



Hormesis effects of chlorantraniliprole on a key egg parasitoid used for management of rice lepidopterans

Xiaojing Wang^{1,2,**}, Lixia Tian^{2,**}, Michele Ricupero³, James D. Harwood²,
Yuyong Liang⁴, Lian-Sheng Zang^{1,*}, and Su Wang^{2,*}

- ¹ State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, PR China
² Institute of Plant Protection, Beijing Academy of Agricultural and Forestry Sciences, Haidian, Beijing, China
³ Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123, Catania, Italy
⁴ Institute of plant Protection, Jiangxi Academy of Agricultural and Forestry Sciences, Haidian, Beijing, China
* Corresponding authors: anthocoridae@163.com, lsz0415@163.com
** These authors contributed equally to this work.

With 3 figures

Abstract: The egg parasitoid *Trichogramma japonicum* (Hymenoptera: Trichogrammatidae) is used for biological control of lepidopteran pests, and it could be used for optimizing Integrated Pest Management (IPM) in rice crop. Chlorantraniliprole is a diamide insecticide broadly applied for pest control in rice cropping systems and it has been generally considered harmless to egg parasitoids. However, the impact of sublethal or low lethal concentrations of chlorantraniliprole on *T. japonicum* remains unclear. In this context, under laboratory conditions we assessed the acute toxicity of chlorantraniliprole on *T. japonicum* as well as sublethal effects of a low lethal concentration (LC₁₀) on key biological traits of the parasitoid. In addition, we also evaluated the impact of chlorantraniliprole on the biocontrol service provided by *T. japonicum* on the pest *Chilo suppressalis* (Lepidoptera: Crambidae) under field conditions. The LC₁₀ of chlorantraniliprole on adult parasitoids through exposure with residues was 0.110 mg/L, and this LC₁₀ induced hormesis in exposed individuals; locomotory activity, respiratory metabolism and mass reproduction of *T. japonicum* were enhanced. Under field conditions, chlorantraniliprole led to increased biocontrol service provided by *T. japonicum* (reduced pest damage). These results can be useful for optimizing further the use of *Trichogramma* parasitoids into IPM in rice crops. It also hints the importance of carrying out careful risk-assessment of pesticide non-lethal effects under field conditions when developing IPM.

Keywords: diamide, insecticide, ecotoxicology, *Trichogramma japonicum*, sublethal effects, side effects

1 Introduction

Rice (*Oryza sativa* L.) is one of the most economically relevant staple food crops in the world, it is widely consumed by the human population, with China and India being the largest producing countries (FAOSTAT 2022). A large number of insect pests, namely lepidopterans (Lepidoptera), frequently threaten plant growth and yield in rice fields. Chemical insecticides are extensively used as they represent the most effective and economic strategy to minimize crop losses.

Although these products are effective in pest control they show multiple issues notably risks of pesticide resistance and outbreaks in pests, as well as multiple side effects on non-target organisms, e.g. through exposure to sublethal and/or low lethal concentrations (Mohammed et al. 2018; Wei et al. 2019; Menail et al. 2020; Ricupero et al. 2020a). Sublethal

concentrations of pesticides are concentrations that do not induce significant mortality in exposed populations, still they can cause multiple side effects in biocontrol agents, notably sublethal physiological and/or behavioral effects in exposed individuals (Desneux et al. 2007) but also hormesis phenomena (Guedes et al. 2016; Cutler et al. 2022). Understanding the impact of sublethal effects of pesticides is key for evaluating their safety for beneficial insects in Integrated Pest Management (IPM) systems (Desneux et al. 2007; Lu et al. 2012).

Chlorantraniliprole is a high-efficiency insecticide frequently applied for pest control. As the first commercial insecticide of the anthranilic diamide class, it has been classified as a ryanodine receptor modulator (Cordova et al. 2006). Chlorantraniliprole induced insect feeding cessation, lethargy, muscle paralysis and death by directly activating

ryanodine receptors which alter calcium release in muscle cells (Sattelle et al. 2008). Due to its high insecticidal activity, chlorantraniliprole is routinely used in rice fields to control various insect pests, although primarily lepidopterans such as *Chilo suppressalis* and *Cnaphalocrocis medinalis* (Zheng et al. 2011; Wei et al. 2019).

Parasitoid wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) can parasitize eggs of various agricultural insect pests (Pizzol et al. 2010; Tabone et al. 2010; Qu et al. 2020). These parasitoids have been used notably for managing lepidopteran pests for more than 100 years and they are routinely released in open fields (Huang et al. 2020; Zang et al. 2021). *Trichogramma japonicum* has been identified as an efficient biological control of lepidopteran pests (Wang et al. 2021; Zhang et al. 2021) and is widely used in rice fields in China (Zang et al. 2021). For this reason, its use could help optimizing IPM programs for rice crops pending it is not negatively affected by pesticides already included in such packages (Chen et al. 2010; Zang et al. 2021).

The selectivity of chlorantraniliprole towards beneficial arthropods has been assessed and yielded contrasting results (Biondi et al. 2012; Abbes et al. 2015; Ricupero et al. 2020b). Previous studies generally considered chlorantraniliprole to be safe for parasitoids of *Trichogramma* genus (Preetha et al. 2009; Brugger et al. 2010; Khan and Ruberson 2017; Yang et al. 2020). Nevertheless, recent studies hinted that low lethal concentrations (LC) of chlorantraniliprole could impact reproductive traits of *T. brassicae* and stressed that this insecticide has to be avoided in IPM (Parsaeyan et al. 2020; Nozad-Bonab et al. 2021). However, the impact of sublethal and low lethal concentrations of chlorantraniliprole on behaviors and biocontrol efficiency of *T. japonicum* in rice fields has not been evaluated yet.

