Article

**Sublethal and Transgenerational Toxicities of Chlorfenapyr on Biological Traits and Enzyme Activities of Paracoccus marginatus (Hemiptera: Pseudococcidae)**

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**Simple Summary:** Papaya mealybug, *Paracoccus marginatus*, is an important invasive pest worldwide, which attacks more than 200 host plants. Chlorfenapyr has been demonstrated to have a significant control effect on *P. marginatus*. To evaluate the long-term sublethal effects of chlorfenapyr on *P. marginatus*, the sublethal and transgenerational effects of chlorfenapyr on the biological traits and changes of enzyme activities of *P. marginatus* were investigated. The results showed that chlorfenapyr had significant effects on the development of subsequent generations of *P. marginatus*, and chlorfenapyr also activated the activities of SOD of *P. marginatus*. The results demonstrated that chlorfenapyr-mediated sublethal effects occur in at least two successive generations of *P. marginatus*. Therefore, it is necessary to reapply the chlorfenapyr prior to emergence of the F3 generation to suppress the population and prevent outbreaks of *P. marginatus*.

**Abstract:** Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is an economically important, invasive insect that is now distributed worldwide. Chlorfenapyr has been demonstrated to have a significant control effect on *P. marginatus*. In order to evaluate the sublethal and transgenerational effects of chlorfenapyr on *P. marginatus*, the life table data of three consecutive generations were collected and analyzed by the age stage, two-sex life table method, and the enzyme activities were assayed using a spectrophotometer. The results showed that exposure to the insecticide had significant effects on the biological traits of subsequent generations of *P. marginatus*, and a higher intrinsic rate of increase (*r*), finite rate of increase (*λ*), net reproductive rate (*R₀*), and a shorter mean generation time (*T*) were observed in the chlorfenapyr-treated F1 mealybugs. Enzyme activity assays showed that chlorfenapyr significantly inhibited the activities of catalase (CAT) and peroxidase (POD) while activating the activities of superoxide dismutase (SOD), which suggested that SOD, CAT, and POD may play an important role in the self-defense of *P. marginatus* against chlorfenapyr. These results conclusively demonstrated that exposure of *P. marginatus* to sublethal concentrations of chlorfenapyr induced hormetic effects on the F1 generation while having negative effects on the F0 and F3 generations.

**Keywords:** *Paracoccus marginatus*; chlorfenapyr; sublethal dose exposure; transgenerational effects; enzyme activities

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1. **Introduction**

The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is an economically important invasive pest in subtropical and tropical regions throughout the world [1,2]. *P. marginatus* is known to damage over 200 plant species from 60 families of plants [1,3], causing serious economic losses and potential
threats to numerous economically important crops as well as a great number of ornamental plants [4]. Chemical control has been the primary strategy used in many integrated pest management (IPM) systems because it is often the fastest and most efficient means of pest control while providing reliable and effective control of targeted pests. Currently, chemical control application of profenophos, chlorpyriphos, buprofezin, dimethoate, imidaclopride, thiametoxam, and acetampride are the insecticides most commonly employed against the papaya mealybug in field situations in India and Sri Lanka [5–7]. Our previous toxicological tests showed that chlorfenapyr had the highest toxicity among the 15 insecticides we tested [8]. Chlorfenapyr, which is a member of the pyrroles class of insecticides, has a broad insecticidal spectrum that functions by disrupting the oxidative phosphorylation of the H proton gradient, resulting in the interruption of ATP and ultimately death of the organism [9,10]. At present, chlorfenapyr is used mainly in controlling Spodoptera litura (Fabr.) (Lepidoptera: Noctuidae) [11], Plutella xylostella (L.) (Lepidoptera: Plutellidae) [12], and Tetranychus cinnabarinus (Boisduval) (Acari: Tetranychidae) [13]. The recommended field dose range of chlorfenapyr is 90–120 g a.i. ha$^{-1}$ based on the British Crop Production Council Pesticide Manual (version 6.0) [14].

