BIOLOGICAL CONTROL OF *XYLOTRECHUS RUSTICUS* (COLEOPTERA: CERAMBYCIDAE) BY RELEASING OF *DASTARCUS HELOPHOROIDES* (COLEOPTERA: BOTHRIDERIDAE) AND ITS EXPRESSION OF CYTOCHROME P450 GENES

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Abstract. *Xylotrechus rusticus* is one of the major forest trunk borers in China, causing a significant threat to afforestation and forestry engineering construction. Controlling *X. rusticus* is challenging because of its hidden life habit. The use of natural enemies against *X. rusticus* is an important strategy for controlling Cerambycidae pests. This is the first study to investigate the effectiveness of *Dastarcus helophoroides* as a biological control against *X. rusticus* under indoor and outdoor environments. The expression patterns of *CYP6BK3* and *CYP6BQ13* genes in various tissues and at different development stages were analyzed to clarify their role in the development of *D. helophoroides*. The results showed that the parasitism rate of *D. helophoroides* was the higher when their eggs were released on the larvae of *X. rusticus* L. indoors. Furthermore, the parasitism rate of *D. helophoroides* was the highest (86.67%) when their eggs were released on the larvae of *X. rusticus* in the ratio of 1:100 (host: *D. helophoroides*) under artificial wood segment simulation. The average parasitism rates of *D. helophoroides* eggs and adults on the exposed wood segment of the Cerambycidae were 81.83% and 83.29%, respectively. Indoor release of *Scleroderma guani* increased their parasitic rate on the aged larvae and pupae of *X. rusticus*. qPCR analysis of CYP450 gene expression showed that *CYP6BK3* and *CYP6BQ13* genes were expressed at all development stages of *D. helophoroides*; however, their expression levels were higher in adults than in the larvae. Notably, *CYP6BK3* expression was the highest in the adipose tissue, whereas *CYP6BQ13* was the highest in the hindgut tissue.
Keywords: *Xylotrechus rusticus, Dastarcus helophoroides, control effect, control parasitism rate, gene expression, cytochrome P450 genes*

Introduction

Dastarcus helophoroides belongs to the Coleoptera order of Bothrideridae. It is an ideal natural enemy insect for Cerambycidae, Buprestidae, Curculionidae, and Xylocopidae (Urano, 2003; Hajek and Eilenberg, 2018; Yang et al., 2018). In the 1980s, China began to study the application of *D. helophoroides* as a biological control. Releasing *D. helophoroides* adults or egg cards in natural or artificial forests can effectively control various longicorn trunk borers, such as *Monochamus alternatus*, *Anoplophora glabripennis*, and *Batocera horsfieldi* (Zhou et al., 2013; Zhang et al., 2022a; Yu and Li, 2000).

Xylotrechus rusticus belongs to the Coleoptera order of Cerambycidae. It is one of the main trunk borers threatening poplar production in northern China. *X. rusticus* lives in the trunk throughout its development stages, including the egg, larva, and pupa; thus, it is difficult to be reached via conventional artificial methods like pesticide application. Besides, the adult stage is short and the generation duration is not very synchronized (Laurentiu and Mitrea, 2019). Therefore, achieving efficient control of *X. rusticus* through the traditional artificial and physical control approaches is challenging. The use

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of chemical pesticides for pest control is discouraged due to their adverse effects on the natural environment. Thus, biological control methods have continued to gain research focus for controlling longicorn beetles. Till now, there is no report on the biological control technology that can effectively control *X. rusticus* population. Therefore, this study aimed to develop biological control strategy against *X. rusticus* using its natural enemy insects. The findings of this study will provide scientific and technological support for controlling longicorn trunk borers. In addition, this study offers a scientific basis for formulating technical regulations of forest pests using natural enemies.

Cytochrome P450 monooxygenase is a form of heme protein widely existing in animals and plants. It is regarded as a vital multifunctional oxidase because of its oxygenase and oxidase activities (James and Xu, 2012; Nelson, 2006). The functions of this enzyme system are very extensive. In insects, P450 is primarily involved in degrading exogenous toxic substances and regulating the synthesis and metabolism of endogenous substances. Due to evolutionary pressure, the cytochrome P450 enzyme system is diverse (Manikandan and Nagini, 2018). So far, 27 families, including *CYP4*, *CYP6*, *CYP9,* and *CYP12* have been identified in insects (Scott et al., 2008; Berenbaum et al., 2021). In this study, we explored the relationship between the growth process of *X. rusticus* L. and cytochrome P450 to gain insights into the natural evolution mechanism of Coleoptera insects. At present, there have been few reports on the control of *X. rusticus* L. and the expression of the *CYP6* gene of the cytochrome P450 enzyme system. The research results will help to evaluate the stress resistance of the *D. helophoroides* at the molecular level, and as an important natural enemy insect in biological control, *D. helophoroide*s has broader application prospects in the biological control of beetle pests.