In this context, we assessed the effects of a low lethal concentration of chlorantraniliprole (LC₁₀) on various key traits of *T. japonicum*. We hypothesized that LC₁₀ of chlorantraniliprole can alter key biological traits of *T. japonicum*, affecting its behavioral and physiological activities. To test this, under laboratory conditions we first estimated the LC₁₀ of chlorantraniliprole by exposing *T. japonicum* adults to dry insecticide residues. Then, the impact of this LC₁₀ was assessed on parasitism rate, locomotion, respiratory activities and mass reproduction under laboratory conditions. Moreover, the impact of LC₁₀ of chlorantraniliprole on the biocontrol efficiency of *T. japonicum* against *C. suppressalis* on rice was also investigated in field conditions.

2 Materials and methods

2.1 Insect rearing

Trichogramma japonicum used for the experiments and laboratory colony were reared in the Institute of Plant

Protection, Beijing Academy of Agricultural and Forestry Sciences. Newly emerged adults of *T. japonicum* were separated by sex and maintained in ventilated plastic boxes (10.0 × 8.0 × 5.0 cm) containing ca. 250–300 females per box. Ten percent of virgin females were selected for establishing the laboratory colony and, the remaining females were used in the following experiments within 2 days. The rearing of *T. japonicum* was conducted according to Wang et al. (2021).

A lab colony of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) was initiated using individuals collected from rice fields at the research station of the Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi Province, China. Fifty pairs of *C. suppressalis* were randomly selected and reared in ventilated plastic cylindrical arenas (D = 25.0 cm and H = 45.0 cm) containing from four to five young shoots (25 cm high) of *Oryza sativa* L. cv. “Liaoxing #1”. Rice plant seeds were provided by the Institute of Rice Crop Research, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning, (China). To obtain fresh eggs, female adults were introduced into plastic Petri dishes (9 cm diameter, covered by a mesh net) containing one fresh rice leaf (5.0–6.0 cm²) as oviposition substrate. Newly laid eggs were collected within 12 h. Insect rearing was maintained with the following conditions: 25±1°C, 65±10% RH, 16L:8D (600 lux light intensity).

2.2 Residual toxicity on adults

The concentration-response mortality relationship of chlorantraniliprole on *T. japonicum* was established by exposing parasitoid adults to dry insecticide residue on glass. Preliminary trials were used to determine the range of concentrations to be tested (Desneux et al. 2004) and five concentrations (4, 2, 1, 0.5, and 0.25 ppm) were then made by dissolving chlorantraniliprole (95% technical grade, Bayer, China) in 99.5% analytical-grade acetone (Sigma-Aldrich, USA) using a magnetic stirrer for 10 min at 300 rpm (pure acetone solution used as control). To obtain a homogeneous insecticide residue layer on the glass tube, 0.5 mL of chlorantraniliprole solution was applied to the inner surface of the tube (height = 7.5 cm, inner diameter = 3.0 cm) and rotated to prevent the formation of solution streaks on glass. Insecticide-treated and control tubes were placed under the fume hood for 1.5 h to allow the complete evaporation of insecticide solution before introducing adult parasitoids. Then, 80 *T. japonicum* female adults (1-day old) were placed per tube (= one replicate). Tubes were covered with nylon gauze to allow air ventilation. A solution of honey-water (0.15:1) was provided as food inside the tube between two layers of Parafilm to avoid potential contamination by the insecticide. Tubes were kept in a climatic cabinet (25±1°C, = 65 ± 1% RH, and 16L:8D) for 24 h. Parasitoid adults were maintained in the tube for 1 h to allow sufficient contact with insecticide residues and then transferred into a clean tube for mortality observation

after 24 h. Parasitoids that showed no movement 24 h after the contact exposure were considered dead. At least five replicates were carried out per each insecticide-concentration and the control.

2.3 Sublethal effects

2.3.1 Effect on parasitism and emergence rate

To evaluate the impact of LC₁₀ chlorantraniliprole on the parasitism of *T. japonicum*, adult females were exposed to insecticide residue on glass (as described in section 2.2). Then, per each replicate, 40 females were placed in a glass tube (2.0 cm diameter and 6.0 cm height) for 24h with a host egg card (1.5 × 4.0 cm). Each host egg card was bearing 1000 newly laid *C. cephalonica* eggs which were previously devitalized with UV light. To evaluate the parasitism rate, the number of eggs that showed black color (i.e. parasitized eggs) over the total number of eggs was counted under a stereomicroscope (Zeiss, SteREO Discovery V20, Germany) for 10 consequent days. The total number of emerged *T. japonicum* adults was counted from the beginning of the experiment until no new *T. japonicum* adults emerged. Each newly emerged parasitoid adult was sex-determined under stereomicroscope. The parasitism rate, emergence rate and the female proportion (female nb / total emerged wasps) were calculated. The experiment was replicated ten times for LC₁₀ chlorantraniliprole and control treatments.

2.3.2 Effect on locomotion

This assay aimed at evaluating the impact of LC₁₀ chlorantraniliprole on the locomotory activity of *T. japonicum*. A single (1-d-old) *T. japonicum* female previously exposed to LC₁₀ of chlorantraniliprole (see Section 2.2) was introduced into a plastic Petri dish arena (D = 9.0 cm) with a cluster of five eggs of *C. cephalonica* and covered by a transparent glass plate (parasitoids exposed to acetone were used as control). The locomotory activity of *T. japonicum* female was recorded for 10 min by means of a 4K video camera (X-T3 with F1.2-56 fixed focus lens, Fujifilm, Japan), placed on the top of the experimental arena. Image recording lasted 10 min and was analyzed to obtain the parameters of locomotion by using insect behavior analyzed using Casonar-Image 1.5 (Casonar, Yunnan, China) according to the following behavioral items:

Creeping speed: confirmed as average speed (mm/s) of continuously creeping (no pause longer than 0.5 s).

Orientation change times: times, recorded as the creeping orientation change angle >30° as one change.

Interval rest times: times, recorded as the wasp kept static > 5 s as one rest.

