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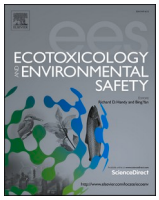

Effects of chlorantraniliprole on the development, fecundity and prey consumption of a non-specific predator, *Rhynocoris fuscipes* (Hemiptera: Reduviidae)

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ABSTRACT

Transplant treatment with chlorantraniliprole (CAP) is a proactive approach to protect transplanted plants from pests during early establishment and has been comprehensively applied in tobacco fields in Guangdong Province, China. However, it is not known whether the high dose of CAP in transplant treatments has lethal or sublethal effects on the generalist predator *Rhynocoris fuscipes* Fabricius (Hemiptera: Reduviidae). To address this concern, the mortalities of *R. fuscipes* were assessed when 2nd instar larvae of *R. fuscipes* were in direct contact with or consuming CAP and when their eggs were exposed to CAP. Furthermore, 2nd instar nymphs *R. fuscipes* were long-term exposed to CAP until they reached adulthood, and their life table parameters were determined. After exposure to CAP, the activity of detoxification enzymes (P450, CaeE and GST) and the functional respond of *R. fuscipes* to their preys *Agrotis ipsilon* larvae were determined. In this study, CAP at all concentrations did not significantly increase the mortality of 2nd instar of *R. fuscipes* nymphs in comparison with the control. The detoxification enzyme (P450, CarE and GST) activities and the number of *A. ipsilon* larvae consumed by *R. fuscipes* in the transplant treatment were not affected by CAP after 3-d or long-term exposure. These results indicated that CAP was harmless to *R. fuscipes* according to IOBC protocols. However, during the treatment of 2nd instar nymphs with a label rate of 15 g AI/ha and a 5× label rate of 75 g AI/ha, CAP significantly prolonged the pre-adult and pre-oviposition periods, and treated adults had lower oviposition. Attention should be given to the time interval between transplant treatment and the release of this biocontrol agent into the field to minimize the impact of CAP on the predator *R. fuscipes*.

1. Introduction

The treatment of transplanted plants with systemic insecticides is a proactive approach to prevent insect pests from damaging crops and is currently widely adopted for rice (*Oryza sativa* L.), cabbage (*Brassica oleracea* L.) and tobacco (*Nicotiana tabacum* L.), among other crops (Liao et al., 2013; Cameron et al., 2015; Liu et al., 2023). It can be more practical, effective and economical than broadcast sprays for managing early-season foliar pests and thus helps establish seedlings in the field (Cameron et al., 2015). Chlorantraniliprole (CAP), an anthranilic diamide, has been used as a transplant treatment because of its xylem mobility and systemic properties for managing numerous lepidopteran moths, including *Agrotis ipsilon* (Hufnagel) and *Chloridea virescens* (Fabricius) in tobacco fields (Liu et al., 2023; Zilnik et al., 2021) and *Chilo suppressalis* (Walker) and *Cnaphalocrocis medinalis* (Guenée) in paddy fields (Cheng, 2013). By activating the ryanodine receptors of insects that control calcium release from muscle cells, CAP results in feeding cessation, paralysis and death in insects (Lahm et al., 2005, 2007).

Systemic insecticides, such as CAP, are considered to only contact pests when they feed on threatened plants, reducing the number of non-target organisms exposed to insecticides (Sánchez-Bayo et al., 2013).

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However, to extend the pest control duration, a high dose of insecticide is applied to plants during transplant treatment (e.g., a 5× label application rate for tobacco transplant plants (Liu et al., 2023)), which raises concerns about the adverse effects of insecticides on crops, nontarget organisms and the environment (Gontijo et al., 2014; Yao et al., 2022).

Although CAP was reported to generally have low toxicity against beneficial insects such as parasitoids, e.g., the parasitoid wasps *Aphelinus mali* (Haldeman), *Dlichogenidea tasmanica* (Cameron) and *Trichogramma* species, and the predatory bugs *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stål) (Brugger et al., 2010; de Castro et al., 2013), several studies revealed that this insecticide increased the mortality of predators belonging to Neuroptera (lacewings) (*Chrysoperla carnea* (Stephens)) (Gontijo et al., 2014), Coleoptera (bettles) (*Cycloneda sanguinea* (L.), *Chauliognathus flavipes* Fabricius, *Hippodamia convergens* (Guérin-Méneville), *H. axyridis*, *Coccinella septempunctata* L., *Paederus fuscipes* Curtis) (Barbosa et al., 2017; Fernandes et al., 2016; He et al., 2019; Khan et al., 2021; Nawaz et al., 2017) and Hemiptera (bugs) (*Cyrtorhinus lividipennis* Reuter, *Orius insidiosus* (Say), *Rhynocoris marginatus* Fabricius) (Fernandes et al., 2016; Patel, 2020; Yang et al., 2012). Insecticides, even at non-lethal levels, may block some physiological or biochemical processes, impacting the survival, growth, development, reproduction and behaviour of natural enemies of insect pests. For example, at sublethal doses, CAP adversely affects long-term life table parameters of the Asian ladybird *Harmonia axyridis* (Pallas) (Nawaz et al., 2017).