After application of insecticides in the field, pests may be exposed to different sublethal doses of insecticides, and such sublethal doses result in different biological outcomes. For example, sublethal effects of pesticides may affect host biology in several ways, such as prolonging the duration of their developmental, reducing their survival rate and fecundity, and, consequently, disrupting the population dynamics of the targeted insects [15,16]. Previous studies have mainly reported the effects of sublethal doses on a single generation of the pest. Some insects, however, may continue to be exposed to sublethal doses for extended periods of time, which may cause transgenerational effects on their offspring [17]. For example, low lethal concentrations of acetamiprid and buprofezin were found to affect the duration of the preadult period, survival rate, reproduction, and population growth rate of Brevicoryne brassicae (L.) (Hemiptera: Aphididae) in the F$_1$ generation [18]. The average fecundity, intrinsic rate of increase ($r$), and finite rate of increase ($\lambda$) of Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) in the F$_4$ generation were higher than those in the F$_0$ and F$_1$ generations after exposure to a sublethal dose of triflumezopyrim [19]. Instances such as these demonstrate the importance of evaluating the transgenerational effects of sublethal doses of pesticides on insects.

Insects often reduce the toxicity of pesticides via activating or inhibiting detoxification by their internal enzyme system [20–22]. Acetylcholinesterase (AchE) and carboxylesterase (CarE) are important detoxifying enzymes, which can metabolize the toxins in insects to maintain the physiological activities [23]. Antioxidative enzymes mainly include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and the combined action of these three enzymes maintains free radicals at low levels in insects to protect the cells from damage [24]. Enzyme activities can be used as a biomarker of organisms exposed to sublethal dose of insecticides [25].

In this study, we evaluated the long-term effects of sublethal exposure and transgenerational effects of chlorfenapyr on P. marginatus using the age-stage two-sex life table and assessed the detoxifying and antioxidative enzymes activities of P. marginatus. The results will contribute important information for the chemical control of P. marginatus, such as determining the most effective pesticide dosage and establishing the application interval.

2. Materials and Methods

2.1. Insects

_Paracoccus marginatus_ were collected from pawpaw trees at the Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China. These insects were continuously reared on potato sprouts in laboratory without exposure to any insecticides for above 20 consecutive generations. The mealybugs were reared in an artificial climate chamber set at 28 ± 1 °C, 70 ± 5% relative humidity, a photoperiod of 14 L:10 D, and a light intensity of 12,000 lx at the Institute of Plant Protection, Fujian Academy of Agricultural Sciences.
2.2. Toxicity of Chlorfenapyr on *P. marginatus*

A 10% chlorfenapyr suspension concentrate was purchased from BASF (China) Co., Ltd. (Shanghai, China). A series of chlorfenapyr solutions with different concentrations were prepared by diluting with distilled water (2.5, 5, 10, 20, 40, 80 mg·L\(^{-1}\)). Distilled water was used as the control group. Each of the treatments was replicated three times. At least thirty nymphs per replicate were used for toxicity test. Newly emerged 2nd-instar *P. marginatus* nymphs were carefully placed on a leaf disc (3 cm diameter) cut from sweet potato leaves. After two hours, the unestablished insects were removed, and the living individuals were left and used for test. The leaf discs containing the control and treatment groups of mealybugs were dipped into either water or one of the above chlorfenapyr solutions for 15 s \[26,27\] and then removed and allowed to dry at room temperature. After thoroughly dry, the leaf discs with the mealybugs were placed in a Petri dish (3.5 cm diameter) containing 2\% (w/v) agar to retain leaf moisture. The leaf discs and mealybugs were then returned to the artificial climate chamber described previously. After 48 h, the mortality of *P. marginatus* was recorded.