Time spent to contact host egg: time spent (s) from the wasp being placed in the dish to contacting the eggs and remaining on the surface of any egg over 5s.

2.3.3 Effect on respiratory activity

The respiratory activity of *T. japonicum* after exposure to LC₁₀ chlorantraniliprole was measured by using an insect respiratory measurement system (Xiao et al. 2017). The system included a CO₂ infrared analyzer (Testo535, Munich, Germany), an InPro-6000 model gas O₂ sensor (Mettler-Toled, Switzerland), a stable air pump (SP200EC-LC, Schwarzer-Precision, Germany), an airflow meter, a CO₂ purification tube, a drying tube, a glass sample chamber (2.0 cm diameter and 5.0 cm height), a data collector (LabPro V, Vernier, USA), and a temperature sensor (TMP-112, Texas Instrument, USA). A terminal analysis program (Sable T, Sable System, Denmark) was used to calculate the average respiratory quotient and the respiratory rate based on the collected raw data. We introduced into the sample chamber 10 *T. japonicum* females previously exposed to LC₁₀ chlorantraniliprole residue on glass (see section 2.2). The air pump was subsequently activated and adjusted to an airflow of 0.05 L/min. All sensors and data collectors were maintained for recording over 30 min, and the respiratory quotients and respiratory rates of 10 wasps were calculated by device-paired software. The tests were replicated 10 times for both LC₁₀ of chlorantraniliprole and the control.

2.3.4 Effect on mass reproduction

The impact of LC₁₀ of chlorantraniliprole on *T. japonicum* mass reproduction was assessed. Briefly, 10,000 *Trichogramma* wasps (1-d-old) were treated with the LC₁₀ of chlorantraniliprole, and following the reproduction procedures described in Li et al. (2019), we measured mass reproduction after 1 week and documented the number of wasps/m²/day (the experimental area was 55 m²). The bioassay was replicated 10 times for both LC₁₀ chlorantraniliprole and control.

2.3.5 Effect on field parasitism

A field evaluation was undertaken to evaluate the effect of LC₁₀ chlorantraniliprole on the biocontrol activity of *T. japonicum* towards its host *C. suppressalis* on rice under field conditions. Two square-shaped rice fields (0.2 hectares) were selected in Nanchang County, Jiangxi Province, China. The distance between rice fields was 200m and each field was surrounded by a 3.0-m border with no plants. Pesticide treatments were excluded from the experimental plots.

According to the population dynamics of *C. suppressalis* recorded in the considered region during 2017–2019, two batches of 60,000 *T. japonicum* were released in each field on June 2nd and June 8th 2020. Parasitoids were exposed to the insecticide through spraying *T. japonicum*-parasitized *C. suppressalis* egg cards (black colored eggs, with 5,000 eggs per card) with the LC₁₀ of chlorantraniliprole. Two parasitized host egg cards were then packed in one degradable spherical vessel (2.0 cm in diameter). Two to three vessels

bearing egg cards were released in five randomly selected locations in the experimental plots (control and chlorantraniliprole plots). After five days, the numbers of parasitized *C. suppressalis* eggs were recorded by collecting five *C. suppressalis* egg clusters (approximately containing 35–80 eggs per cluster, as one replicate) from the five randomly selected sites in the chlorantraniliprole + *T. japonicum* and the *T. japonicum* (control) rice fields for calculating the parasitizing proportion as “parasitized *C. suppressalis* eggs number / total *C. suppressalis* eggs number” for each selected site. Fifteen days after the last wasps had been released, we randomly selected 10 sites within the rice fields and observed 40 rice plants in each site as a single replicate in both the LC₁₀ chlorantraniliprole and the control. The following parameters were recorded: n. total dead shoots, n. total of damaged plants by *C. suppressalis* and rice plants with white ear-head. Then the percentage of damaged plants by *C. suppressalis* was calculated as: (total dead shoots number + total damage plant number + number of rice plants with white ears) / 40 plants * 100%.

2.4 Statistical analysis

Datasets were subjected to Levene and Kolmogorov-Smirnov tests for checking checked homogeneity and normality distribution, respectively. Lethal concentrations of chlorantraniliprole against *T. japonicum* through residual contact were determined through probit analysis using PoloPlus 2.0. The concentration–mortality relationship was considered valid when no significant deviation between the observed and the expected data occurred (at $P < 0.05$). A series of independent t-tests were carried out to compare the significance of the biological traits of *T. japonicum*, including parasitism, emergence rate and emerged female rate), locomotion parameters (creep speed, orientation change times, interval rest times, and time spent to contact host eggs), respiration traits (respiratory rate and respiratory quotient), field-parasitized *C. suppressalis* rate and damage proportion on rice plants. All t-tests were performed between the two independent factors LC₁₀ chlorantraniliprole treatment and control treatment at $P = 0.05$ (using SPSS V23).

3 Results

3.1 Acute toxicity on adults

The LC₅₀ and LC₁₀ of chlorantraniliprole on *T. japonicum* after exposure to insecticide residues were 0.741 mg/L (95% confidence limit: 0.590–0.924) and 0.110 mg/L (95% confidence limit: 0.064–0.161), respectively ($\chi^2=1.819$, $df=4$, $P=0.770$, slope=1.57±0.19).

3.2 Sublethal effects

Chlorantraniliprole in LC₁₀ by contact exposure significantly increased the host parasitism by *T. japonicum* ($t = 3.563$, $df = 18$, $P < 0.001$) as well as the parasitoid emergence rate

($t = 2.707$, $df = 18$, $P = 0.014$) when compared to control group (Fig. 1). By contrast, no effect of the LC₁₀ of chlorantraniliprole was observed on the sex ratio of emerging *T. japonicum* ($t = 0.865$, $df = 18$, $P = 0.399$, Fig. 1).