Materials and methods

X. ruticus

The study was conducted from May to July 2019 at the Heilongjiang Cold Region Wetland Ecology and Environment Research Key Laboratory, Harbin University, School of Geography and Tourism (Harbin, Heilongjiang Province, China). The 30-day-old *X. ruticus* larvae (four instars larvae) were collected from infested small black poplar in Tangfang Town, Bin County, Harbin. The infested poplar was cut down, and the parts with larvae were divided into wood segments of about 1 ~ 1.5 m and transported back to the laboratory. After splitting the wood segments, the *X. rusticus* larvae were collected and put into an insect rearing box. The larvae in the box were maintained under artificial feeding in an incubator at 25℃ and RH75% ± 10% in the dark for later use. The two ends of the remaining wood section were coated with paraffin to keep moisture, and then the wood sections were covered with insect-proof net and placed in an open space to collect the adults of *X. rusticus*. The aged larvae and pupae with uniform size and normal posture were selected and stored at 4℃ in a refrigerator for later use.

D. helophoroides

The adult and egg cards of *D. helophoroides* were provided by the Institute of Forest Ecological Environment and Protection, Chinese Academy of Forestry Sciences. A total of 10 adults were randomly placed in each finger tube (12 mm × 75 mm) regardless of

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gender. Water absorbent cotton was placed at the tube mouth to maintain humidity. The tubes were then placed in a dark constant temperature incubator at 12℃, RH75% ± 10, for later use. Each egg card was a 5 cm × 10 cm kraft paper, folded in half; about 50 insect eggs were placed on the inner side of the egg cards and stored at 4℃ in a refrigerator for later use.

Indoor parasitism experiment involving the adults and eggs of D. helophoroides

Putting larvae in insect rearing box indoor

The larvae of *X. rusticus* and the adults of *D. helophoroides* were put in an 1000 ml indoor insect rearing box in the ratio of 2:1, 1:1, 1:2, and 1:3 under artificial feeding. The box was covered with a 4-mesh insect-proof net to prevent insects from escaping and placed in a dark room (25℃ ± 1, RH70%). Parasitism was observed and recorded daily. Each treatment consisted of 10 larvae and was replicated thrice. Meanwhile, the larvae of *X. rusticus* and the eggs of *D. helophoroides* were placed in an insect rearing box in the ratio of 1:10, 1:25, 1:50, and 1:100. The hatching and parasitism of the eggs were observed and recorded every 5 days. Each treatment consisted of 10 larvae and was replicated thrice. The parasitism rate was scored using the method described by Tang: if the larvae of *X. rusticus* were parasitized by *D. helophoroides* adults or larvae, or the larvae of *X. rusticus* were bitten on the body surface or dead, then *X. rusticus* was regarded as parasitized, and the parasitism rate was determined.

Indoor simulated wood section release

First, 10 cm x 20 cm poplar tree sections were cut. Next, the midpoints of the wood sections were cut into two halves, and the center of each half was dug out using a carving knife at a depth of 5 cm × 5 cm for later use. The experiment consisted of five treatments: the larvae of *D. helophoroides* and *X. rusticus* were randomly selected (regardless of gender) and put into the groove of the wood sections in the ratio of 2:1, 1:1, 1:2, and 1:3. The wood sections were externally bound firmly with a rubber band and placed in a dark place at room temperature (25℃ ± 1, RH70%). Another group of *D. helophoroides* adults was set as the control. One *X. rusticus* larva was put in each wood section, and three repeats were set. Parasitism was observed and recorded daily for 15 days. Meanwhile, the pore passages of longicorn beetle on the treated and untreated wood sections were simulated by drilling into the pit and putting 10 larvae of *X. rusticus* in the wood section. The external surfaces of the wood sections were fixed with a rubber band, and the *D. helophoroides* egg card was put in the ratio of 1:10, 1:25, 1:50, and 1:100 outside the wood sections. Each card was fixed with 3 cm long nails and placed in a dark place in a greenhouse (25℃ ± 1, RH70%). The hatching and parasitism of insect eggs were observed and recorded every 5 days. Each treatment was repeated thrice for 30 days.

