

Research Progress on the Innate Immunity of *Bactrocera dorsalis* (Hendel) and the Regulation Mechanism of Parasitic Wasp Venom Protein

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Abstract [Objectives] The use of natural enemies of living insects and their derivatives can effectively avoid the problems of pesticide residues, pest resistance, biodiversity decline, control effect weakening and so on. [Methods] Parasites inject various parasitic factors into hosts to inhibit the development of hosts, adjust the immunity of hosts, interfere with the growth and development of hosts, and reduce the nutrition metabolism of hosts, so as to ensure the growth and development of the offspring. Host pests can escape or conquer the parasitism of parasitic wasps through immune defense system in order to reproduce their own offspring. [Results] Under intense and strong selection pressure, in order to effectively ensure the success rate of parasitism, the adaptive diversity of parasitism strategies of parasitic wasps is finally caused. In the process of evolution and under the pressure of directional selection, the innate immunity and acquired immunity gradually evolve. [Conclusions] In-depth research on parasitic factors of parasitic wasps and their interaction with crop pests' immunity and development can not only improve theoretical understanding of insect immunity and development biology, pest biological control and other disciplines, but also be expected to enable the application of some components of parasitic factors to agriculture, medicine and pharmacy. *Bactrocera dorsalis* is a destructive fruit and vegetable pest. This paper summarized the venom protein of *B. dorsalis* parasitoids and the immune interaction with hosts, in order to provide theoretical basis for biological control of plant pests by using parasitic natural enemies.

Key words *Bactrocera dorsalis* (Hendel); Wasp; Immune response; Venom protein

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Parasitic wasps are an important group of insects in Hymenoptera with high diversity, as well as the main actants of biological pest control and natural control factors^[1]. Through continuous biological evolution, a stable interaction system has been formed between parasitic wasps and their hosts^[2-3], and such interaction relationship has gradually become a research focus in insect immunology and parasitology^[4-5]. In order to successfully parasitize and reproduce their offspring, parasitic wasps generally adopt specific strategies and methods to completely inhibit the development of host pests (idiobiont parasitism), or allow the hosts to continue to develop (koinobiont parasitism)^[6]. However, host pests will avoid or conquer the parasitism of parasitic wasps depending on their own immune defense systems^[7]. Under the pressure of such complex ecological selection, in order to effectively ensure the success rate of parasitism, parasitic wasps have evolved various strategies suitable for parasitism^[8], and organisms have gradually evolved innate immunity and acquired immunity in the process of evolution and under the pressure of directional selection. Insects and other invertebrates lack adaptive immune response, but in the

process of long-term resistance to pathogen invasion, they have evolved multiple innate immunity, also known as natural immunity, as well as non-specific immunity^[9]. In the study on the interaction mechanism between parasitoids and their hosts, it was found that parasitoids could not successfully parasitize without their own parasitic factors, such as polydnavirus (PDV)^[10], venom proteins^[11], virus like particles (VLPs)^[12], teratocyte^[13-15], ovarian proteins and other factors^[16]. These parasitic factors are injected into hosts' body with host eggs, and they act on hosts' immune system, and ensure the growth and development of parasitic wasp offspring by regulating the development and the ganglions and behaviors of host pests and other means. The research on parasitic factors of parasitic wasps mostly focuses on two major categories: venom and polydnavirus. The composition of venom protein is more complex than that of Hymenoptera social insects (such as *Apis mellifera* and *Solenopsis invicta*) and toxic arthropods (such as scorpions and spiders). The components of venom protein are mainly enzymes, followed by proteins and peptides rich in Cys residue domains^[17]. According to literature reports, there are significant differences in the venom protein composition of parasitic wasps among closely related species, and even within the same parasitic wasp species, there are significant differences between different geographic populations or individuals within the population. It may be related to the diversity of the "parasitic wasp-host pest" system and the long-term synergistic evolution between the two^[18-21]. *Bactrocera dorsalis* belongs to Tephritidae of Diptera,

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and is a destructive fruit and vegetable pest^[22]. The parasitic wasps of *B. dorsalis* can parasitize at different developmental stages such as eggs, larvae, and pupae, and even obey inter-stage parasitism. They have a variety of parasitic modes and are important regulatory factors in the ecosystem. In recent years, there have been many studies on indoor mass rearing and biological characteristics of *B. dorsalis* parasitoids, but few studies on their venom protein components. In this paper, the venom protein of *B. dorsalis* parasitoids and the immune interaction with hosts were summarized, in order to provide theoretical basis and support for biological control of plant pests by using parasitic natural enemies.