Adult female *T. japonicum* after being exposed to LC₁₀ of chlorantraniliprole increased significantly their creeping speed in comparison to control ($t = 9.117$, $P < 0.001$) and showed more frequent changes in the orientation behavior ($t = 3.061$, $P = 0.01$) (Fig. 2). In addition, significantly fewer interval rest times ($t = -6.682$, $P < 0.001$) and shorter time spent for contacting host eggs ($t = -3.441$, $P < 0.001$) were

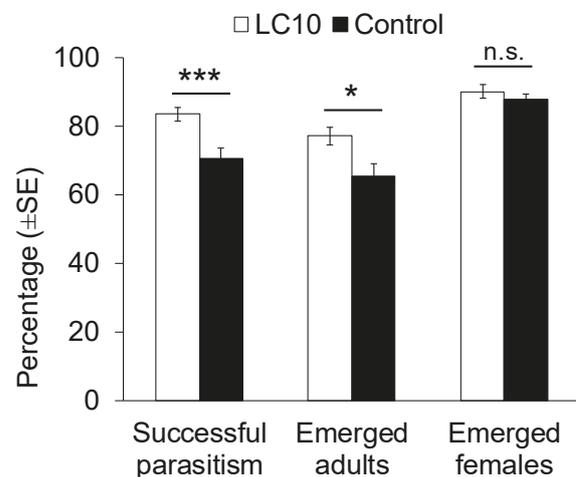


Fig. 1. Mean percentages (\pm SE) of parasitized hosts, successfully emerged adults and females of *T. japonicum* when exposed to LC₁₀ of chlorantraniliprole. ***: $P < 0.001$, *: $P < 0.05$, n.s.: not significant (t-test).

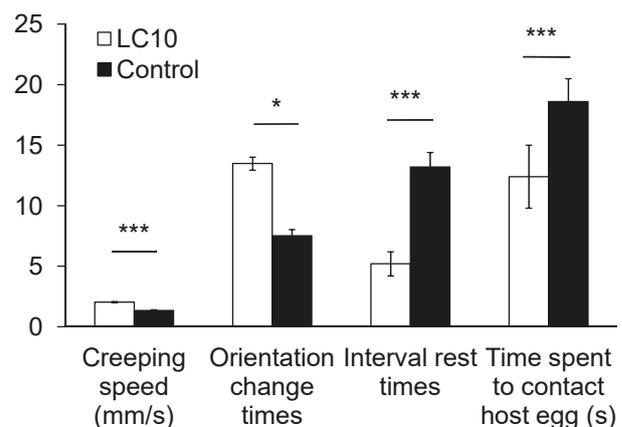


Fig. 2. Mean values (\pm SE) of locomotory performance parameters recorded for *T. japonicum* females exposed to LC₁₀ chlorantraniliprole and for control ($n=10$ for each group). ***: $P < 0.001$, *: $P < 0.05$ (t-test).

observed for *T. japonicum* females that had been exposed to insecticide residues (vs. control).

The respiratory metabolism of *T. japonicum* adults was assessed by measuring the respiratory rate and respiratory quotient. When *T. japonicum* females were exposed to LC₁₀ of chlorantraniliprole there were significant increases in both the respiratory rate and respiratory quotient (7.26±0.23 J/g min and 1.9±0.17, respectively) when compared to the control (3.94±0.14 J/g min and 1.4±0.09, respectively) (respiratory rate: $t = 14.214$, $P < 0.001$, respiratory quotient: $t = 12.344$, $P < 0.001$).

3.3 Effects on mass reproduction

There was a significant increase in *T. japonicum* mass reproduction when parasitoids were exposed to dry residue of LC₁₀ chlorantraniliprole (35766.9 ± 1127.3 parasitoids/day/m²) when compared to the control group (21514.4 ± 2354.2 parasitoids/day/m²) ($t = 5.46$, $d.f. = 18$, $P < 0.001$).

3.4 Effect on field parasitism

Chlorantraniliprole-treated *T. japonicum* applied to suppress *C. suppressalis* in the field showed higher biocontrol service when compared to the control ($t = 3.204$, $d.f. = 4.568$, $P < 0.001$, Fig. 3A). As a result, less plant damage was observed in rice field where chlorantraniliprole-treated parasitoids were released ($t = -8.27$, $d.f. = 18$, $P < 0.001$, Fig. 3B).

4 Discussion

Chlorantraniliprole is one of the most used insecticides against lepidopteran pests in rice fields (Khan & Ruberson 2017) as it is generally considered as safe compound for *Trichogramma* parasitoids that are used for biological control applications in rice cropping systems (Yang et al. 2020). Parasitism and emergence rates are key physiological traits for parasitoid reproduction and biocontrol service that they usually provide. For this, they are routinely considered for assessing sublethal impact of insecticides on insect parasitoids (Abbes et al. 2015; Ricupero et al. 2020a). In our study, the LC₁₀ of chlorantraniliprole increased both parasitism by *T. japonicum* and the emergence rate of parasitoid offspring. In addition, no alteration of the sex ratio was observed with similar number of females offspring in control group vs. LC₁₀ chlorantraniliprole group. By contrast, Parsaeyan et al. (2020) showed that *T. brassicae* emergence rate was reduced by 24% when parental generation was exposed to field rate of chlorantraniliprole. By contrast, no alteration was recorded on the emergence rate of *T. chilonis* after the exposure of egg cards to field concentration of chlorantraniliprole (Brugger et al. 2010).