Parasitism experiment involving the adults and eggs of D. helophoroides in an outdoor environment

Poplar tree segments (diameter; 25-30 cm) infested by *X. rusticus* were selected and divided into three groups according to their external damage level: noncracking bark, cracking bark, falling bark, and bare xylem. A total of 50 adults of *D. helophoroides* were released into each wood section. The adults of *D. helophoroides* were placed on

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the surface of the wood section and allowed to crawl freely to find the host. The wood sections were covered with a 4-mesh insect-proof net to prevent the insects from escaping. Both ends of the insect-proof net were wrapped and bound with adhesive tape. Each treatment was repeated three times and placed in an outdoor shade. The experiment was conducted for 20 days. Finally, the wood sections were cut to count and the parasitism rate was determined.

The poplar tree sections with cracked bark and bare xylem were selected. The egg card was fixed with small nails in the ratio of 1:50. The wood sections were covered with a 4-mesh insect prevention net to prevent the insects from escaping and placed in a cool place outside the room for feeding and observation. Each treatment was repeated three times for a total of 30 days. At the end of the experiment, the invaded wood segments were split, and the parasitic conditions were counted and recorded.

Analysis of cytochrome P450 gene expression in D. helophoroides

Total RNA extraction and reverse transcription

The 1st, 2nd, 3rd, and 4th instar larvae and adults of *D. helophoroides* were selected. Nine tissue samples, including body wall, foregut, midgut, gastric cecum, hindgut, martensitic duct, fat body, muscle, hemolymph, and brain, were dissected and quickly frozen in liquid nitrogen for RNA extraction. Total RNA of each sample was extracted using Trizol reagent, according to the manufacturer's instructions. Extracted RNA samples were stored in the refrigerator at -80℃ for later use. Each treatment was repeated three times. cDNA was synthesized using PrimeScriptTM RT reagent kit with gDNA Eraser (Takara, Japan), according to the manufacturer's instructions.

Real-time fluorescence quantitative PCR

Fluorescent quantitative PCR primers were designed using beacon Designer 7.0 software based on the sequences of *CYP6BK3* and *CYP6BQ13* (NCBI accession numbers I3QII8_9CUCU and I7D7Q6_9CUCU, respectively) published on the GenBank. Primers were synthesized by Shanghai Sangon. *β-actin* and *RPL4* were used as reference genes. The relative expression level of the *P450* gene was determined using the ABI 7500 real-time PCR system (Applied Biosystems, US) with SYBR green as a dye and 5-fold diluted cDNA as the template. Reaction system: SYBR Premix Ex TaqTM (2×) 10 μL, cDNA template 1.0 μL, each of upstream and downstream primers 0.8 μL, ROX Reference Dye2 (50×) 0.4 μL. Water without ribozyme was used to adjust the total volume to 20 μL. The PCR procedure included two steps: 95℃ for 30 s; 95℃ for 5 s; 60℃ for 34 s, 40 cycles in total, and then 95℃ for 15 s; 60℃ for 60 s; 95℃ for 15 s was used to record the melting curve. Each sample was analyzed in triplicate.

Statistical analyses

DPS and Excel software were used to process and analyze the experimental data and 95% confidence intervals. SPSS17.0 software was used to conduct analyses of variance and data significance analysis using Tukey in SPSS method; these results were subjected to multiple mean comparisons. q-PCR data were analyzed using Bio-Rad IQ-5 2.0 software.

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Results

External morphological changes in X. rusticus after the parasitism by D. helophoroides

The external morphological changes in *X. rusticus* following *D. helophoroides* parasitism are shown in *Figure 1*. The adults of *D. helophoroides* rely on their strong pereiopoda to actively look for the larvae of *X. rusticus*. When they find the larvae, they inject a paralytic agent to make the host faint and start feeding. *D. helophoroides* adults first bite the weak parts of the larva body wall and eat all the nutrients (*Fig. 1a*). The larvae of *D. helophoroides* mainly cause damage to the host by biting the heads with their jaws and laying the eggs on the larva. The adults of *X. rusticus* were eaten within a short time after being parasitized by the larvae of *D. helophoroides*. *Figure 1b* shows *D. helophoroides* larva feeding on the adults of *X. rusticus*. After the larvae of *D. helophoroides* were hatched in the field, they would find the host in the trunk insect path and parasitize on them rapidly. As shown in *Figure 1c*, the larvae ate the adults of *X. rusticus*.