Innate Immune Response of *B. dorsalis*

When insects are infected by pathogens, they will first trigger the natural immune system, which recognizes pathogens through pattern recognition receptor (PRRs), and triggers the activation of serine protease (SPs) and the extracellular cascade reaction of serine protease inhibitors (SPIs), which further lead to the activation of intracellular signal pathways and induce the up-regulation of gene expression of effector molecules such as antibacterial peptide AMP, thereby clearing pathogens in the body^[23]. Like other insects, *B. dorsalis* lacks acquired immunity and only has innate immunity^[24]. The innate immune response of *B. dorsalis* can be divided into cellular innate immunity response and humoral innate immunity response, which work together to resist the invasion of foreign organisms^[25–26]. Cellular innate immunity response also includes the spreading, proliferation, differentiation, phagocytosis, encapsulation and other processes of blood cells, while humoral innate immunity response includes melanization and three major antibacterial immune pathways of Toll, Imd and Jak/Stat^[27].

Cellular innate immunity

The study of cellular innate immune response began with the first discovery of phagocytosis in starfish larvae by Russian scientist Metchnikoff in 1884^[28]. In the 1960s, scientists found circulating blood cells with phagocytic ability in the body of *Drosophila melanogaster*, and explored their role in defense against microorganisms^[29]. The blood cells in the hemolymph of insects are the main part of the immune system, which mainly comes from the stem cells of the mesoblast. These blood cells float in the blood cavity, or attach to various tissues and organs. Blood cells participate in a variety of physiological functions, playing a vital role in insect metabolism, metamorphosis and innate immunity. They have phagocytosis and immunity, and can protect the insect body from the harm of parasites^[30]. Among them, prohemocytes, granulocytes, plasmatocytes, oenocytoids, lipohemocytes and spherulocytes^[31] are the main types of hemocytes involved in cellular immune response. In the process of limiting the spread of external pathogens and eliminating invading parasites, the immune functions of insect blood cells mainly include: phagocytosis, nodulation and encapsulation^[32]. When insects are invaded by exogenous substances such as pathogenic fungi, the blood cells that play

phagocytosis engulf or synthesize antimicrobial peptides to eliminate them, which will also be accompanied by melanization reaction. When the pathogens are larger, such as protozoa, nematodes and parasites, and the cells cannot engulf them, they must be cleared by forming nodules and cysts. Therefore, a significant phenotype of insect phagocytosis and cyst reaction is melanization, and pigments mainly appear on the surfaces of hosts' stratum corneum, or on the surfaces of organisms invading the blood cavity or near them. The two immune responses are closely related and collaborate with each other to jointly defend against the invasion of foreign organisms or pathogenic microorganisms.

There are three types of blood cells in the haemolymph of *B. dorsalis* larvae. The first type of blood cells has smooth membrane and clear cytoplasm and nucleus under the microscope; for the second type of blood cells, the surface of the cell membrane is relatively rough, with pseudopodia extending out; and as to the third type of blood cells, although the surface of the cell membrane is smooth, the contents are condensed into a ball, and this type accounts for a very small proportion in all types of blood cells. The blood cell concentration of *B. dorsalis* larvae is about $(15 - 40) \times 10^6$ cells/ml, and with the continuous increase of insect age, the concentration of blood cells shows an upward trend, rising from 16.53×10^6 cells/ml at the 2nd instar and 30.14×10^6 cells/ml at the 3rd instar, to 35.94×10^6 cells/ml at the prepupal stage. In the three developmental stages, the concentration of the first type of blood cells is significantly lower than that of the second type of blood cells (with pseudopodia). The concentration of the first type of cells decreases first and gradually increases then, but the differences are not significant among different instars, with values of 3.13×10^6 , 2.87×10^6 and 4.87×10^6 cells/ml, respectively. The concentration of the second type of cells shows an upward trend, from 13.4×10^6 cells/ml at the 2nd instar, slowly increasing to 27.27×10^6 cells/ml at the 3rd instar, finally to 31.07×10^6 cells/ml at the prepupal stage, and the difference between the latter two is not significant^[34].

