize variance and improve the normality of residuals. Data on emergence rate were arcsine transformed to address variance heterogeneity and meet the assumptions of ANOVA. The developmental duration of larvae, pupation rate, developmental duration of pupa, abnormality rate, pupal mass and emergence rate of *S. frugiperda* were analyzed by one-way ANOVA, and means were differentiated by Duncan's test. Differences in antioxidant enzyme activities and nutrients content of *S. frugiperda* among different treatments were compared using *t*-test. Data are represented as mean ± standard error (SE). Statistical significance was set at $p < 0.05$. Significant differences between groups are indicated by different letters. Graphs were generated using Graphpad Prism 5 (Graphpad, La Jolla, CA, USA) to visually summarize the data and aid in the interpretation of results.

3. Results

3.1. Non-Consumptive Effects of H. axyridis on the Biology of S. frugiperda

The developmental duration of larvae of *S. frugiperda* 1st–3rd instar treatments were longer than those of the control. Non-consumptive effects had a significantly negative impact on the developmental duration when exposed in the 1st and 2nd instars larvae ($F_{(3,8)} = 6.68$, $p = 0.0143$) (Figure 2A). Moreover, there was a remarkable effect on pupal developmental duration ($F_{(3,8)} = 6.17$, $p = 0.0178$), and 1st instar treatment was evidently prolonged (Figure 2B). When the 1st instar was kept in the presence of the predator, they had significantly increased pupal abnormalities ($F_{(3,8)} = 4.79$, $p = 0.034$) (Figure 2C). The pupal mass was obviously decreased when exposed in the 1st and 2nd instars' larvae ($F_{(3,8)} = 46.36$, $p < 0.0001$) (Figure 2D); meanwhile emergence rate of pupa decreased by non-consumption effect when larvae were exposed to the predator in the 1st instar ($F_{(3,8)} = 5.69$, $p = 0.022$) (Figure 2E), but there were no obvious changes when 2nd and 3rd instars were exposed to the predator.

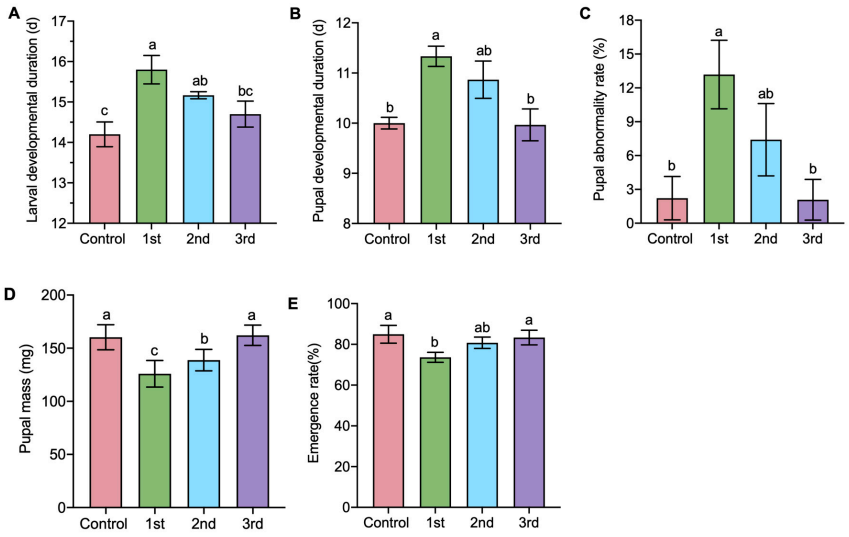

Figure 2. Non-consumptive effects of *H. axyridis* on the growth and development of *S. frugiperda*. (**A**) Larval developmental duration, (**B**) pupal developmental duration, (**C**) pupal abnormality rate, (**D**) pupal mass, (**E**) emergence rate. Data are means ± SE and different letters above the bars indicated significant difference (Ducan's test, $p < 0.05$). Control group without non-consumption effect, 1st: exposed to the predator at the first instar, 2nd: exposed to the predator at the second instar, 3rd: exposed to the predator at the third instar. (d), days.