The reduviid bug *Rhynocoris fuscipes* Fabricius (Hemiptera: Reduviidae) is a generalist predator that is abundant in tobacco, cotton and paddy fields in subtropical and tropical areas (Deng et al., 2014; Tomson et al., 2017). It has been reported to predate upon more than 42 insect species (Sahayaraj and Selvaraj, 2003) and has been used as a biocontrol agent to suppress populations of pests such as *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) (Deng et al., 2012), *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) (Deng et al., 2012) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Deng et al., 2015) in tobacco fields. Since CAP is widely used in tobacco fields (Liu et al., 2023), to maintain and conserve predator populations in the field, the compatibility of *R. fuscipes* with CAP needs further study.

Therefore, the present study evaluated the acute toxicity and ecotoxicological risks of CAP to *R. fuscipes* under laboratory conditions. Subsequently, the effects of long-term exposure to field-recommended CAP doses on the growth, development, survival, longevity, fecundity, and population parameters of *R. fuscipes* were determined using an age-stage, two-sex life table. Finally, the functional response of *R. fuscipes* to *A. ipsilon* and the activity of three detoxification enzymes (glutathione S-transferase (GST), cytochrome P450 monooxygenase (P450) and carboxylesterase (CarE)) were tested under both 3-d and long-term exposure. These experiments were conducted to obtain information on the compatibility of CAP and the predator *R. fuscipes* in agrosystems and thus improve the integrated pest management (IPM) of lepidopteran pests such as *A. ipsilon*.

2. Materials and methods

2.1. Insects and transplanted seedlings

The colony of *R. fuscipes* used in the experiments was originally established using adults collected in Nanxiong city, China (25°6' 12" N, 114°17'12" E), in 2020. The sex of adults was determined based on the methodology of Huang (2007). Females and males were grouped into pairs in mesh-covered cages (28 × 18 × 9.5 cm) and fed mealworm larvae (*Tenebrio molitor* L.). Tobacco leaves were used as spawning substrates. Eggs were collected and hatched in new cages. Newly hatched nymphs were fed mealworm larvae and mass reared to the 2nd instar stage. Then, they were placed and reared individually in plastic vials (dia. 3 × 3 cm) to adult emergence. The 2nd instar nymphs used in our experiment were all molted within the previous 24 h.

The colony of *A. ipsilon* used in the experiments was originally established using larvae collected in Guangzhou City in China (23°10'12" N, 113°16'12" E), in 2020 and then maintained in the laboratory on an artificial diet (Zhang et al., 2012). After larval maturation, pupae were transferred to a plastic container (dia. 12.5 × 10.5 cm) for adult emergence, and the adult moths were then fed 10% sucrose water. Pieces of cabbage (*B. pekinensis*) leaves were used as the oviposition substrate.

The insects were reared, and all experiments were conducted at 28 ± 1 °C, 70 ± 5% relative humidity (RH) and a light:dark photoperiod (L:D) of 16:8 h in incubators (QX-256, Jiangnan Instrument Factory, Ningbo).

The tobacco variety Yueyan 97 was supplied by the Guangdong Institute of Tobacco Science. Seeds of tobacco were sown in a moist commercial mix composed of 45% vermiculite, 35% peat moss and 20% organic matter (Xianghui Agriculture Technology, Hunan, China) and grown at 26 ± 1 °C, 70% RH and a 16:8 h (L:D) photoperiod in a growth chamber. After 16 d, each seedling was transferred from the tray to a plastic pot (diam. 9 cm ×10 cm) filled with the same commercial mix and watered as needed. The tobacco plants used in our experiment had 7–8 fully expanded true leaves.

2.2. Acute toxicity of CAP to R. fuscipes nymphs and A. ipsilon larvae

The contact toxicity of CAP to *R. fuscipes* nymphs was determined as described by Snodgrass (1996) with slight modifications. In the preliminary experiment, none of the tested concentrations (0.1, 10, 100, or 1000 mg AI/L) caused significant mortality of *R. fuscipes*. Therefore, 1000 mg of AI/L CAP in acetone was used in this experiment and was diluted to 5 concentrations (62.5, 125.0, 250.0, 500.0, and 1000.0 mg AI/L). In brief, CAP (99.3%, Shanghai Pesticide Research Institute Co., Ltd., China) was dissolved in acetone at 1 g AI/L, and the solutions were diluted with acetone. One millilitre of the above solution was transferred to a glass tube (dia. 2.7 × 4.2 cm, plugged with cotton), and the glass tube was rotated until the solvent evaporated to form a uniform film. Acetone only was used as a control. The 2nd instar nymphs were randomly selected and introduced into the treated glass tube. After 3 d, the nymphs were transferred to new glass tubes and provided with adequate mealworm larvae. For the contact toxicity of CAP to 2nd instar *A. ipsilon* larvae, the concentrations of CAP in acetone were 1.55, 3.10, 6.20, 12.40, 24.80, 49.60 and 82.66 mg AI/L, and the assay procedure was the same as that described above.

To assess the ingestion toxicity of CAP to *R. fuscipes* nymphs, the active ingredients of CAP were dissolved in acetone at 2 g AI/L, and the solutions were diluted with 30% sucrose in 0.1% Triton X-100 water solution to obtain a series of five concentrations: 12.5, 25, 50, 100 and 200 mg AI/L. According to the preliminary experiment, the maximum concentration that the *R. fuscipes* nymphs were willing to consume was 200 mg AI/L CAP in 30% sucrose in a 0.1% Triton X-100 water solution. Thirty mL of the solution was placed on a piece of parafilm (1 × 1 cm), and the parafilm was placed on the bottom of a Petri dish (dia. 6 cm). Control was acetone: above solution = 1: 9 in volume. The 2nd instar nymphs were randomly selected and starved for 3 d individually. Then, they were introduced to the Petri dish with sucrose solution as a food source. The solution was renewed daily, and after 72 h, the nymphs were provided with sufficient mealworm larvae, and the parafilm was removed.