2.3. Transgenerational and Sublethal Effects of Chlorfenapyr on *P. marginatus*

We contrasted the life history parameters of the F\(_0\), F\(_1\), and F\(_3\) generations from the control and treatment groups to determine the transgenerational effects of chlorfenapyr. One hundred newly oviposited eggs laid within a 24 h period of F\(_0\) generation were randomly selected for life table study. After eggs hatched into nymphs and grew to 2nd-instar nymphs, the nymphs were treated with LC\(_{30}\) of chlorfenapyr as described in Section 2.2. Forty-eight hours after treatment, the survivors (F\(_0\)-chlorfenapyr) were numbered and individually transferred to new untreated sweet potato leaf discs for life table study. Until growth to adults, males and females were paired in centrifuge tubes (1.5 mL) and reared with fresh sweet potato leaves. The development duration, survival, and fecundity of the individuals were observed and recorded daily until the death of all individuals. For the F\(_1\) generation, one hundred eggs from F\(_0\) adults were randomly collected. The newly hatched nymphs were individually transferred to plastic cases (3.5 cm diameter) containing sweet potato leaves, and the growth and survival of the nymphs were observed daily. When adults emerged, they were paired in centrifuge tubes. The feeding method and the data to be recorded were the same as those for the F\(_0\) adults. The life table research method of the F\(_3\) generation was the same as that for the F\(_1\) generation. All experiments were conducted in the artificial climate chamber described above.

2.4. Enzyme Activity Assay

In this experiment, activities of five enzymes, AChE, CarE, CAT, SOD, and POD, were assayed. There were two treatments of each enzyme: F\(_0\)-chlorfenapyr and F\(_0\)-CK. Additionally, there were three replicates per treatment for each enzyme. The 2nd-instar nymphs of *P. marginatus* were treated with chlorfenapyr at concentrations of LC\(_{30}\) for 48 h, and the survivors (F\(_0\)-Chlorfenapyr) were picked up. The control groups (F\(_0\)-CK) were treated with distilled water. Then, 8 mg of survivors of each replicate were collected in a 1.5 mL centrifuge tube and treated with liquid nitrogen and stored in \(−80^\circ\text{C}\). According to the kit instructions, the absorbance of AChE, CarE, CAT, SOD, and POD were read at 412 nm, 450 nm, 405 nm, 550 nm, and 420 nm using a spectrophotometer (TU-1900, Beijing Purkinje General Instrument Co., Ltd., Beijing, China), respectively.

2.5. Data Analysis

The LC\(_{30}\), LC\(_{50}\), and LC\(_{90}\) values and the 95% confidence intervals of chlorfenapyr were calculated using the probit regression analysis program of SPSS version 25.0 (IBM company, Stanford, CA, USA).

We treated *P. marginatus* males in the subpupal and pupal stages as 3rd-instar nymphs to facilitate analysis of the life table parameters because females and males of *P. marginatus* have different instar durations. The raw data from the *P. marginatus* cohorts were analyzed...
using the TWOSEX-MSChart program (Hsin Chi, Taizhong, China) [28–30]. The variances and standard errors of developmental duration, longevity, fecundity, and population life table parameters were calculated using the bootstrap method, and significant differences were compared with the paired bootstrap test [31]. Population prediction was performed using the TIMING-MSChart program (Hsin Chi, Taizhong, China) [32]. Figures of the age-stage survival rate \( (s_{ij}) \), age-specific survival rate \( (l_x) \), age-specific fecundity \( (m_x) \), age-specific maternity \( (l_xm_x) \), reproductive value \( (v_{ix}) \), age-stage-specific life expectancy \( (e_{ij}) \), and total population size were generated using Sigmaplot 12.2 software (Systat Software, Inc., San Jose, CA, USA).