3.2. Non-Consumptive Effects of *S. frugiperda* on Antioxidant Enzyme Activities

Exposure to *H. axyridis* markedly influenced H_2O_2 concentration and antioxidant enzymes (CAT, SOD and POD) activities in *S. frugiperda*. When exposed in the 1st and 2rd *S. frugiperda*, H_2O_2 concentration increased 34.39% ($t_4 = -5.91$, $p = 0.0041$), 22.45% ($t_4 = -6.51$, $p = 0.0029$), respectively, whereas no significant differences were observed in the 3rd instar ($t_4 = -1.56$, $p = 0.19$) (Figure 3A). CAT activity significantly increased by 38.33% ($t_4 = -2.92$, $p = 0.043$) when exposed in the 1st instar; however, the 2rd ($t_4 = -2.3$, $p = 0.083$) and 3rd ($t_4 = -1.64$, $p = 0.18$) instars did not show significant differences (Figure 3B). POD activity exhibited marked variation, with significant increases of 55.22% ($t_4 = -5.4$, $p = 0.0057$), 27.38% ($t_4 = -4.46$, $p = 0.011$) and 21.31% ($t_4 = -3.06$, $p = 0.038$) in the 1st, 2rd and 3rd instar, respectively (Figure 3C). SOD activity in prey significantly increased by 19.94% ($t_4 = -3.47$, $p = 0.026$) in the 1st instar, and by 33.67% ($t_4 = -4.37$, $p = 0.012$) in the 2nd instars, but not in the 3rd instar ($t_4 = -2.23$, $p = 0.09$) (Figure 3D). The presence of *H. axyridis* induced a stress response of several antioxidant enzymes in *S. frugiperda* larvae that depended on the instar exposed to the predator.

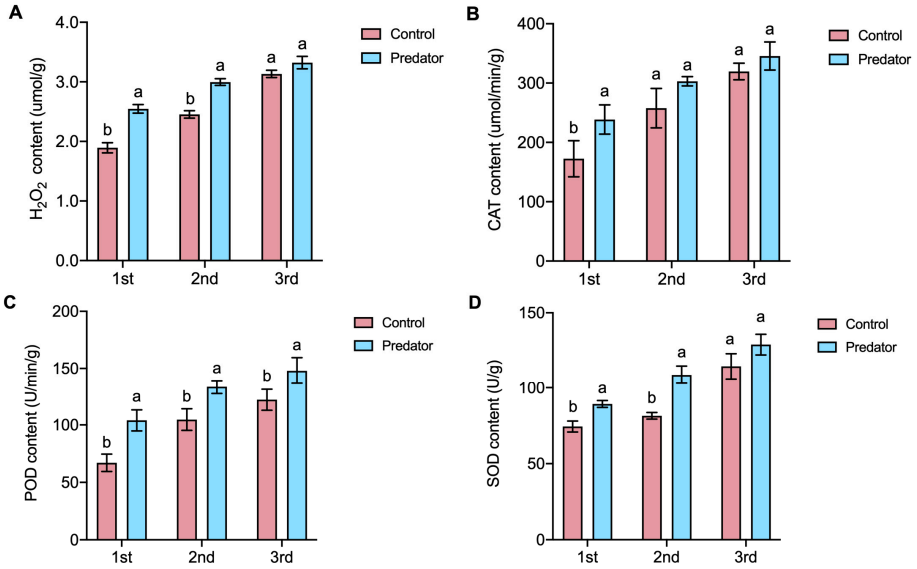

Figure 3. Non-consumptive effects of *H. axyridis* on antioxidant enzyme activities of the *S. frugiperda*. (**A**) H_2O_2, (**B**) CAT, (**C**) POD, (**D**) SOD. Data were mean ± SE and different letters above the bars indicated significant differences for pairwise comparisons within each instar (*t*-test, $p < 0.05$). Control group without exposure to the predator, 1st: exposed to predator at the first instar, 2nd: exposed to predator at the second instar, 3rd: exposed to predator at the third instar.

3.3. Non-Consumptive Effects on Nutrients in *S. frugiperda* Larvae

After exposure to non-consumptive effects, glycogen content in prey was significantly reduced by 29.51% ($t_4 = 3.58$, $p = 0.023$) and 12.5% ($t_4 = 3.61$, $p = 0.032$) when exposed in the 1st instar and 2nd, respectively, yet the 3rd instar did not result in significant reductions ($t_4 = 0.51$, $p = 0.64$) (Figure 4A). Protein content was not affected significantly in any instar (Figure 4B). Trehalose content was significantly reduced by 21.56% ($t_4 = 3.01$, $p = 0.04$) when exposed in the 1st instar; however, the 2nd ($t_4 = 2.5$, $p = 0.067$) and 3rd ($t_4 = 2.28$, $p = 0.085$) instars did not result in significant differences (Figure 4C). Triglyceride content in prey significantly increased by 47.25% ($t_4 = -5.05$, $p = 0.0072$) in the 1st instar and by 17.24% ($t_4 = -3.36$, $p = 0.0284$) in the 2nd instar, but not in the 3rd instar ($t_4 = -0.73$, $p = 0.51$) (Figure 4D). These findings indicate that non-consumptive effects influenced some aspects of nutrient metabolism in *S. frugiperda* larvae.