The development and differentiation of blood cell types, the increase of mitosis, the release of blood cells from hematopoietic organs and the migration of fixed blood cells are affected by endocrine activities. Some factors produced by trauma or invasion of pathogenic microorganisms and multicellular microorganisms can cause the nervous system to produce more hormones, which further affects the mitotic index and the release of blood cells. It is precisely because the nerves and endocrine system of insects control the development, differentiation, and release of blood cells, as well as the activity of the insect immune system, the endocrine changes induced by parasitic wasps will inevitably affect the immune potential of the hosts^[35].

Humoral innate immunity

Humoral immunity mainly includes melanization reaction mediated by prophenoloxidase activating system (PPO-AS) and synthesis of antimicrobial peptides (AMPs) mediated by Toll, immune deficiency (IMD), JAK/STAT and other signal pathways. AMPs

are mainly synthesized in the fat body. AMPs are synthesized and then released into the hemolymph. Toll pathway is mainly activated by gram-positive bacteria and fungi, and IMD pathway is mainly triggered by Gram-negative bacteria. Microbial recognition represents the first step of immune response, and different microbial populations are recognized by different pattern recognition receptors, which will also activate specific signal pathways^[36]. Peptidoglycan recognition proteins (PGRPs) play a major role in recognizing bacterial infection in insects, and PGN (peptidoglycan) can be recognized by the conservative host PGN recognition proteins (PGRPs)^[37].

According to Dong *et al.*^[38], two PGRP genes (BdPGRP-SA and BdPGRP-SD, encoding 192 and 196 amino acid residues, respectively) were cloned from female adults of *B. dorsalis*. The expression of BdPGRP-SA in different developmental stages and tissues of *B. dorsalis* was determined by qRT-PCR, indicating that it was expressed at all developmental stages. BdPGRP-SA exhibited high expression during larval development. The expression of BdPGRP-SD was more pronounced and higher in adults. In different tissues of adult *B. dorsalis*, BdPGRP-SA was highly expressed in the fat body and hindgut, while BdPGRP-SD was only highly expressed in the fat body and was low in other tissues. When attacked by the immune triggers PGN-EB (*Escherichia coli* O111: B4) and PGN-SA (*Staphylococcus aureus*), these two genes were up-regulated. RNA interference (RNAi) inhibits the transcriptional expression of any gene, which will show increased sensitivity to PGN of Gram-negative *E. coli* and Gram-positive *S. aureus*. The inhibition of RNAi on the expression of BdPGRP-SA and -SD resulted in weak expression of four AMPs after injection of *E. coli* or *S. aureus*. BdPGRP-SA and -SD participated in independent recognition of Gram-negative and Gram-positive bacteria to activate downstream AMP responses to bacterial infections. Shi cloned a homolog of NF- κ B transcription factor Relish from *B. dorsalis*, named BdRelish^[39]. Analysis of the transcriptional profiles of BdRelish in different tissues of *B. dorsalis* found that the gene was expressed in the fat body, midgut, and head of *B. dorsalis*, with the highest expression level being found in the fat body. Therefore, the high transcription level of BdRelish in the fat body might indicate that BdRelish is closely involved in the activation and regulation of immune defense in *B. dorsalis*. In addition, scientists have also discovered the cDNA of phycin gene (BdPho) in *B. dorsalis*. Real time quantitative PCR analysis showed that the transcription level of BdPho was highest in the adult stage, and it was mainly expressed in the fat body of the abdomen of the adult. The mRNA level of BdPho significantly increased to 7.46 and 14.53 times at 3 and 6 h, possibly exhibiting antibacterial activity against Gram-negative microorganisms due to infection with *E. coli* (PGN-EB). Furthermore, the expression level of BdPho significantly increased to 3.83 times after mating, indicating that female adults enhanced their immunity by increasing the expression level of BdPho during mating^[39].

Epidermal tanning also plays an important role in maintaining

normal development and protecting insects from external physical damage. According to Erhu Chen's research, real-time quantitative PCR of TH cDNA (BdTH) cloned from *B. dorsalis* confirmed that it was significantly expressed in the epidermis of larvae at the 3rd instar, and its expression gradually increased before pupation, indicating its role in the tanning of the stratum corneum in larvae and pupae. When injected with dsBdTH or 3-iodo-tyrosine (3-IT) as TH inhibitor or fed with 3-IT, the tanning of the stratum corneum of larvae was significantly damaged, which seriously hindered the emergence of adults and caused a large number of adult deaths. Moreover, when *E. coli* was injected into the larvae fed 3-IT, the mortality rate was 92%, and the expression of four antimicrobial peptide genes was significantly reduced. These results indicate that BdTH may play a key role in the pupation of larvae and the immune response of *B. dorsalis*, and can serve as a potential new target for pest control^[40].