The differences in the activities of the three antioxidative enzymes between the treatment and control groups were analyzed using t-test, and the graphs were plotted using GraphPad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Toxicity of Chlorfenapyr on \( P. \) marginatus

The LC\(_{30}\), LC\(_{50}\), and LC\(_{90}\) of chlorfenapyr in 2nd-instar \( P. \) marginatus nymphs were 5.55 mg L\(^{-1}\) (95% confidence limit, CL: 4.53–6.57 mg L\(^{-1}\)), 11.64 mg L\(^{-1}\) (95% CL: 10.09–13.34 mg L\(^{-1}\)), and 71.17 mg L\(^{-1}\) (95% CL: 56.69–94.56 mg L\(^{-1}\)), respectively.

3.2. Life History Traits

The sublethal concentration of chlorfenapyr had significant effects on the developmental time of the F\(_0\) generation of \( P. \) marginatus (Table 1). The duration of the 2nd-instar nymphs \( (p = 0.006) \) of the F\(_3\) individuals were significantly prolonged by chlorfenapyr treatment. Compared to the control group of mealybugs, the F\(_1\) individuals exposed to chlorfenapyr had a significant increase in the durations of the egg and 1st- and 2nd-instar nymphal stages \( (p < 0.001) \), while the duration of 3rd-instar nymphs and adult longevity of F\(_1\) individuals \( (p < 0.001) \) were significantly decreased. In the F\(_3\) generation of the treated group, the duration of the 1st-instar nymphs \( (p = 0.038) \) was significantly shortened, while the adult preoviposition period (APOP) \( (p < 0.001) \) was extended compared to the control group.

In F\(_3\) individuals from the chlorfenapyr treatment group, the durations of the egg and nymphal stages, and total preoviposition period (TPOP) were all significantly higher than they were in the F\(_0\) and F\(_1\) generations. The trend of F\(_3\) from the chlorfenapyr-treated group with higher duration than F\(_0\) and F\(_1\) was the same in the control group. These same parameters also differed significantly between the F\(_0\) and F\(_1\) generations \( (p < 0.001) \). However, the adult longevity and APOP in F\(_1\) individuals from the chlorfenapyr treatment group were significantly lower than they were in the F\(_0\) and F\(_3\) generations. The duration time of the egg, 1st- and 2nd-instars, and TPOP in the F\(_0\), F\(_1\), and F\(_3\) generations were all significantly different than equivalent values in the control group \( (p < 0.001) \), whereas no significant differences were observed for APOP in the different generations (Table 1). To sum up, the development durations of the preadult were prolonged.

3.3. Population Parameters

The transgenerational effects of chlorfenapyr (LC\(_{30}\)) on the population parameters of the F\(_1\) and F\(_3\) generations were evaluated based on life table data (Table 2). Compared to the control group, the intrinsic rate of increase \( (r) \), finite rate of increase \( (\lambda) \), net reproductive rate \( (R_0) \), and mean generation time \( (T) \) in the F\(_0\) generation of \( P. \) marginatus were not significantly affected by chlorfenapyr. Only the ratio of female adults to total individuals \( (N_f/N) \) in the F\(_0\) generation was significantly decreased after the sublethal chlorfenapyr treatment \( (p = 0.026) \). However, other than the fecundity \( (F, F_I) \), the values for the \( r, \lambda, R_0 \), and ratio of reproductive females \( (N_f/N_f) \) in the F\(_1\) were significantly decreased by chlorfenapyr. In contrast to the F\(_1\), in F\(_3\) individuals, only the \( N_f/N \) was significantly increased in the chlorfenapyr treatment \( (p < 0.001) \) when compared with the control, with no significant differences found in the other F\(_3\) parameters.
Table 1. Duration of each development stage, longevity, APOP, TPOP and oviposition days of *P. marginatus* in different generation exposed to LC30 of chlorfenapyr.