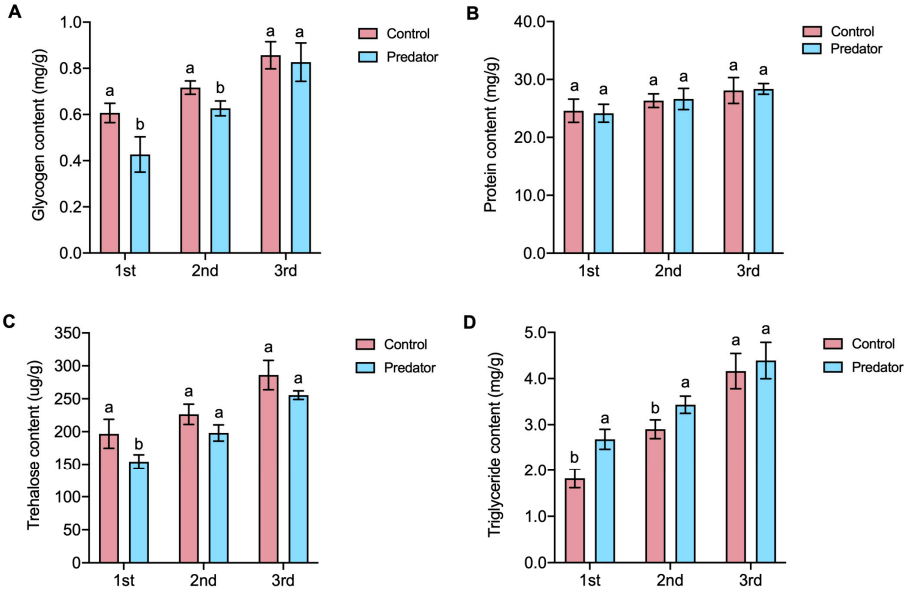

Figure 4. Non-consumptive effects of *H. axyridis* on nutrients in the *S. frugiperda*. (**A**) glycogen, (**B**) protein, (**C**) trehalose, (**D**) triglyceride. Data were mean ± SE and different letters above the bars indicated significant differences for pairwise comparisons within each instar (*t*-test, $p < 0.05$). Control group without exposure to the predator, 1st: exposed to the predator at the first instar stage, 2nd: exposed to the predator at the second instar stage, 3rd: exposed to the predator at the third instar stage.

4. Discussion

As biological control research progresses and more findings are validated, predators control prey populations not only through direct consumption but also through non-consumptive effects that significantly influence prey growth and development [33,34]. Non-consumptive effects are also recognized as an equally important component of pest biological management strategies [35]. In thrips exposed to predators, the presence of predator eggs alone can increase prey mortality and reduce the number and quality of eggs laid [35]. Dragonfly nymphs would reduce foraging when stressed by the natural enemy, leading to lower nutrient intake, slower developmental rate, smaller insect size and decreased fecundity [33]. Aphids [36] and whiteflies [37] adjusted their ecological strategies, such as reducing foraging and slowing down metabolic rate, to reduce the risk of predation when faced with natural enemy stress, but it also affects their developmental rate and fecundity. These are all ecological trade-offs between reproduction or development, when facing natural enemy coercion [34,38]. Our study contributes to this body of knowledge by demonstrating that non-consumptive effects significantly affected the biology of *S. frugiperda* larvae, with stronger effects observed in younger stages.

The antioxidant enzyme system includes catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD), which synergize to accomplish intracellular antioxidant effects [39,40]. For example, Slos et al. found that CAT and SOD were upregulated in *Lestes viridis* larvae under predator pressure [39]. Reactive oxygen species (ROS) are usually increased when insects are stressed [40]. Increased levels of ROS can damage the cellular structure and muscle tissue, and can also impair the mobility and cardiac function of the organism [39]. Higher levels of SOD and CAT and lower levels of POD in *Bemisia tabaci* nymphs exposed to non-consumptive effects suggests that mediation of the negative impacts caused by ROS may be advantageous [37]. Accordingly, increased SOD and CAT activity, which buffer the negative effects of ROS, redirects more energy to the muscles to increase the body's maneuvering performance [41,42]. Our results align with these studies that *S. frugiperda* larvae showed an increased H_2O_2 concentration under non-consumptive effects, which indicated oxidative damage; CAT, POD and SOD activities also increased. When *S. frugiperda* larva were at risk of

predation, the coping strategy adopted induced mitigation of damage caused by intracellular generation of ROS to individuals by controlling the level of antioxidant enzymes.