Insect phenoloxidase activating proteinase (PAP) is an important part of the humoral innate immunity system in insects, and it is also a key enzyme in the process of prophenoloxidase activation. The invasion stimulation of foreign organisms or pathogenic microorganisms can induce the upstream proteins of the cascade reaction to cleave and activate the precursor of PAP, while the activated PAP can cleave and activate inactive prophenoloxidase (PPO) to the active prophenoloxidase (PO), ultimately catalyzing the formation of quinones and melanin. Meanwhile, quinones can trigger the production of reactive oxygen radicals (ROS), such as superoxide anion (O_2^-) and hydroxyl radical (OH). Excessive reactive oxygen species can help kill pathogens and parasites, but also cause damage to organisms^[41-42]. Therefore, in order to reduce and prevent the damage of excessive reactive oxygen species in insects, oxidative stress mechanisms such as protective enzymes can be used to promptly eliminate excessive reactive oxygen species in the body. Antioxidant enzymes in organisms mainly include catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), which play an important role in scavenging reactive oxygen species^[43]. Generally, when host larvae are parasitized, the POD activity significantly increases, leading to the inhibition of prophenoloxidase (PO) activity, which is very beneficial for the normal development of host insects and the prolonging of their lifespan, and also for parasitic wasps to obtain more nutrients from the hosts and ensure successful parasitism^[44].

The immune function of insects requires high energy consumption to achieve optimal performance, while also resisting the invasion of pathogens or parasites. Therefore, insects need to balance their resource allocation between immune function and other adaptive components^[33]. The primary cascade activation of prophenoloxidase mediated by serine protease is a process of constantly amplifying signals. In order to prevent self-injury caused by excessive activation of the pathway, invertebrates control the immune response within a certain time and space range through a rigorous regulatory mechanism^[45]. The mating, reproduction and immunity of *B. dorsalis* are also the same. According to the study of Shi

et al.^[46], it was found that the PO activity and antimicrobial peptide activity of male and female adults of *B. dorsalis* were significantly enhanced after mating, and the male showed significantly more than the female. It might be because females need to fly frequently to find suitable spawning sites, while males tend to stay in place to gain more opportunities for mating. The immune upregulation caused by mating in males is more considered from the perspective of reproduction and evolution, while that in female adults is more considered from effectively combating the potential risks at new spawning sites. In addition, the immune response level is highest in the pupal and mature larval stages, followed by adults and juvenile larvae. Research has found that the immune defense of *B. dorsalis* is conditionally expressed to maximize its suitability in different situations.

Venom Protein of *B. dorsalis* Parasitoids

Parasitic wasps control their population by inhibiting the growth and development of host pests, and rely on a single or multiple parasitic factors to synergistically exercise their biological functions to ensure the survival and reproduction of their offspring. Some parasitic factors can inhibit hosts' natural immunity and regulate hosts' development, neural reflex and behavioral phenotype. In addition to containing DNA viruses such as PDV, parasitic wasps may also carry RNA viruses. RNA viruses can regulate parasitic wasps themselves and also participate in interactions between parasitic wasps and their hosts, making them a potential class of parasitism-related active factors^[47]. For example, when the larvae of *B. dorsalis* are attacked by *Psytalia incisi* (Silvestri), they continuously twist their bodies to increase the difficulty of piercing by parasitic wasps' ovipositors, thereby prolonging parasitic wasps' parasitism time on the hosts and ultimately reducing parasitic wasps' parasitism efficiency. However, once *B. dorsalis* larvae are successfully parasitized, they only need 2–4 s to be anesthetized, and their behavior is extremely slow, which is related to polydisperse DNA virus, polydnavirus (PDV) secreted by parasitic wasps during egg laying. At present, existing research at home and abroad has shown that polydnavirus is an important factor for parasitic wasps to break through hosts' immune system and regulate hosts' growth and development, playing an irreplaceable role in the parasitic process of parasitic wasps^[45].