The toxicity of CAP to the eggs of *R. fuscipes* was evaluated in a dipped surface assay. CAP in acetone at 2 g AI/L was diluted with 0.1% Triton X-100 water to create a series of solutions according to the preliminary experiment and the reasons abovementioned. Acetone: 0.1% Triton X-100 water = 1:9 in volume was used as a control. *R. fuscipes* egg clusters containing 9–20 eggs each were then dipped in the solutions for 10 s. After hatching, the number of nymphs was recorded, and their mortalities were recorded for 2 weeks.

There were 3 replicates per concentration and 10 nymphs or 25–32 eggs in each replicate. Both acute toxicity assays were performed under the same conditions used for rearing. Mortalities were recorded daily for

2 weeks. Nymphs were considered dead if they remained motionless when poked with a fine paint brush. The mortality and hatching rate were calculated as: mortality (%) = the number of dead nymphs/total number of treated nymphs × 100%. Hatching rate (%) = the number of hatched nymphs/the number of treated eggs × 100%.

2.3. Life table of R. fuscipes exposed to 15 and 75 g AI/ha CAP

Because the results of the acute toxicity assays showed that CAP did not affect the mortality of *R. fuscipes* nymphs, the life tables of *R. fuscipes* were investigated after they were exposed to 15 and 75 g AI/ha CAP from 2nd instar nymphs, which are the label rates and recommended doses to protect tobacco plants against *A. ipsilon* according to our previous study (Liu et al., 2023). Given that arthropod predators may be exposed to insecticides in the field by topical contact with sprayed transplant plants, 2nd instar nymphs were exposed to CAP according to the contact toxicity assay procedure described in Section 2.2, except that the nymphs were reared in glass tubes until adulthood. The nominal application dosages per glass tube were 15 and 75 g AI/ha, and acetone only was used as a control. There were 60 nymphs per treatment. The mortality and stage of *R. fuscipes* nymphs were recorded daily until the adult stage. Then, the sex ratio was recorded. Newly emerged *R. fuscipes* adults in each treatment group were randomly paired, transferred to a clean container and reared under the rearing procedure. The survival rate and quantity of eggs were recorded daily. Tobacco leaves with *R. fuscipes* eggs were kept in an incubator, and the hatching rate was recorded.

2.4. Detoxification enzyme activity assays

Second instar *R. fuscipes* nymphs were treated the same as in the contact toxicity assay described in Section 2.2. After 72 h of exposure or adult emergence (within 48 h), live *R. fuscipes* were collected, frozen in liquid nitrogen and stored at -80 °C. For the nymphs exposed to insecticide for 3 d, there were 5 nymphs in each biological replicate, and for those under long-term exposure, a single adult was treated as a biological replicate. Each treatment consisted of 7 biological replications. The procedures for the activity assays of each enzyme followed the manufacturer's instructions for the relevant kits. The total protein quantity was determined by an assay kit (Product ID: BL521A, Biosharp®, Anhui, China), and the detoxification enzyme activity was tested using a CYP450 ELISA Kit (Product ID: JM-12141O1), GST ELISA Kit (Product ID: JM-00073O1) and CarE ELISA Kit (Product ID: JM-00067O1) purchased from Jiangsu Jingmei Biotechnology Co., Ltd. (Jiangsu, China). The optical density was recorded using a microplate reader (Thermo Varioskan Flash, Thermo, USA). The activity of all enzymes was analysed and is presented as nmol min^{-1} mg^{-1} protein.

2.5. Functional response tests

To assess the impacts of transplant treatment on the effectiveness of *R. fuscipes* as a biocontrol agent, the functional response of *R. fuscipes* to two CAP exposure intervals during transplant treatments was investigated. The 2nd instar nymph was fed with adequate 2nd instar *A. ipsilon* larvae for 24 h. Then, each nymph was placed on a tobacco seedling that was treated 24 h in advance with CAP at 15, 75 g AI/ha (Coragen®, FMC®, US) or water only (control) in a cage (mesh sizes 0.15 mm).

First, after 72 h of exposure (starved), the *R. fuscipes* nymphs were transferred to experimental plastic Petri dishes (dia. 18 cm) containing 2nd instar *A. ipsilon* larvae at different densities (10, 20, 40, 80, 100 prey/predator).

Second, the *R. fuscipes* nymphs were fed an adequate amount of *A. ipsilon* larvae and reared in a cage. After adults emerged (previous 48 h), *R. fuscipes* adults were individually transferred to plastic Petri dishes and starved for 72 h. The predator was then provided with 4th instar *A. ipsilon* larvae at different densities (5, 10, 15, 30, 50, 70 prey/predator).

For both functional response tests, the amount of prey consumed by the predator was recorded after 24 h. All of the predators were employed for the test only once. Moreover, a control treatment was carried out without predators. Each density in the treatments included 5 replicates.

2.6. Data analysis

The data on CAP acute toxicity were analysed based on a log-rank test comparing the Kaplan—Meier survival curves using SPSS 21 software (IBM, New York, USA).