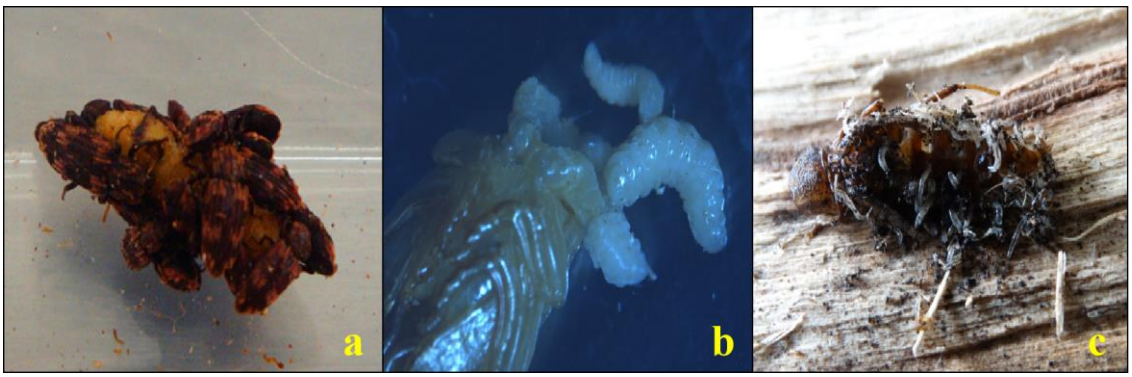

***Figure 1**. D. helophoroides parasitizing on the X. rusticus. (a) Parasitism of D. helophoroides on adult of X. rusticus in indoor environment. (b) Parasitism of D. helophoroides on larva of X. rusticus in indoor environment. (c) Parasitism of D. helophoroides on larva of X. rusticus adult in outdoor environment*

Effect of D. helophoroides parasitism indoor

The direct indoor or wood section releasing methods were used to release the eggs or adults of *D. helophoroides*. The parasitic effects on the larvae of *X. rusticus* are shown in *Table 1*. Significant difference were observed in the parasitic rate of *D. helophoroides* on *X. rusticus* larvae between the direct releasing and wood section releasing methods. The parasitic rate was positively related to the proportion of the egg card put into the insect box. That is, the larger the proportion of egg cards, the higher the parasitic rate. Under the direct releasing method, the parasitic rate was 100% when the proportion was 1:100 and 30% when the proportion was 1:10. When the egg card was placed outside the simulated pest wood section, the parasitic rate was 86.67% when the feeding ratio was 1:100, and only 23.33% when the feeding ratio was 1:10. These results show that larger proportion of *D. helophoroides* eggs facilitate parasitism of *X. rusticus* larvae. Besides, directly releasing *D. helophoroides* eggs near the bare host was more effective than releasing the eggs outside the wood section.

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The parasitic rate of *X. rusticus* by *D. helophoroides* adults was significantly different between the methods of direct release and simulated damaged wood segments. The parasitic rate of *X. rusticus* larvae increased with the increase in the proportion of released natural enemies. Specifically, the parasitism rate was 66.67% when the ratio of adults was 1:3 and only 26.67% when the ratio was 2:1, showing a significant difference ($P < 0.05$). Meanwhile, the parasitism rate was 72.22% when the ratio of adults released from the wood section was 1:3 and 38.89% when the ratio was 2:1.