Time spent for host searching and feeding activities is an important behavioral trait that should be considered when parasitoids are exposed to pesticide residues (Desneux et al. 2004). According to the results of the behavioral assay, *T.*

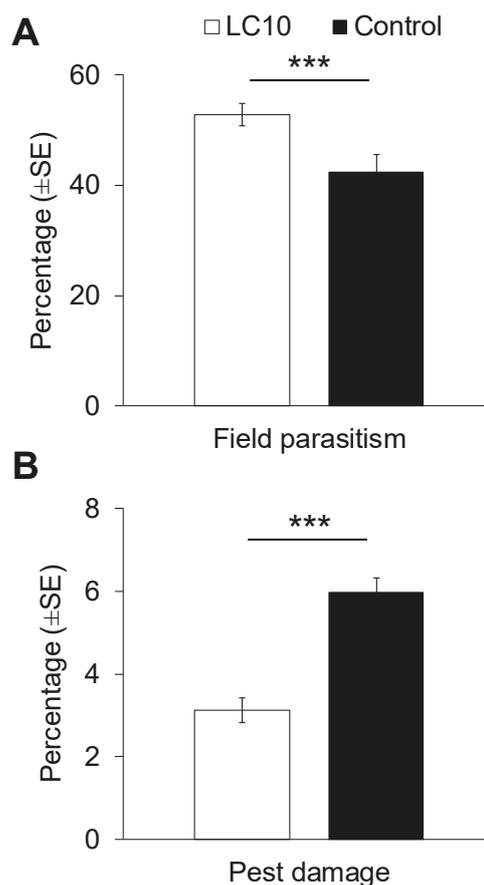


Fig. 3. Mean percentages (±SE) of parasitism on *Chilo suppressalis* by *T. japonicum* when exposed to LC₁₀ of chlorantraniliprole (**A**) and pest damage on rice fields (**B**). ***: $P < 0.001$ (t-test).

japonicum females exposed to LC₁₀ chlorantraniliprole had faster locomotion and spent a shorter time with their hosts. Behavioral changes are the result of toxic compounds that can reduce or increase insect mobility (Desneux et al. 2007). A reduction in insect locomotion after exposure to anthranilic diamides is generally observed as a result of impaired targeted mechanisms in insect body (Hannig et al. 2009; Plata-Rueda et al. 2019; Williams et al. 2020). By contrast, chlorantraniliprole residues at recommended label field rate on plant and inert substrates had no effect on the behavior of the parasitoid *Copidosoma truncatellum* (Hymenoptera: Encyrtidae) (Ramos et al. 2018).

We highlighted for the first time a positive sublethal effect, i.e. an hormesis effect, of chlorantraniliprole on the locomotion of *T. japonicum*. Low pesticide concentrations proved to have both negative and positive effects on natural enemies (Rix and Cutler, 2020). For instance, spinosad LC₂₀ caused a significant decrease in male locomotor activity of *T. chilonis* (Wang et al. 2017), while a sublethal dose of beta-cypermethrin increased the flying activity and walking capacity of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Xiao et al. 2017).

The respiratory results showed an increase in the respiratory metabolism and respiratory quotient of *T. japonicum* adults that were exposed to LC₁₀ of chlorantraniliprole. The increase in respiratory activity might be linked to the increase in locomotion since higher levels of locomotory activity would result in energy demand and, as a consequence, a higher respiration rate (Xiao et al. 2017). By contrast, a reduction of respiratory rate has been associated to a decrease in locomotion for *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) when exposed to chlorantraniliprole residues under laboratory conditions (Plata-Rueda et al. 2019). Nevertheless, insect respiration could be also influenced by metabolic functions that are involved in defense mechanisms against toxicants (Pimentel et al. 2012).

A general increase in mass reproduction of *T. japonicum* adults exposed to LC₁₀ of chlorantraniliprole was demonstrated in our study. Moreover, adults of *T. japonicum* that were preliminary exposed to chlorantraniliprole effectively controlled *C. suppressalis* in the field and less rice plant damage was recorded. These results can be considered as a consequence of an increased parasitism capacity in *T. japonicum* through exposure to the low lethal concentration of chlorantraniliprole. Although predation/parasitism capacity of insect natural enemies is generally considered to be negatively affected when exposed to sublethal or low lethal concentrations of pesticides (He et al. 2012; Yao et al. 2015; Dai et al. 2020; Palma-Onetto et al. 2021), the outcomes of our investigations provided evidence of a chlorantraniliprole-induced hormesis in the parasitoid *T. japonicum*.

Hormesis is used to describe a biphasic model of a dose-response, in which high doses are inhibiting or toxic and low doses are stimulatory or beneficial (Cutler et al. 2022). Previously, positive hormesis effects have been documented as improving physiological responses which could help insect detoxification from contaminated environments (Guedes et al. 2016). However, parasitoids may prolong their pre-adult development, with extended pupal stages of the parasitoid *T. pretiosum* exposed to some chemical pesticides (Carvalho et al. 2003). Considering the positive hormesis effects on natural enemies by different chemicals is therefore essential and should be thoroughly investigated on a case-by-case basis. The mechanisms of hormesis have been widely investigated, potentially stimulating an organism to generate physiological homeostasis overcompensation or trade-off stress adaptation to reform its physiological stability balance (Calabrese 2009). Hormesis responses may also regulate biological or physiological characteristics, which may counteract the inhibitory effects of toxic stressors. The mechanism of action behind hormesis seems to activate detoxification process and energy pathways (Ullah et al. 2020).

Hormesis may trigger the fecundity-longevity trade-off in insects and their relative hormone responses (Yu et al. 2010; Haddi et al. 2016). As other hormesis environmental

stressors (e.g. humidity, salinity, and temperature), sublethal and/or low lethal concentrations of pesticides may influence gene expression in physiological feedback pathways, which could affect fitness-adapted traits (Forbes, 2000; Cutler et al. 2022). Therefore, hormesis effect need to be investigated at multiple tiers, including transgenerational and metabolic levels (Dai et al. 2021; Zhu et al. 2022).

The use of insecticides to control insect pests is not only toxic to beneficial arthropods (Desneux et al. 2007) but also may result in insecticide resistance (Wei et al. 2019; Pires Paula et al. 2021; Zhu et al. 2022). A sustainable and environment-sound use of insecticides should be the key point of future research for optimizing IPM programs (Ragsdale et al. 2011; Han et al. 2022; Thomine et al. 2022); integrating hormesis effect could provide an innovate way for optimizing insecticide applications in IPM (Guedes et al. 2016; Tang et al. 2019).