The LC_{50} of CAP to *A. ipison* was calculated via probit analysis and a χ^2 analysis was applied to test whether the probit lines were parallel and to test the heterogeneity of the residuals as described by Barbosa et al. (2017) and Wu et al. (2021) using SPSS 21.

The life table parameters were calculated according to two-sex life table theory (Chi and Liu, 1985; Chi, 1988; Chi and Su, 2006; Tuan et al., 2014a, b) using TWOSEX-MSChart (Chi, 2016).

The functional response data were analysed in two steps using SAS. The first step was to determine the shape of the functional response. To discriminate between type II and type III functional responses, a polynomial logistic regression was performed between the proportion of prey consumed (N_e/N_0) and the initial prey density (N_0) (Juliano, 2001; Timms et al., 2008): $N_e/N_0 = a+bN_0+cN_0^2+dN_0^3+e$, where N_e = number of prey consumed; N_0 = initial number of prey; a = intercept; b = linear coefficient; c = quadratic coefficient; and d = cubic coefficient. The sign of the linear coefficient (b) can be used to distinguish the type of functional response: if $b < 0$, the functional response is type II; if $b > 0$ and $c < 0$, the functional response is type III. Then, the handling time and attack rate were estimated using non-linear least squares regressions. Since prey items were not replaced during the experimental period, the random predator equation of Rogers was used to describe the type II functional response parameters (Rogers, 1972): $N_a=N_0\{1\text{-}\exp[\alpha(T_hN_a\text{-}T)]\}$, where N_a = number of prey consumed, N_0 = initial number of prey, α = attack rate, T_h = handling time and T = experimental time.

The life table parameters, weight of *R. fuscipes* adults and metabolic enzyme activities of *R. fuscipes* were analysed using SPSS 21 and first tested for normality. For normally distributed data, one-way ANOVA was used to determine the effect of treatment, and the means of the treatments were compared using Duncan's multiple range test. For the non-normally distributed data, a non-parametric method, the Kruskal—Wallis test with Bonferroni corrections, was used for multiple comparisons.

3. Results

3.1. Acute toxicity of CAP to R. fuscipes

In the acute toxicity assay, the log-rank test showed no significant differences between the cumulative survival curves of 2nd instar *R. fuscipes* exposed to different CAP concentrations for 3 d by contact (df = 1, χ^2 = 0.6619, p = 0.4159; Fig. 1). The highest mortalities occurred at 200 mg/L and 1 g/L, which was 6.67% overall during the 2-week observation. Moreover, the LC_{50} of CAP for 3rd instar larvae of *A. ipsilon* was 16.67 mg AI/L after 3 d of exposure (Table S1).

Similarly, in the ingestion toxicity assay, CAP did not affect the survival rate of 2nd instar *R. fuscipes* nymphs (df = 1, χ^2 = 0.9389, p = 0.3326; Fig. S1) during the 2-week observation period. No nymphs died in the control, and the mortality of the insecticide treatment group ranged from 3.33–6.67%. The hatching rates of *R. fuscipes* eggs after exposure to insecticide solution ranged from 89.00–91.40%. Exposure to CAP had no effect on the hatching rate of *R. fuscipes* eggs ($F_{5,12}$ = 0.095, p = 0.991; Fig. S2A) or the survival rate of nymphs after hatching (df = 1, χ^2 = 0.4525, p = 0.5012; Fig. S2B). The mortality of nymphs was 3.80% in the control treatment and 3.85–6.67% in the CAP

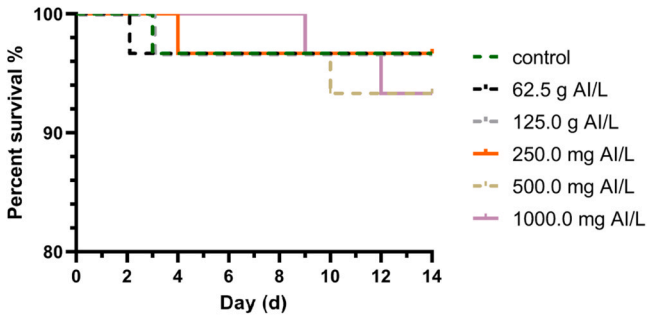

Fig. 1. Survival curves of 2nd instar nymphs of *R. fuscipes* exposed to five CAP concentrations by contact for 3 d. n = 30.

treatment.

3.2. Age-stage, two-sex life table of R. fuscipes with long-term exposure to CAP

When the *R. fuscipes* nymphs were exposed to two field-recommended CAP doses from the 2nd instar to the adult stage, the insecticide treatments affected the 4th and 5th instar duration, pre-oviposition period of adult females and fecundity (Table 1, 4th instar: U = 28.706, p = 0.009; 5th instar: U = 28.706, p = 0.009; pre-oviposition: $F_{2,66}$ = 8.784, $p < 0.0001$; fecundity: $F_{2,66}$ = 5.867, p = 0.005). In particular, treatment with 75 g AI/ha prolonged the 5th instar duration by an average of 2.8 d and the pre-oviposition duration by 1.7 d (Table 1). Compared with the control, female *R. fuscipes* exposed to CAP laid 15.8 fewer eggs at 15 g AI/ha and 11.0 fewer eggs at 75 g AI/ha. There was a significant difference in nymph stage duration and fecundity between the control and insecticide treatments (Fig. 2, Fig. S3, Fig. S4). However, there was no significant effect of insecticide treatment on the early stage of development (2nd or 3rd instar), the body weight of adults or the longevity of adult females or males (Table 1, $p > 0.05$). The female ratios of the control, 15 g AI/ha and 75 g AI/ha treatments ranged from 0.43–0.44, and the offspring hatching rates were 95.92%, 95.66% and 96.48%, respectively.