Table 1. *Parasitism rate of X. rusticus on larva by releasing eggs or adults of D. helophoroides in indoor environment*

Treatments	Release rate	Eggs	Release rate	Adults
	X. rusticus to D. helophoroides	**Parasitism rate/%**	***X. rusticus to D. helophoroides***	**Parasitism rate/%**
Directly release	1:10	30 ± 10 c	2:1	26.67 ± 9.62 b
	1:25	43.33 ± 5.77 c	1:1	36.67 ± 9.62 b
	1:50	70 ± 10 b	1:2	56.67 ± 9.62 a
	1:100	100 a	1:3	66.67 ± 9.61 a
Release by logs	1:10	23.33 ± 5.77 c	2:1	38.89 ± 5.77 b
	1:25	36.67 ± 11.58 b	1:1	44.44 ± 5.77 b
	1:50	60 ± 10 ab	1:2	50 ± 0.00 ab
	1:100	86.67 ± 5.77 a	1:3	72.22 ± 5.77 a

Different letters at the table on the same column indicate significant differences ($p < 0.05$)

Effect of D. helophoroides parasitism outdoor

The effect of parasitism by *D. helophoroides* adults on the larvae of *X. rusticus* in the outdoor environment are shown in *Table 2*. The lowest parasitism rate (58.63%) was observed in the wood section invaded by *X. rusticus*; however, this was not significantly different from the rate observed in the cracked bark state. Meanwhile, the highest parasitism rate (81.83%) was observed in the bare xylem state. Notably, the parasitism rate did not depend on the state of the wood segment xylem (i.e., bare or not). The average parasitic rate was 72.32% when the bark was cracked and 83.29% when the xylem was bare. Of note, the release of eggs from the shed bark effectively improved the parasitic rate.

Table 2. *Parasitism rate of X. rusticus on larva by releasing adults of D. helophoroides under outdoor environment*

Treatments	Adults			Eggs		
	Numbers of *X. rusticus* larva	**Parasitism rate/%**	**Average parasitic rate/%**	**Numbers of *X. rusticus* larva**	**Parasitism rate/%**	**Average parasitic rate/%**
Exposures xylem of the tree	23	82.6	81.83 ± 2.64 a	12	85.71	83.29 ± 2.11 a
	19	78.9		14	82.35	
	25	84		9	81.82	
Bark crack of the tree	18	55.56	61 ± 6.49 b	12	61.9	72.32 ± 9.02 b
	22	68.18		17	77.27	
	27	59.26		14	77.78	
Bark not crack of the tree	18	50	58.63 ± 9.46 b	-	-	-
	14	57.14		-	-	
	16	68.75		-	-	

Different letters at the table on the same column indicate significant differences ($p < 0.05$)

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Relative expression of CYP6B3 and CYP6BQ13 in the larvae and adults of D. helophoroides

CYP6B3 and *CYP6BQ13* genes were expressed at all growth stages of *D. helophoroides*. However, the expression of the two genes in the adults was higher than in the larvae. Besides, *CYP6B3* expression in the 4th instar larvae was higher than in the other instars, whereas *CYP6BQ13* expression in the 2nd instar larvae was higher than in the other instars (*Fig. 2*).

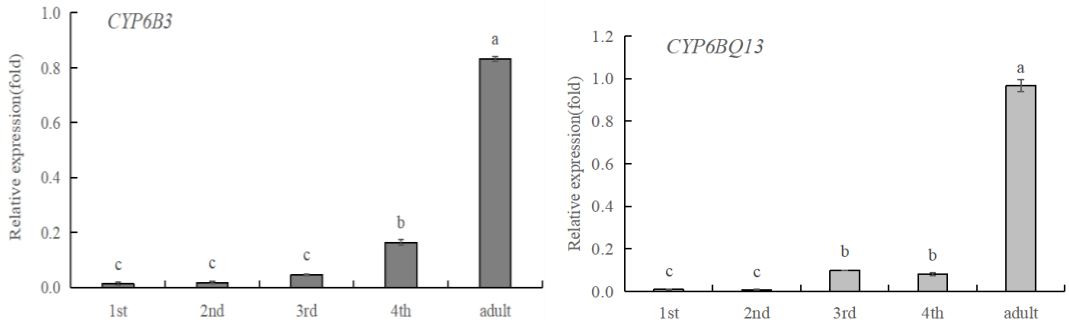

Figure 2. *The relative m RNA quantities of CYP6B3 and CYP6BQ13 in larvae and adults of D. helophoroides*

The m RNA quantity is expressed relative to the life stages (1st, 2nd, 3rd, 4th and adult) with the highest level of transcript: the adult sample. The different lowercase letters denote significant differences between the tissues (Tukey's HSD test, $p < 0.05$).