To conclude, we demonstrated that a low concentration of chlorantraniliprole could induce hormesis effect on multiple biological traits of *T. japonicum*. Therefore, our finding could be helpful to optimize the use of this parasitoid into IPM programs for rice cropping systems. However, further risk assessment of the sublethal impact of chlorantraniliprole on other beneficial arthropods are still needed to comprehensively understand the impact of this selective toxicant in the arthropod community in rice.

Acknowledgments: This work was funded by the National Natural Science Foundation of China (32001904), the Technical Innovation Program of Beijing Academy of Agriculture and Forestry Sciences (KJCX20200110), and the Beijing Technology Program (Z201100008020014).

References

- Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G., ... Zappala, L. (2015). Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. *PLoS One*, *10*(9), e0138411. <https://doi.org/10.1371/journal.pone.0138411>
- Biondi, A., Desneux, N., Siscaro, G., & Zappalà, L. (2012). Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere*, *87*(7), 803–812. <https://doi.org/10.1016/j.chemosphere.2011.12.082>
- Brugger, K., Cole, P., Newman, I., Parker, N., Scholz, B., Suvagia, P., ... Hammond, T. G. (2010). Selectivity of chlorantraniliprole to parasitoid wasps. *Pest Management Science*, *66*(10), 1075–1081. <https://doi.org/10.1002/ps.1977>
- Calabrese, E. (2009). Hormesis: Why it is important to toxicology and toxicologists. *Environmental Toxicology and Chemistry*, *27*(7), 1451–1474. <https://doi.org/10.1897/07-541.1>
- Carvalho, G. A., Reis, R. B., Rocha, L. C. D., Moraes, J. C., Fuiñi, L. C., & Ecole, C. C. (2003). Side-effects of insecticides used

- in tomato field on *Trichogramma pretiosum* (Hymenoptera, Trichiogrammatidae). *Acta Scientiarum. Agronomy*, 25(2), 275–279. <https://doi.org/10.4025/actasciagron.v25i2.1771>
- Chen, H., Huang, S., Zhang, Y., Zeng, X., & Huang, Z. (2010). Control efficacy of *Trichogramma japonicum* against *Chilo suppressalis* and *Chilaraea auricilia*. *Journal of Applied Ecology*, 21(3), 743–748.
- Cordova, D., Benner, E., Sacher, M., Rauh, J., Sopa, J., Lahm, G., ... Tao, Y. (2006). Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pesticide Biochemistry and Physiology*, 84(3), 196–214. <https://doi.org/10.1016/j.pestbp.2005.07.005>
- Cutler, G. C., Amichot, M., Benelli, G., Guedes, R. N. C., Qu, Y., Rix, R. R., ... Desneux, N. (2022). Hormesis and insects: Effects and interactions in agroecosystems. *The Science of the Total Environment*, 825, 153899. <https://doi.org/10.1016/j.scitotenv.2022.153899>
- Dai, C., Ricupero, M., Puglisi, R., Lu, Y., Desneux, N., Biondi, A., & Zappalà, L. (2020). Can contamination by major systemic insecticides affect the voracity of the harlequin ladybird? *Chemosphere*, 256, 126986. <https://doi.org/10.1016/j.chemosphere.2020.126986>
- Dai, C., Ricupero, M., Wang, Z., Desneux, N., Biondi, A., & Lu, Y. (2021). Transgenerational effects of a neonicotinoid and a novel sulfoximine insecticide on the harlequin ladybird. *Insects*, 12(8), 681. <https://doi.org/10.3390/insects12080681>
- Desneux, N., Decourtye, A., & Delpuech, J. (2007). The Sublethal Effects of Pesticides on Beneficial Arthropods. *Annual Review of Entomology*, 52(1), 81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440>
- Desneux, N., Wajnberg, E., Fauvergue, X., Privet, S., & Kaiser, L. (2004). Oviposition behaviour and patch-time allocation in two aphid parasitoids exposed to deltamethrin residues. *Entomologia Experimentalis et Applicata*, 112(3), 227–235. <https://doi.org/10.1111/j.0013-8703.2004.00198.x>
- Forbes, V. (2000). Is hormesis an evolutionary expectation? *Functional Ecology*, 14(1), 12–24. <https://doi.org/10.1046/j.1365-2435.2000.00392.x>
- Guedes, R., Smagghe, G., Stark, J., & Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annual Review of Entomology*, 61(1), 43–62. <https://doi.org/10.1146/annurev-ento-010715-023646>