| Stage                  | Generation | Control |                |                | LC30     |                |
|------------------------|------------|---------|----------------|----------------|----------|----------------|
|                        |            | n       | Mean ± SE      | n              | Mean ± SE|
| Egg (d)                | F0         | -       | -              | 97             | 4.78 ± 0.06 bB  |
|                        | F1         | 97      | 6.23 ± 0.12 aA | 100            | 6.04 ± 0.12 aA|
| 1st instar (d)         | F0         | -       | -              | 90             | 5.88 ± 0.12 bB  |
|                        | F1         | 90      | 6.11 ± 0.15 aB | 82             | 6.11 ± 0.15 aB|
| 2nd instar (d)         | F0         | -       | -              | 85             | 3.93 ± 0.12 bC  |
|                        | F1         | 85      | 4.45 ± 0.14 bB | 73             | 4.45 ± 0.14 bB |
| 3rd instar (d)         | F0         | -       | -              | 68             | 4.28 ± 0.08 aB  |
|                        | F1         | 68      | 4.97 ± 0.16 aA | 49             | 6.57 ± 0.37 aA  |
| Adult longevity (d)    | F0         | -       | -              | 68             | 12.89 ± 0.93 aB |
|                        | F1         | 68      | 16.87 ± 1.09 aA| 45             | 10.96 ± 1.11 aB|
| APOP (d)               | F0         | 49      | 6.14 ± 0.36 aA | 35             | 6.11 ± 0.25 aA  |
|                        | F1         | 51      | 5.96 ± 0.25 aB | 9              | 5.11 ± 0.42 aB  |
|                        | F3         | 21      | 5.48 ± 0.48 bA | 36             | 7.11 ± 0.62 aA  |
| TPOP (d)               | F0         | 49      | 24.69 ± 0.38 aC| 35             | 25.57 ± 0.40 aC|
|                        | F1         | 51      | 26.04 ± 0.41 aB| 9              | 27.56 ± 0.84 aB|
| Oviposition days (Od) (d) | F0        | 49      | 7.37 ± 0.41 aB | 35             | 8.37 ± 0.59 aA  |
|                        | F1         | 51      | 9.31 ± 0.61 aA | 9              | 9.11 ± 1.54 aA  |
|                        | F3         | 21      | 8.43 ± 0.61 aA | 36             | 8.19 ± 0.69 aA  |

Standard errors (SE) were estimated by using the bootstrap technique with 100,000 re-samplings. Significant differences at \( p < 0.05 \) between two different treatments and generations were compared with the paired bootstrap test implemented in the TWOSEX-MSChart program. The lower-case letters show significant differences between control and chlorfenapyr treatments in the same generation, while the capital letters indicate significant differences among the F0, F1, and F3 generations within the same treatment (\( p < 0.05 \)).

The \( r, \lambda, R_0, \) and \( N_f/N \) values were significantly lower in the F1 than in the F0 and F3 generations, and the length of \( T \) in the F1 was significantly increased from the treated F0 to F3 generations (\( p < 0.05 \); Table 2). The \( r, \lambda, \) and \( N_f/N \) values in the F1 were significantly lower than they were in the F0 and F3 generations, while the \( T \) and \( F \) values of the F0 were significantly lower than those of the control F1 to F3 generations. No significant difference was observed in the \( N_f/N_j \) and \( F \) values among the F0, F1, and F3 generations of the chlorfenapyr treatment group.

3.4. Survival and Fecundity

The age-stage-specific survival rate (\( s_{xj} \)) curves indicated the chance that a mealybug egg survives to age \( x \) and stage \( j \) and showed separately survival rate in different life stages of *P. marginatus* (Figure 1). Variations in peaks showed differences in the developmental stages. The probability of an egg surviving to the female adult stage in the F1 after being treated with chlorfenapyr was extremely low (0.14, 14 individuals) compared to eggs in the F0 (0.40, 40 individuals) and F3 (0.44, 44 individuals) generations.
Table 2. Sublethal effects of chlorfenapyr on the population parameters of *P. marginatus* in different generations.