Relative expression of CYP6B3 and CYP6BQ13 in various tissues of adult D. helophoroides

The mRNA levels of the *CYP6B3* and *CYP6BQ13* genes were analyzed in the foregut, midgut, gastric caecum, hindgut, Markovian duct, fat body, muscle, hemolymph, and brain tissues of *D. helophoroides* adults. The results showed that the two genes were expressed in all the tissues. Specifically, *CYP6B3* expression was highest in the fat body, midgut, gastric caecum, and hemolymph, followed by the brain. Meanwhile, *CYP6BQ13* expression in the hindgut was significantly higher than in the other tissues but less in the foregut and midgut (*Fig. 3*).

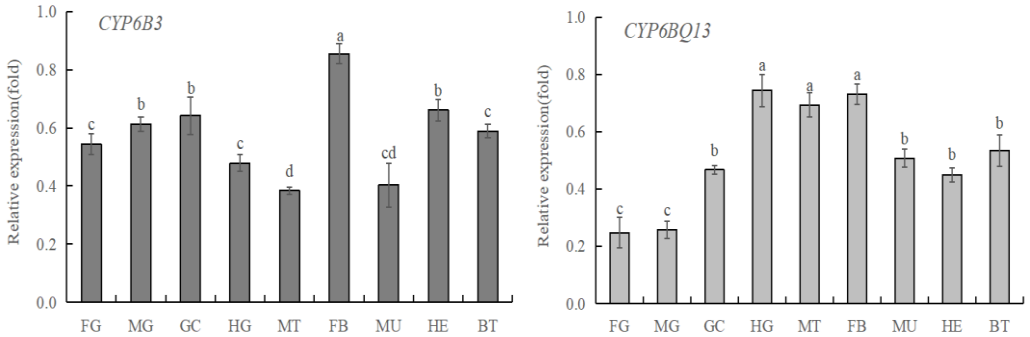

Figure 3. *The relative expression levels of CYP6B3 and CYP6BQ13 in various tissues and organs of adult D. helophoroides. FG, foregut; MG, midgut; GC, gastric caecum; HG, hindgut; MT, Markovian duct; FB, fat body; MU, muscle; HL, hemolymph; BT, brain tissues*

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Discussion

D. helophoroides is among the leading natural enemies of forest stem borers in China. It is widely distributed in most forests in China and is considered a multi-host insect (Jiang et al., 2023). The adults of *D. helophoroides* can live for up to six years. The adults bite and lay eggs around the host, and the larvae can eat up the host and cocoon (Yi et al., 2023; Yang. 2004). This study showed that the parasitism control effect of *D. helophoroides* on the larvae and adults of *X. rusticus* was significant. The evolution of the external morphological characteristics of *D. helophoroides* is closely related to its biological habit; therefore, it is optimally adapted to live in the tunnel of trunk boring pests. These adaptations include expanded body surface with strong bristles and six short and thick feet that can be hidden under the abdomen. These adaptations also shield them from the attack by large trunk pest larvae (such as longicorn beetle larvae). Additionally, the front edge of the sheath wing and the middle chest side plate of *D. helophoroides* can form a close shutting structure. These adaptive structures cooperate with the hard body wall to resist counterattack by the larvae of large trunk borers and ensure smooth completion of parasitic behaviors, such as oviposition on the host surface (Zhang et al., 2014; Lee et al., 2017). In this study, *D. helophoroides* parasitism exerted external morphological changes on the larvae and adults of *X. rusticus*. Notably, this is the first study to characterize parasitism-induced morphological changes on *X. rusticus*.

Most development stages of *X. rusticus* occur in hidden wood tunnels. Besides, there are no pathogenic microorganisms and natural enemies of *X. rusticus*, making it relatively challenging to control (Skryknyk et al., 2023). In this study, the parasitic effects of *D. helophoroides* were studied under three egg and adult releasing approaches, including indoor direct releasing, simulated releasing in the environment of the damaged wood section, and outdoor semi-natural release. In the indoor experiment, the parasitism rate of adult insects under the simulated damaged wood section method was higher than in the direct releasing method. The parasitism rate reached 100% when *D. helophoroides* eggs were directly released in the ratio of 1:100, indicating that controlling the feeding ratio can effectively increase the parasitism rate of natural enemies. Lu et al. (2011) found that *Apriona swainsoni* could feed on the eggs of *D. helophoroides*, but this phenomenon was not observed in this study. The larvae of *X. rusticus* in the tunnel of the damaged poplar tree section were easily accessed and parasitized by the released *D. helophoroides* eggs and adults in the outdoor semi-natural environment. The parasitic rate was highest when the xylem was exposed and the egg card was used. The egg card release method is simpler and easier to operate. In the actual operation, the egg card was placed in the shade above the damaged part of the trunk. This study confirms that *D. helophoroides* exhibit effective parasitic effects against the larvae of *X. rusticus*, providing a theoretical basis for the actual release of *D. helophoroides* in the forest to control *X. rusticus*. However, further studies should be conducted to determine the specific forest operation and control effect of *D. helophoroides*. Also, it is important to examine whether *D. helophoroides* can survive the winter in the cold north.