- Haddi, K., Mendes, M. V., Barcellos, M. S., Lino-Neto, J., Freitas, H. L., Guedes, R. N. C., & Oliveira, E. E. (2016). Sexual success after stress? Imidacloprid-induced hormesis in males of the neotropical stink bug *Euschistus heros*. *PLoS One*, 11(6), e0156616. <https://doi.org/10.1371/journal.pone.0156616>
- Han, P., Lavoie, A.-V., Rodriguez-Saona, C., & Desneux, N. (2022). Bottom-up forces in agroecosystems and their potential impact on arthropod pest management. *Annual Review of Entomology*, 67(1), 239–259. <https://doi.org/10.1146/annurev-ento-060121-060505>
- Hannig, G. T., Ziegler, M., & Marcon, P. G. (2009). Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Management Science*, 65(9), 969–974. <https://doi.org/10.1002/ps.1781>
- He, Y., Zhao, J., Zheng, Y., Desneux, N., & Wu, K. (2012). Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. *Ecotoxicology (London, England)*, 21(5), 1291–1300. <https://doi.org/10.1007/s10646-012-0883-6>
- Huang, N. X., Jaworski, C. C., Desneux, N., Zhang, F., Yang, P. Y., & Wang, S. (2020). Long-term and large-scale releases of *Trichogramma* promote pesticide decrease in maize in north-eastern China. *Entomologia Generalis*, 40(4), 331–335. <https://doi.org/10.1127/entomologia/2020/0994>
- Khan, M. A., & Ruberson, J. R. (2017). Lethal effects of selected novel pesticides on immature stages of *Trichogramma pretiosum* (Hymenoptera: Trichiogrammatidae). *Pest Management Science*, 73(12), 2465–2472. <https://doi.org/10.1002/ps.4639>
- Li, L. J., Lu, X., Zhang, G. H., Zhou, S. X., Chang, X., & Ding, Y. (2019). Study on the nest proportion of female *Trichogramma* to host eggs and time for inoculation aiming at propagation *Trichogramma japonicum* with rice moth eggs. *Journal of Northeast Agricultural Sciences*, 44(5), 34–37.
- Lu, Y. H., Wu, K. M., Jiang, Y., Guo, Y. Y., & Desneux, N. (2012). Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature*, 487(7407), 362–365. <https://doi.org/10.1038/nature11153>
- Menail, H., Boutefnouchet-Bouchema, W., Haddad, N., Taning, C., Smagghe, G., & Wahida, L. (2020). Effects of thiamethoxam and spinosad on the survival and hypopharyngeal glands of the African honey bee (*Apis mellifera intermissa*). *Entomologia Generalis*, 40(2), 207–215. <https://doi.org/10.1127/entomologia/2020/0796>
- Mohammed, A. A., Desneux, N., Fan, Y., Han, P., Ali, A., Song, D., & Gao, X. (2018). Impact of imidacloprid and natural enemies on cereal aphids: Integration or ecosystem service disruption? *Entomologia Generalis*, 37(1), 47–61. <https://doi.org/10.1127/entomologia/2017/0471>
- Nozad-Bonab, Z., Hejazi, M. J., Iranipour, S., Arzanlou, M., & Biondi, A. (2021). Lethal and sublethal effects of synthetic and bio-insecticides on *Trichogramma brassicae* parasitizing *Tuta absoluta*. *PLoS One*, 16(7), e0243334. <https://doi.org/10.1371/journal.pone.0243334>
- Parsaeyan, E., Saber, M., Safavi, S. A., Poorjavad, N., & Biondi, A. (2020). Side effects of chlorantraniliprole, phosalone and spinosad on the egg parasitoid, *Trichogramma brassicae*. *Ecotoxicology (London, England)*, 29(7), 1052–1061. <https://doi.org/10.1007/s10646-020-02235-y>
- Pires Paula, D., Lozano, R. E., Menger, J. P., Andow, D. A., & Koch, R. L. (2021). Identification of point mutations related to pyrethroid resistance in voltage-gated sodium channel genes in *Aphis glycines*. *Entomologia Generalis*, 41(3), 243–255. <https://doi.org/10.1127/entomologia/2021/1226>
- Palma-Onetto, V., Oliva, D., & González-Teuber, M. (2021). Lethal and oxidative stress side effects of organic and synthetic pesticides on the insect scale predator *Rhyzobius lophanthae*. *Entomologia Generalis*, 41(4), 345–355. <https://doi.org/10.1127/entomologia/2021/1045>
- Pimentel, M. A. G., Faroni, L. R. A., Corrêa, A. S., & Guedes, R. N. C. (2012). Phosphine-induced walking response of the lesser grain borer (*Rhyzopertha dominica*). *Pest Management Science*, 68(10), 1368–1373. <https://doi.org/10.1002/ps.3314>
- Pizzol, J., Pintureau, B., Khoualdia, O., & Desneux, N. (2010). Temperature-dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hymenoptera: Trichiogrammatidae). *Journal of Pest Science*, 83(4), 447–452. <https://doi.org/10.1007/s10340-010-0327-0>