Long-term CAP exposure had an effect on the net reproduction rate (R_0), mean generation time (T), intrinsic rate of increase (r) and finite rate of increase (λ) (Table 2, R_0: $F_{2,299997}$ = 21690.4, $p < 0.0001$; T: $F_{2,299997}$ = 860131.9, $p < 0.0001$; r: $F_{2,299997}$ = 109573.9, $p < 0.0001$; λ: $F_{2,299997}$ = 109741.5, $p < 0.0001$). The net reproduction rate (R_0) of the control was greater than that of the insecticide treatments, but the lowest R_0 value was observed at 15 g AI/ha. The mean generation time (T) was prolonged when the insecticide dose increased and was extended by 1.18 and 5.15 d at 15 g AI/ha and 75 g AI/ha, respectively. The intrinsic rate of increase (r) decreased from 0.0575 to 0.0519 d^{-1} with increasing doses of CAP, as did the finite rate of increase (λ), which decreased from 1.0592 to 1.0533 d^{-1}.

Table 1
Age-specific life table of *R. fuscipes* exposed to two field-recommended CAP concentrations from the 2nd instar stage. The values are the means ± SEs. Different letters in the same row indicate significant differences among treatment groups according to the Bonferroni correction post hoc test or Duncan's multiple range test ($p < 0.05$).

Parameter	Control		15 g AI/ha		75 g AI/ha	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Egg duration (d)	60	5.00 ± 0.00 a	60	5.00 ± 0.00 a	60	5.00 ± 0.00 a
1st instar duration (d)	60	7.00 ± 0.00 a	60	7.00 ± 0.00 a	60	7.00 ± 0.00 a
2nd instar duration (d)	58	6.60 ± 0.14 a	59	6.25 ± 0.15 a	58	6.07 ± 0.16 a
3rd instar duration (d)	56	7.25 ± 0.17 a	55	7.55 ± 0.18 a	57	7.30 ± 0.16 a
4th instar duration (d)	56	7.91 ± 0.17 b	52	8.83 ± 0.25 a	55	8.58 ± 0.24 a
5th instar duration (d)	56	10.70 ± 0.27 b	52	10.48 ± 0.35 b	53	13.45 ± 0.37 a
Pre-oviposition period (d)	23	6.65 ± 0.31 b	23	8.65 ± 0.29 a	24	8.30 ± 0.46 a
Female ratio	23	0.43	23	0.44	24	0.44
Adult body weight (mg)	53	53.70 ± 0.82 a	52	55.01 ± 0.89 a	54	54.62 ± 0.05 a
Fecundity (eggs/female)	23	113.48 ± 3.55 a	23	97.70 ± 3.73 b	24	102.48 ± 2.64 b
Offspring hatching rate (%)	1130	95.92	967	95.66	1023	96.48
Female adult longevity (d)	23	49.22 ± 1.37 a	23	47.09 ± 1.72 a	24	50.91 ± 0.89 a
Male adult longevity (d)	30	40.40 ± 1.27 a	29	39.17 ± 1.72 a	30	38.60 ± 0.93 a

3.3. Activities of detoxification enzymes

To determine the potential role of detoxification enzymes in tolerance to CAP, the activities of P450, GST and CarE in *R. fuscipes* were analysed. The results showed that the mean activity values of the three tested detoxification enzymes were lower in the insecticide treatments than in the control (Table 3). However, the activities of all three detoxification enzymes did not significantly differ between the two recommended field dose groups and the control group after 3 d of exposure (P450: $F_{2,18}$ = 0.552, p = 0.585; GST: $F_{2,18}$ = 0.616, p = 0.551; CarE: $F_{2,18}$ = 0.256, p = 0.777) or after long-term exposure (P450: $F_{2,18}$ = 0.540, p = 0.594; GST: $F_{2,18}$ = 0.127, p = 0.882; CarE: $F_{2,18}$ = 0.407, p = 0.673).

3.4. Functional response of R. fuscipes to A. ipsilon

The consumption of *A. ipsilon* by *R. fuscipes* in the control group was fitted to a type II functional response (Table S2), and exposure to CAP in the transplant treatment group did not change the shape of the functional response, as revealed by the negative values of the linear parameter (all b < 0, $p > 0.05$). The number of *A. ipsilon* consumed by *R. fuscipes* increased with increasing density of *A. ipsilon* (Fig. 3). Based on the overlapping 95% CIs (Table 4), the attack rate (a) and handling time (T_h) of *R. fuscipes* were not significantly different between treatments with different exposure durations or CAP doses and the control. When 2nd instar *R. fuscipes* were exposed to CAP for 3 d and fed on 2nd instar *A. ipsilon* larvae, the maximum attack rates (T/T_h) were 87.29, 74.07 and 67.11 larvae for the control and 15 and 75 g AI/ha treatments, respectively. When adults of *R. fuscipes* (which emerged within 48 h) were exposed to CAP from the 2nd instar and fed 4th instar *A. ipsilon* larvae, the maximum attack rates were 18.98, 33.90 and 21.98 larvae for the control and 15 and 75 g AI/ha treatments, respectively. The search rate of the predator decreased with increasing *A. ipsilon* density, which showed that the predator needed to spend more time searching for prey (Fig. S5).