Venom plays an important role in the interaction between parasitic wasps and host pests, and this conserved parasitic factor is present in female wasps in different types of parasitic wasps^[3]. In addition to paralytic toxins, antimicrobial active substances, proteases and their inhibitors, parasitic wasp venom protein also contains other protein components that inhibit hosts' immune response, regulate hosts' growth and development, paralyze and block hosts' nerves, and affect the development of wasp eggs. Among these active substances in the venom, there are not only organic small molecular substances such as amines, aromatic amino acids and terpenoids, but also key factors for successful parasitism of parasitic wasps, that is, proteins and enzymes^[48–49]. At

present, there are more than 10 kinds of enzymes identified in the protein components of parasitic wasp venom, including metalloproteinase, serine protease, phenoloxidase, peptidase, trehalase, chitinase, and acid or alkaline phosphatase.

Venom organs of *B. dorsalis* parasitoids

The venom organs of *B. dorsalis* parasitoids are located in the ectoderm, formed by the invagination of the 8th or 9th sternum, connected with the ovipositor, and located at the proximal back of the female's abdomen^[50]. Different types of parasitic wasp venom organs differ in morphology, but are generally composed of venom sacs, glands, and venom ducts. After the synthesis of parasitic wasp venom in the venom glands, it is secreted into the venom sacs for storage, and then injected into hosts' body with parasitic wasp eggs through venom ducts connected to the ovipositor, achieving multiple effects on hosts and further improving the survival rate of parasitic wasps' offspring within hosts. In addition, some parasitic wasp venom organs are also attached with Duvernoy's glands, which, together with the venom glands, are two types of glands in the female reproductive organs of Hymenoptera insects. Duvernoy's glands are often in the form of unbranched tubes or sacs, and the glands are filled with oil^[51]. The venom organs of the two species, *Fopius arisanus* (Sonan) and *Fopius vandenboschi* (Fullaway), are composed of venom sacs, venom glands and venom ducts connecting the ovipositor. The venomous sacs are yellow in color, surrounded by a layer of membrane, and divided into two areas that are oval or cone-shaped, with slight longitudinal folds visible on their surface. The ends of the venomous sacs are connected to the venom glands. The venomous glands are branched, with the base of each branch expanding at the confluence and the end enclosed in a circular arc shape, free from the body fluids of parasitic wasps. Duvernoy's glands are attached to the ovipositor, and have a thin base, which is cystic. The lengths of venom sacs of *F. arisanus* are about 0.31 and 0.29 mm; the lengths of venom sacs of *F. vandenboschi* are about 0.25 and 0.17 mm; and the lengths of venom sacs of *Diachasmimorpha longicaudata* are about 0.54 and 0.48 mm. The venom organ is composed of oval yellow venom sacs, two slender venom glands, and venom ducts, and has two forked branches and narrowed ends. The venom organ of *P. incisi* consists of a nearly spherical yellow venom sac, a branched transparent venom gland, and venom ducts. Each venom gland branch is relatively thin at the base, and has a rounded and closed long handle-shaped end, and the convergence at the base is not expanded. The venom sacs are about 0.34 mm long, nearly orange yellow, and have obvious longitudinal wrinkles and clear lines on the surface^[52]. As of now, only a small proportion of parasitic wasps have been studied on the ultrastructure of venomous organs. In China, only the ultrastructures of venom organs in several parasitic wasps such as *Scleroderma guani*, *Pteromalus puparum*, *Macrocentrus cingulum*, *Opius caricivora* Fischer, *Diadegma semilausum*, and *Cotesia plutellae* (Kurdjumov), have been studied^[53]. We can gain a deeper understanding on the evolution and classification of insects, as well as the physiological

defense mechanisms of parasitic wasps, by exploring and studying the venom organs of parasitic wasps. For example, the eggs of *D. longicaudata* can escape hosts' immunity, and they are mainly protected by the fiber layer structure on the surface of mature eggs and not damaged by hosts' passive immune system.