Cytochrome P450 (CYP450) enzyme system is one of the oldest known enzyme systems in organisms; however, its precise functions have not been fully elucidated (Zhao et al., 2023). The roles of the known P450 enzyme system are closely related to human life (Hu et al., 2023). Research on the function of insect CYP450 genes has gradually shifted from their role in drug resistance to their influence on growth,

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development, and reproduction (Nauen et al., 2022; Scott, 2008). The expression patterns of insect P450 genes vary significantly between different organs. For example, the expression levels of some P450 genes (such as *CYP6BQ13v2*) are higher at the larval stage than at the adult stage, while others (such as *CYP6L1*) are highly expressed at the adult stage than at the larval stage (Hu et al., 2021; Wen and Scott, 2001; Rewitz et al., 2007). The diverse expression patterns of P450 genes reflect their functional differences (Cahlíková et al., 2011). For instance, *CYP6BQ13v2*, which is highly expressed in the larvae of *Tribolium castaneum* has been shown to participate in ecdysone synthesis (Jones et al., 2011). Meanwhile, *CYP6L1* is believed to regulate sex and reproduction because of its high expression in male *Blattella germanica* adults. Furthermore, studies have shown that *CYP6L1* gene exists specifically in the testis and accessory glands of male *Blattella germanica* adults, indicating that it may be related to reproductive development (Feyereisen, 2006). Here, we employed qPCR to examine the mRNA levels of *CYP6BK3* and *CYP6BQ13* genes in the larvae and adults of each *D. helophoroides* instar. The expression of the two genes was higher in the adults than in the larvae, suggesting that they may be adult-specific genes with special roles in physiological metabolism. The expression characteristics of genes in developmental stages and tissues are closely related to their functions (Zhang et al., 2022b). CYP450 genes are expressed in almost all species because of their diverse functions; however, their expression patterns vary in different organs, CYP450 gene was found in the mammalian liver for the first time (Feyereisen et al., 2015). Several CYP450 genes in the liver are mainly involved in drug metabolism (Li et al., 2024). *CYP6A1* is highly expressed in the foregut, midgut, gastric caecum, and Markovian duct (Ullah et al., 2023; Dunkov et al., 1997). The expression patterns of *CYP4C69*, *CYP4C73*, and *CYP4DH1* in different tissues showed that they are highly expressed in the midgut, gastric caecum, martensitic duct, and fat body (Ibrahim et al., 2015). The expression patterns of *CYP4C69*, *CYP4C73*, and *CYP4DH1* in different tissues of *Locusta migratoria* showed that they are highly expressed in the midgut, gastric caecum, Malpighian duct, and adipose tissue (Zhu et al., 2017; Zhang et al., 2013). Midgut, gastric caecum, Malpighian duct, and adipose tissue play an important role in insects' growth, development, and reproduction (Dunkov et al., 1997). The midgut is the main place for digestion and nutrient absorption, and its derivative gastric cecum assists in further absorption of food and water. Martensitic tubules are essential in the metabolism and detoxification of foreign substances. Fat bodies can metabolize sugars, lipids, and proteins; they are also the target tissues of hormones (Feyereisen, 2006). The expression patterns of CYP450 genes in the above tissues suggest that they might play a crucial role in the metabolism of endogenous and exogenous substances in *D. helophoroides*.

Conclusion

The findings of this study show that *D. helophoroides* can be used as a natural enemy for controlling *X. rusticus*. Applying egg cards of *D. helophoroides* in the field can effectively control *X. rusticus*. During operation, the egg cards should be placed under the shade where the bark falls off, and the xylem is exposed to increase the parasitic rate. The expression patterns of cytochrome P450 genes in different tissues and developmental stages were examined using qPCR. *CYP6BK3* and *CYP6BQ13* genes play an essential role in the growth, development, and reproduction of *D. helophoroides*.

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