4. Discussion

Pesticide hazards to non-target organisms have been a growing global problem for decades (Sánchez-Bayo et al., 2013). To our knowledge, only one reference has evaluated the effect of insecticides on *R. fuscipes* (George and Ambrose, 1999), and no studies have evaluated the effect of CAP on *R. fuscipes*. The results of our acute toxicity assays showed that regardless of the exposure route (contact, ingestion or predator egg application) or duration (3 d or until adult), CAP did not cause mortality in *R. fuscipes* (Fig. 1, S1, S2, Table 1). In our study, the maximum concentration of CAP to which *R. fuscipes* was exposed was 1 g AI/L (Fig. 1), whereas the LC_{50} values of two important tobacco

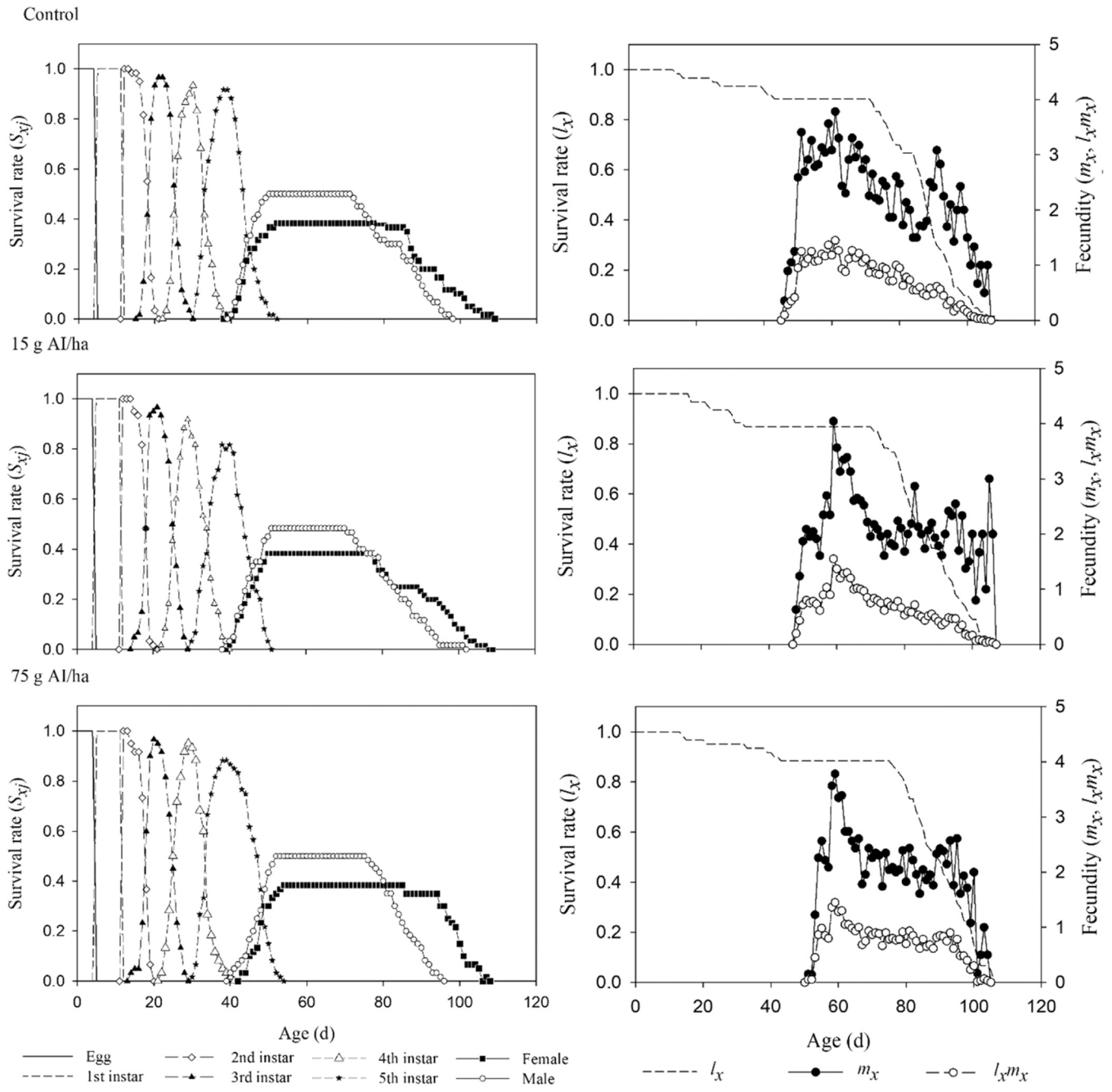

Fig. 2. Age-stage survival rate (s_{xj}), survival rate (l_x) and age-specific fecundity (m_x) of *R. fuscipes* exposed to two field-recommended CAP concentrations from the 2nd instar stage.

Table 2
Population parameters of *R. fuscipes* exposed to two field-recommended CAP concentrations from the 2nd instar stage. Values = mean ± SE. Different letters in the same row indicate significant differences among treatment groups according to Duncan's multiple range test ($p < 0.05$).