In addition to biological changes, physiological responses to non-consumptive effects include shifts in nutrient allocation. In situations where the prey perceives a potential threat, sugars in the prey's body were converted to fats to increase their resilience [37,38]. This is corroborated by observations in hornworm, Colorado beetle and cabbage looper moths, which increase lipid content while reducing food intake when exposed to predators [43]. Similarly, Anisoptera larvae at risk of predation reduce their food intake, resulting in slower growth but increased metabolism along with lower glucose levels [44]. Additionally, the red-legged grasshopper was found to develop a stress response with a decrease in feeding and a concomitant increase in metabolic energy expenditure under the stress of natural enemies of the hunting spider [16]. We found that *S. frugiperda* larvae converted saccharides into fat in order to mitigate the risk of predation and maintain minimum growth and developmental requirements in response to the threat of *H. axyridis*, reflecting a similar adaptive response.

The ability to detect and respond to predators is an innate behavior fundamental to survival [45]. Multiple sensory systems are involved in the sensing of predators in vertebrates and invertebrates, including olfaction, audition and vision [46–48]. For example, *Drosophila* larvae utilize olfaction to detect the presence of parasitoids *Leptopilina boulardi* and produced a defensive response [49]. *Drosophila melanogaster* adults can identify natural and non-natural enemies visually and in the presence of the parasitoid *Leptopilina boulardi* depress egg production to protect offspring from being parasitized [50]. In our study on predator detection mechanisms, *S. frugiperda* larvae detect *H. axyridis* through chemical and visual signals. Although our experimental setup did not allow us to determine the dominant signal, this highlights an important area for future research. Understanding how prey utilize different sensory systems to detect predators could lead to innovative pest management strategies. For example, integrating knowledge of predator detection cues into pest control could improve the effectiveness of biological control by leveraging these sensory responses.

Some studies have found a trade-off between prey stress and immune function under non-consumptive effects. For example, the tobacco caterpillar shows reduced reproductive behavior and longevity due to energy allocation to immunity under bat predation [51]. Janssens and Stoks found increased antioxidant defense under non-consumptive effects, slowing escape speeds [52]. In our study, *S. frugiperda* increased antioxidant enzymes activity to mitigate oxidative stress, requiring significant energy for enzyme synthesis. This led to enhanced carbohydrate consumption and fat storage for survival, potentially hindering growth and development in predator environments.

Over the past few decades, research in biological control has predominantly centered on the direct lethal impacts of natural enemies on pests, primarily via consumption, parasitism or infection. However, in recent years, there has been a growing acknowledgment that natural insect enemies can exert significant influence not only through direct consumption but also through non-consumptive effects. Non-consumptive effects can amplify the consumptive effects of predators, thereby enhancing the efficacy of biological control strategies [53]. Notably, non-consumptive effects can also be directly utilized for pest control. For example, the presence of cues from *Paederus fuscipes* has been shown to significantly reduce the longevity and fecundity of small brown planthoppers, *Laodelphax striatellus* adults, indicating their potential application in pest management [54]. Additionally, pheromones from live spined soldier bugs *Podisus maculiventris* have been observed to effectively mitigate the damage caused by the Colorado potato beetle *Leptinotarsa decemlineata* to plants [55]. In conclusion, these findings not only deepen our understanding in this field but also provide crucial insights for developing novel biological control approaches. Future research could explore the use of extracts from *H. axyridis* for the biological control of *S. frugiperda*. Overall, our study enhances the understanding of non-consumptive effects and their implications

for pest management. Future research should focus on leveraging predator detection mechanisms and exploring novel control strategies to optimize pest management.

5. Conclusions

Our study revealed significant non-consumptive effects of *H. axyridis* on *S. frugiperda* larvae. Exposure to *H. axyridis* extended developmental duration, particularly in the 1st and 2nd instar larvae, and increased pupal developmental time and abnormalities. Pupal mass and emergence rates decreased for larvae exposed during the 1st instar, with no notable effects observed in later instars under non-consumptive effects. Elevated hydrogen peroxide (H_2O_2) levels in response to predator presence in earlier instars, indicated stress, accompanied by significant changes in catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) across different larval stages. Nutrient analysis revealed reduced glycogen and trehalose in the 1st instar larvae, while the protein content remained unaffected, and triglycerides increased in the 1st and 2nd instars. These findings underscore the impact of non-consumptive effects on *S. frugiperda*, influencing its development, physiological stress response and nutrient allocation. This study suggests that non-consumptive effects can be strategically utilized to enhance biological control measures, potentially reducing reliance on chemical insecticides and mitigating pest populations in agricultural systems. However, the study has limitations, such as the focus on a single predator–prey interaction and the controlled laboratory conditions that may not fully replicate field scenarios. Future research should explore the non-consumptive effects of other natural enemies, assess these interactions under more variable environmental conditions and investigate the practical applications of these findings in diverse agricultural settings.

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