- Plata-Rueda, A., Martínez, L. C., Costa, N. C. R., Zanuncio, J. C., de Sena Fernandes, M. E., Serrão, J. E., ... Fernandes, F. L. (2019). Chlorantraniliprole-mediated effects on survival, walking abilities, and respiration in the coffee berry borer, *Hypothenemus hampei*. *Ecotoxicology and Environmental Safety*, *172*, 53–58. <https://doi.org/10.1016/j.ecoenv.2019.01.063>
- Preetha, G., Stanley, J., Suresh, S., Kuttalam, S., & Samiyappan, R. (2009). Toxicity of selected insecticides to *Trichogramma chilonis*: Assessing their safety in the rice ecosystem. *Phytoparasitica*, *37*(3), 209–215. <https://doi.org/10.1007/s12600-009-0031-x>
- Qu, Y., Chen, X., Monticelli, L., Zhang, F., Desneux, N., Dai, H. J., ... Wang, S. (2020). Parasitism performance of the parasitoid *Trichogramma dendrolimi* on the plum fruit moth *Grapholitha funebrana*. *Entomologia Generalis*, *40*(4), 385–395. <https://doi.org/10.1127/entomologia/2020/1059>
- Ragsdale, D. W., Landis, D. A., Brodeur, J., Heimpel, G. E., & Desneux, N. (2011). Ecology and management of the soybean aphid in North America. *Annual Review of Entomology*, *56*(1), 375–399. <https://doi.org/10.1146/annurev-ento-120709-144755>
- Ramos, R. S., de Araújo, V. C., Pereira, R. R., Martins, J. C., Queiroz, O. S., Silva, R. S., & Picanço, M. C. (2018). Investigation of the lethal and behavioral effects of commercial insecticides on the parasitoid wasp *Copidosoma truncatellum*. *Chemosphere*, *191*, 770–778. <https://doi.org/10.1016/j.chemosphere.2017.10.113>
- Ricupero, M., Abbes, K., Haddi, K., Kurtulus, A., Desneux, N., Russo, A., ... Zappalà, L. (2020b). Combined thermal and insecticidal stresses on the generalist predator *Macrolophus pygmaeus*. *The Science of the Total Environment*, *729*, 138922. <https://doi.org/10.1016/j.scitotenv.2020.138922>
- Ricupero, M., Desneux, N., Zappalà, L., & Biondi, A. (2020a). Target and non-target impact of systemic insecticides on a polyphagous aphid pest and its parasitoid. *Chemosphere*, *247*, 125728. <https://doi.org/10.1016/j.chemosphere.2019.125728>
- Rix, R., & Cutler, G. (2020). Low Doses of a Neonicotinoid Stimulate Reproduction in a Beneficial Predatory Insect. *Journal of Economic Entomology*, *113*(5), 2179–2186. <https://doi.org/10.1093/jee/toaa169>
- Sattelle, D., Cordova, D., & Cheek, T. (2008). Insect ryanodine receptors: Molecular targets for novel pest control chemicals. *Invertebrate Neuroscience*, *8*(3), 107–119. <https://doi.org/10.1007/s10158-008-0076-4>
- Tabone, E., Bardon, C., Desneux, N., & Wajnberg, E. (2010). Parasitism of different *Trichogramma* species and strains on *Plutella xylostella* L. on greenhouse cauliflower. *Journal of Pest Science*, *83*(3), 251–256. <https://doi.org/10.1007/s10340-010-0292-7>
- Tang, S., Liang, J., Xiang, C., Xiao, Y., Wang, X., Wu, J., ... Cheke, R. (2019). A general model of hormesis in biological systems and its application to pest management. *Journal of the Royal Society, Interface*, *16*(157), 20190468. <https://doi.org/10.1098/rsif.2019.0468>
- Thomine, E., Mumford, J., Rusch, A., & Desneux, N. (2022). Using crop diversity to lower pesticide use: Socio-ecological approaches. *The Science of the Total Environment*, *804*, 150156. <https://doi.org/10.1016/j.scitotenv.2021.150156>
- Ullah, F., Gul, H., Tariq, K., Desneux, N., Gao, X., & Song, D. (2020). Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. *Pesticide Biochemistry and Physiology*, *165*, 104557. <https://doi.org/10.1016/j.pestbp.2020.104557>
- Wang, D., Lü, L., & He, Y. (2017). Effects of two conventional insecticides on male-specific sex pheromone discrimination and mate choice in *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Environmental Entomology*, *46*(2), 328–334. <https://doi.org/10.1093/ee/nvw172>
- Wang, P., Li, M.-J., Bai, Q.-R., Ali, A., Desneux, N., Dai, H.-J., & Zang, L.-S. (2021). Performance of *Trichogramma japonicum* as a vector of *Beauveria bassiana* for parasitizing eggs of rice striped stem borer, *Chilo suppressalis*. *Entomologia Generalis*, *41*(2), 147–155. <https://doi.org/10.1127/entomologia/2021/1068>
- Wei, Y., Yan, R., Zhou, Q., Qiao, L., Zhu, G., & Chen, M. (2019). Monitoring and Mechanisms of Chlorantraniliprole Resistance in *Chilo suppressalis* (Lepidoptera: Crambidae) in China. *Journal of Economic Entomology*, *112*(3), 1348–1353. <https://doi.org/10.1093/jee/toz001>
- Williams, J. R., Swale, D. R., & Anderson, T. D. (2020). Comparative effects of technical-grade and formulated chlorantraniliprole to the survivorship and locomotor activity of the honey bee, *Apis mellifera* (L.). *Pest Management Science*, *76*(8), 2582–2588. <https://doi.org/10.1002/ps.5832>
- Xiao, D., Tan, X., Wang, W., Zhang, F., Desneux, N., & Wang, S. (2017). Modification of Flight and Locomotion Performances, Respiratory Metabolism, and Transcriptome Expression in the Lady Beetle *Harmonia axyridis* through Sublethal Pesticide Exposure. *Frontiers in Physiology*, *8*, 33. <https://doi.org/10.3389/fphys.2017.00033>
- Yang, Y., Wang, C., Xu, H., Tian, J., & Lu, Z. (2020). Response of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) to insecticides at concentrations sublethal to *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, *113*(2), 646–653. <https://doi.org/10.1093/jee/toz325>
- Yao, F., Zheng, Y., Zhao, J., Desneux, N., He, Y., & Weng, Q. (2015). Lethal and sublethal effects of thiamethoxam on the whitefly predator *Serangium japonicum* (Coleoptera: Coccinellidae) through different exposure routes. *Chemosphere*, *128*, 49–55. <https://doi.org/10.1016/j.chemosphere.2015.01.010>
- Yu, Y., Shen, G., Zhu, H., & Lu, Y. (2010). Imidacloprid-induced hormesis on the fecundity and juvenile hormone levels of the green peach aphid *Myzus persicae* (Sulzer). *Pesticide Biochemistry and Physiology*, *98*(2), 238–242. <https://doi.org/10.1016/j.pestbp.2010.06.013>
- Zang, L., Wang, S., Zhang, F., & Desneux, N. (2021). Biological control with *Trichogramma* in China: History, present status, and perspectives. *Annual Review of Entomology*, *66*(1), 463–484. <https://doi.org/10.1146/annurev-ento-060120-091620>
- Zhang, X., Wang, H. C., Du, W. M., Zang, L. S., Ruan, C. C., Zhang, J.-J., ... Desneux, N. (2021). N. Multi-parasitism: A promising approach to simultaneously produce *Trichogramma chilonis* and *T. dendrolimi* on eggs of *Antheraea pernyi*. *Entomologia Generalis*, *41*(6), 627–636. <https://doi.org/10.1127/entomologia/2021/1360>
- Zheng, X., Ren, X., & Su, J. (2011). Insecticide Susceptibility of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) in China. *Journal of Economic Entomology*, *104*(2), 653–658.
- Zhu, W., Wang, J., & Zhang, Y. (2022). The Mechanism of Chlorantraniliprole Resistance and Detoxification in *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Journal of Insect Science*, *22*(4), 7.

Manuscript received: 7 April 2022

Revisions requested: 15 May 2022

Modified version received: 27 July 2022

Accepted: 6 October 2022