Parameter	Control	15 g AI/ha	75 g AI/ha
R_0 (offspring/individual)	43.50 ± 7.23 a	37.45 ± 6.29 c	39.28 ± 6.49 b
T (d)	65.60 ± 0.78 c	66.78 ± 1.19 b	70.75 ± 0.73 a
r (d^{-1})	0.0575 ± 0.0028 a	0.0543 ± 0.0027 b	0.0519 ± 0.0026 c
λ (d^{-1})	1.0592 ± 0.0029 a	1.0558 ± 0.0029 b	1.0533 ± 0.0027 c

lepidopteran moths were 16.70 mg AI/L (contact toxicity, Table S1), 0.28 mg AI/L for *A. ipsilon* (digestion toxicity) (Liu et al., 2023) and 28.4–102.5 μg/L for *S. litura* (Su et al., 2012), which were much lower than the tested concentrations in our study. According to the International Organization for Biological Control (IOBC) protocols, the hazards of CAP to *R. fuscipes* can be classified as harmless (classes: 1 = harmless (E < 30%), 2 = slightly harmful (30 < E < 79%), 3 = moderately harmful (80 < E < 99%), 4 = harmful (E > 99%)) (Stark et al., 2007), which indicates that CAP is potentially compatible with the predator *R. fuscipes*. This finding was consistent with previous works showing that CAP did not cause mortality in other Hemiptera predator bugs, such as *O. laevigatus* (Hemiptera: Anthocoridae), *M. pygmaeus* (Rambur), *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) and *Eocanthecona furcellata* (Wolff)

Table 3
Activity of detoxification enzymes in *R. fuscipes* exposed to two field-recommended CAP doses from the 2nd instar stage for 3 days or until adulthood (emerged within 48 h). Values = mean ± SE. There were no significant differences ($p > 0.05$) in the mean activity values in the same column.

Treatment (g AI/ha)	P450 activity (nmol min^{-1} mg^{-1} protein)	Ratio[a]	GST activity (nmol min^{-1} mg^{-1} protein)	Ratio	CarE activity (nmol min^{-1} mg^{-1} protein)	Ratio
3 d after treatment						
Control	45.887 ± 10.439	-	0.352 ± 0.078	-	113.540 ± 22.479	-
15	34.561 ± 10.562	0.753	0.258 ± 0.064	0.732	92.205 ± 23.026	0.812
75	34.029 ± 4.818	0.742	0.347 ± 0.060	0.986	101.820 ± 17.321	0.897
Adult						
Control	12.732 ± 2.932	-	0.060 ± 0.012	-	21.825 ± 4.176	-
15	12.732 ± 1.985	1.000	0.052 ± 0.010	0.878	17.730 ± 2.304	0.812
75	9.518 ± 2.196	0.748	0.056 ± 0.008	0.941	19.98 ± 3.051	0.916

[a] Ratio = mean enzyme activity of treatment/mean enzyme activity of control.

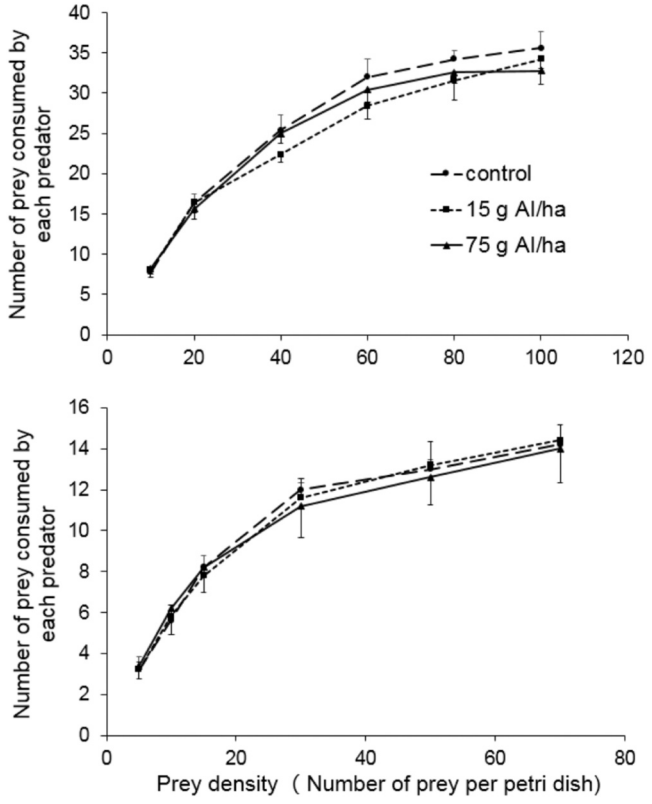

Fig. 3. Number of *Agrotis ipsilon* larvae consumed by *R. fuscipes*. (A) Second instar *R. fuscipes* exposed to CAP for 3 d and fed 2nd instar *A. ipsilon* larvae. (B) Adult *R. fuscipes* (emerged within 48 h) exposed to CAP from 2nd instar and fed 4th instar *A. ipsilon* larvae. Values = mean ± SE. n=5.

(Hemiptera: Pentatomidae) (Biondi et al., 2012; Gontijo et al., 2018; Martinou, et al., 2014; Martinou and Stavrinides, 2015; Yao et al., 2022).