Venom protein components of *B. dorsalis* parasitoids

According to SDS-PAGE gel electrophoresis of the venom protein of four species of *B. dorsalis* parasitoids and the analysis of electrophoretograms, the composition of the venom protein of the two species of parasitoids of the same genus *Fopius*, *F. arisanus* and *F. vandenboschi*, were different in the molecular weight range. The molecular weight of the venom protein from *F. arisanus* is mainly concentrated below 100 KDa, with only one large molecule protein of 249.1183 KDa, and the abundance of its small molecule proteins is higher than that of *F. vandenboschi*. The molecular weight range of venom protein from *F. vandenboschi* is relatively wide, with four large molecular proteins greater than 100 KDa, and the maximum protein molecular weight being 415.994 KDa. The molecular weight range of the concentrated distribution of venom protein in the black strain of *D. longicaudata* is lower than that in the red strain, and the venom protein in the red strain of parasitic wasps lack small molecule proteins with a molecular weight lower than 17 KDa. The black strain has more large molecule proteins than the red strain. The molecular composition of venom protein from *P. incisi* is closer to that of *D. longicaudata*, and it also lacks small molecule proteins with a molecular weight lower than 14 KDa, and shows greater differences from other three parasitic wasps in venom protein, which is related to the different stages of parasitism of parasitic wasps. According to the different stages of parasitism of parasitic wasps, *F. arisanus* is an egg-pupa parasitoid, while other three species are all larva-pupa interphase parasitic wasps, and the composition of venom protein is also relatively similar^[52].

Parasitic Wasp Venom Protein Regulates the Immune Response of *B. dorsalis*

Parasitic wasps can directly regulate the transcription and expression levels of PAP and other related genes in the phenoloxidase activation cascade reaction through parasitic factors. In addition, certain components contained in parasitic factors, such as the expression products of PDVs or active components of venom, can also regulate the activity of PAPs and other related proteins to suppress hosts' innate immune response^[42].

Effects of parasitic behavior on larval hemolymph

The differentiation and proliferation of blood cells in hosts are influenced by the transcription of signal transduction factor genes regulated by parasitic wasps. Parasitic wasps use parasitic factors to disrupt the extension and adhesion ability of blood cells, thereby inhibiting the encapsulation reaction of cells in hosts and damaging the cellular immune system^[54]. The number of blood cells to some extent reflects the immune ability of insects and is closely related to their immune response. According to Shao Tun's research,

the parasitism preference of *D. longicaudata* is towards the larvae of the 2nd and 3rd instars, because the blood cell concentration of *B. dorsalis* during the 2nd instar is the lowest. Based on this, it is speculated that when choosing a host, *D. longicaudata* will parasitize during a period with physiological maturity and relatively low blood cell concentration, in order to avoid the encapsulation of the host's blood cells as much as possible. Parasitic effects can affect the number and composition of blood cells, which are one of the most important factors for recognition and encapsulation of foreign substances in hosts. After being parasitized by *D. longicaudata*, there are no significant changes in the morphology and behavior of blood cells in the larvae of *B. dorsalis*, but there are changes in the concentration and proportion of various types of cells, especially in the second type of cells. Parasitic behavior can lead to a significant decrease in the concentration of the second type of cells with pseudopodia, which to some extent inhibits the extension ability of hosts' blood cells^[35].

Effects of parasitic behavior on protein metabolism of larvae

The process of protein metabolism is also affected by parasitism. Parasitic wasps can adjust the level of protein concentration to make it change in a direction conducive to their own development and even cause changes in the protein types of the hemolymph in hosts. At the initial stage of parasitism, the eggs of parasitic wasps have just developed and cannot feed normally on their own, so they need to take nutrients from hosts' hemolymph to meet their own development. At this time, the concentration of proteins in hosts' hemolymph will decline. When the hosts are parasitized for 22 h, the concentration of hemolymph proteins begin to be higher than that of proteins in non-parasitized insects, which is mainly because parasitoids need to regulate the nutrient flow of hosts independently, so as to accumulate more energy for later development. Previous studies have shown that the eggs of *D. longicaudata* gradually expand after 24 h of production, and embryonic development is basically completed after 48 h^[35]. During the period of 32 – 48 h after production, the key period for the eggs of *B. dorsalis* to be hatched to larvae, the concentration of hemolymph protein drops rapidly, mainly because the growth of parasitic wasp consumes a lot of nutrients.

Effects of parasitic behavior on enzyme activity in larvae

Parasitic effects can effectively inhibit the activity of PO. According to reports by Wang *et al.*^[55], the difference between *B. dorsalis* larvae parasitized by *D. longicaudata* for 24 h and non-parasitized ones was extremely significant. The parasitism significantly increased the activity of antioxidant enzymes, and the dominant antioxidant enzymes also varied at different time periods. They found that the activity of PO and SOD was higher at 24 h, while the activity of CAT and POD significantly increased at 48 h, and the activity of PO and SOD showed a decreasing trend, indicating that the activity of CAT showed a parallel relationship with that of POD, but a complementary relationship with the activity of POD. Liang *et al.*^[44] applied the guaiacol method to measure the changes of peroxidase activity in the body of *B. dorsalis* larvae.