When insects are under insecticide pressure, metabolic energy is diverted to detoxification rather than being used for growth and development (Simelane et al., 2008). Population parameters, including the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (l) and mean generation time (T), can provide useful information about insect population dynamics (Chi and Liu, 1985; Nawaz et al., 2017). CAP may have no lethal effect on hemipteran predators but may affect their development, fecundity or behaviour to some extent. For example, CAP was reported to be harmless to *P. nigrispinus* when they were exposed to 10× the recommended field concentration on soybean leaves for 72 h (mortality < 10%) (de Castro et al., 2013). CAP was also shown to have no effect on the survival curves of *P. nigrispinus* plants grown on treated seeds (100 mL/100 kg seeds (Dermacor®, x-100)), but it prolonged the life expectancy of adult females (Gontijo et al., 2018). The exposure route of pesticides to predatory insects largely involves contact with residual deposits (Hassan et al., 2010). Our results indicated that although apparently harmless to nymphs after short-term exposure to 1× and 5× the label application rates (15 and 75 g ai/ha), CAP adversely affected *R. fuscipes* population levels after long-term exposure (Tables 1–2, Fig. 2).

Table 4
Functional response of *R. fuscipes* exposed to two field-recommended CAP concentrations from the 2nd instar stage for 3 days or until adulthood (emerged within 48 h). Values = mean ± SE.

Treatment (g AI/ha)	Attack rate (a) (95% CI)	Handling time (Th) (95% CI)	Maximum attack rate (T/Th)
3d after treatment			
Control	0.9177 (0.4086–1.4267)	0.0112 (0.00117–0.0212)	89.29
15	0.9004 (0.5015–1.2994)	0.0135 (0.00559–0.0214)	74.07
75	1.0001 (0.4597–1.5404)	0.0149 (0.00603–0.0238)	67.11
Adult			
Control	0.9989 (0.6736–1.3241)	0.0527 (0.0457–0.0597)	18.98
15	0.4890 (0.2246–0.7535)	0.0295 (0.00588–0.053)	33.90
75	0.7062 (0.4136–0.9988)	0.0455 (0.0330–0.0581)	21.98

To obtain rudimentary knowledge of the reason for *R. fuscipes* tolerance to CAP, the changes in detoxification enzyme activities induced by CAP were assayed. The evaluated levels of detoxification enzymes have been considered important for the metabolism of CAP in insects (Mao et al., 2019; Wang et al., 2018). In the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae), GST is likely the main detoxification mechanism responsible for resistance to CAP, and P450 and CarE are involved to some extent (Hu et al., 2014). After 24 h of CAP exposure, the activities of the P450 and GST enzymes in the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) significantly increased, with the P450 enzyme responding fastest (Mao et al., 2019). Our results showed that detoxification enzymes were not responsible for the tolerance of *R. fuscipes* to CAP (Table 3). However, CAP may suppress detoxification enzyme activity. In addition to elevated levels of detoxification enzymes, differences in the structure and function of the target site *RyR* (Qi and Casida, 2013) and reduced insecticide penetration (Pang et al., 2023) may be responsible for the tolerance of *R. fuscipes* to CAP, which requires further study.

The functional response of predators can be influenced by several factors, including insecticide exposure (He et al., 2019; Martinou and Stavrinides, 2015; Yao et al., 2022). Exposure of *M. pygmaeus* and *N. tenuis* to sublethal concentrations of CAP affects the handling time and attack rate of predators (Martinou and Stavrinides, 2015). Our results showed that the presence of CAP in the transplant treatment did not affect the functional response of *R. fuscipes* (Table S2) or its effectiveness as a biological control agent (Fig. 3, Table 4). *R. fuscipes* displayed a type II functional response to *A. ipsilon* (Table S2), which was similar to its functional response on *A. ipsilon* reported by You et al. (2023) and on other prey (Ambrose and Nagarajan, 2010; Deng et al., 2012, 2015).

Pesticides are extensively used for arthropod pest suppression in

modern agriculture and are effective and economical for enhancing yield quality and quantity (Sharma et al., 2019). However, in IPM programs, the incorporation of natural enemies is possible only when they are safe from insecticides used against insect pests. Although CAP had negligible effects on the life table parameters of *R. fuscipes* after long-term exposure in our study, it was harmless after *R. fuscipes* exposure for 72 h. Several studies have shown that CAP is the most selective insecticide for beneficial hemipteran insects among the tested insecticides (de Castro et al., 2013; Fernandes et al., 2016; Martinou et al., 2014; Patel, 2020). Compared with those in the untreated control plots, Whalen et al. (2016) reported no significant differences in the abundance of total predators in CAP-treated field plots and significantly decreased populations of lepidopteran pests. In our study, the number of *A. ipsilon* larvae consumed by *R. fuscipes* after exposure to CAP in the transplant treatment was not affected (Fig. 3, Table 4, S2). Overall, these results suggest that the use of CAP as a transplant treatment has a minimal effect on *R. fuscipes*, but a time interval between transplant treatment and the release of *R. fuscipes* into the field should be set to minimize the impact of CAP on *R. fuscipes*. Additionally, more studies should be performed to assess the compatibility of CAP in transplant treatment with the predator *R. fuscipes* and other predators under field conditions.

CRediT authorship contribution statement

Zepeng Chen: Project administration, Investigation. **Xiaoming Pu:** Software, Investigation, Formal analysis. **Jingxin Zhang:** Writing – review & editing, Validation, Methodology, Data curation. **Huifang Shen:** Methodology, Investigation. **Birun Lin:** Methodology, Funding acquisition. **Haibing Deng:** Writing – review & editing, Supervision. **Qiyun Yang:** Resources, Funding acquisition. **Dayuan Sun:** Project administration, Formal analysis. **Pingping Liu:** Writing – original draft, Visualization, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting informati

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116491.

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