The POD activity in the body of 2-day-old *B. dorsalis* larvae after being parasitized by the parasitoid *P. incisi* was significantly higher than the control, and parasitism could induce the increase of POD activity. As the age of host larvae increased, the absorption value also showed a significant fluctuation phenomenon, and the POD activity was at the most active level when the larvae aged from 72 to 120 h, with the highest value appearing at 72 h; and after entering the pupal stage, the enzyme activity level decreased. According to analysis, the parasitism of *P. incisi* induced an increase in POD activity in the body of *B. dorsalis* larvae, which is a typical physiological defense behavior.

In addition, parasitic effects can also affect changes in host developmental duration, growth and development, as well as changes in nutrient metabolism. For example, after being parasitized by *D. longicaudata*, *B. dorsalis* larvae will show prolonged development period, and the quality of parasitic wasp larvae will gradually increase. It may be because parasitism promotes the consumption of nutrients in hosts and the absorption of nutrients by parasitic wasps, leading to their rapid growth within the host body.

Discussion and Conclusions

B. dorsalis is an important kind of fruit borer in southern China. The study on the interaction between parasitoids and *B. dorsalis* has important theoretical and practical significance for biological pest control using parasitic natural enemies. In recent years, Chinese scholars have made some achievements in the field of interaction between model insects and parasitic wasps, but there is still a lack of in-depth and systematic exploration in the study of non-model agricultural insects. At present, with the rapid development of modern science and technology, how to fully utilize research methods such as molecular biology, modern omics, physiology and biochemistry, and cell biology, as well as modern technologies such as biomacromolecule mass spectrometry identification, gene editing, and transgenic technology, to deeply analyze the natural immune response system of non-model crop pests, identify the composition of different parasitic factors in parasitic wasps and the interaction mechanism between different parasitic factors and different components and pest physiological targets, and reveal the synergistic evolution law between different parasitic factors and pest immune pathways, is worthy of further in-depth consideration and exploration research^[19].

Plant disease and pest resistance has always been a hot research topic in the field of plant protection. Currently, the active ingredients in the production of insecticides are mainly chemical substances. With the increase of pest resistance and drug dosage, problems such as drug residues, environmental pollution and drug tolerance are becoming increasingly prominent. Therefore, the urgent need to search for and develop new harmless and pollution-free insecticides has become a new goal in the field of plant protection research. Research has found that some parasitic factors carried by parasitic wasps possess the characteristics of green, environmentally-friendly, safe, and effective insecticidal active

substances, and the coding genes can be introduced into insect pathogenic microorganisms or plants through certain genetic manipulation methods to improve the anti-insect activity of microorganisms and plants^[1].

There are a wide variety of parasitic wasps, estimated to exceed 150 000 species, accounting for almost 20% of all insect species^[2]. Although some achievements have been made in biological control of pests with parasitic wasps, with the coevolution of parasitic wasps and host pests, the two have produced effective mutual defense strategies. Therefore, carrying out relevant research and revealing its mechanism is of great significance for better carrying out biological control and for improving the theoretical understanding in the field of insect immunity and development biology, and it is expected that some parasitic factor components will be applied to prospective fields such as agriculture, medicine and pharmacy. However, there are still many shortcomings in the research on the regulation of the natural immune response of *B. dorsalis* by parasitic wasp venom protein, and there are still many challenges that need to be further addressed. Three points were mainly summarized. First, domestic scholars have only preliminarily studied the parasitic mechanism of some parasitic wasps on *B. dorsalis* from the perspective of morphology, physiology and biochemistry. There is no research on the interaction between the parasitic wasps and the natural immune response of *B. dorsalis* in the pupal stage, and the interaction mechanism is still unknown. Secondly, the co-evolutionary mechanism between *B. dorsalis* and its parasitic wasps is still unclear. Thirdly, current research has mostly focused on the regulatory effect of a single venom protein component on the natural immune response of *B. dorsalis*, while it is still unclear how multiple components can regulate hosts' immune mechanism through synergistic interactions.

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