| Population Parameter                  | Generation | Control          | LC30            |
|---------------------------------------|------------|------------------|-----------------|
| Intrinsic rate of increase, *r* (day\(^{-1}\)) | *F*\(_0\)  | 0.18 ± 0.01 aA   | 0.16 ± 0.01 aA  |
|                                       | *F*\(_1\)  | 0.18 ± 0.01 aA   | 0.11 ± 0.02 bC  |
|                                       | *F*\(_3\)  | 0.13 ± 0.01 aB   | 0.15 ± 0.01 aB  |
| Finite rate of increase, *λ* (day\(^{-1}\)) | *F*\(_0\)  | 1.19 ± 0.01 aA   | 1.18 ± 0.01 aA  |
|                                       | *F*\(_1\)  | 1.20 ± 0.01 aA   | 1.11 ± 0.02 bC  |
|                                       | *F*\(_3\)  | 1.14 ± 0.01 aB   | 1.16 ± 0.01 aB  |
| Net reproductive rate, *R*\(_0\)* (offspring) | *F*\(_0\)  | 137.10 ± 22.22 aB | 109.48 ± 18.34 aA |
|                                       | *F*\(_1\)  | 212.85 ± 29.45 aA | 31.62 ± 13.05 bB |
|                                       | *F*\(_3\)  | 96.91 ± 23.10 aB | 148.14 ± 27.12 aA |
| Mean generation time, *T* (day)       | *F*\(_0\)  | 27.77 ± 0.40 aC  | 28.65 ± 0.48 aC |
|                                       | *F*\(_1\)  | 29.04 ± 0.25 bB  | 31.25 ± 0.52 aB |
|                                       | *F*\(_3\)  | 34.67 ± 0.65 aA  | 34.22 ± 0.48 aA |
| Ratio of female adults in total individuals (*N*\(_f\)/*N*\(_j\)) | *F*\(_0\)  | 0.56 ± 0.05 aA   | 0.40 ± 0.05 bA  |
|                                       | *F*\(_1\)  | 0.54 ± 0.05 aA   | 0.14 ± 0.03 bB  |
|                                       | *F*\(_3\)  | 0.22 ± 0.04 bB   | 0.44 ± 0.05 aA  |
| Ratio of reproductive females (*N*\(_f\)/*N*\(_j\)) | *F*\(_0\)  | 0.88 ± 0.04 aA   | 0.88 ± 0.05 aA  |
|                                       | *F*\(_1\)  | 0.94 ± 0.03 aA   | 0.64 ± 0.13 bA  |
|                                       | *F*\(_3\)  | 0.95 ± 0.05 aA   | 0.82 ± 0.06 aA  |
| Fecundity (*F*) (eggs/female)         | *F*\(_0\)  | 244.82 ± 33.28 aB | 273.70 ± 31.78 aA |
|                                       | *F*\(_1\)  | 394.17 ± 41.19 aA | 225.86 ± 77.39 aA |
|                                       | *F*\(_3\)  | 440.50 ± 66.18 aA | 336.68 ± 49.23 aA |
| Fecundity (*F*) (eggs/reproductive female) | *F*\(_0\)  | 279.80 ± 35.31 aB | 312.80 ± 31.05 aA |
|                                       | *F*\(_1\)  | 417.35 ± 41.36 aA | 351.33 ± 98.12 aA |
|                                       | *F*\(_3\)  | 461.48 ± 65.83 aA | 411.50 ± 52.53 aA |

Standard errors (SE) were estimated by using the bootstrap technique with 100,000 re-samplings. Significant differences at *p* < 0.05 between two different treatments and generations were compared with the paired bootstrap test implemented in the TWOSEX-MSChart program. The lower-case letters show significant differences between control and chlorfenapyr treatments in the same generation, while the capital letters indicate the significant differences among the *F*\(_0\), *F*\(_1\), and *F*\(_3\) generations within the same treatment (*p* < 0.05).

**Figure 1.** Age-stage-specific survival rate (*s*\(_xj\)) of *P. marginatus* (*F*\(_0\), *F*\(_1\), and *F*\(_3\)) exposed to LC\(_{30}\) of chlorfenapyr. CK represents the control.
The age-specific survival rates \( (l_x) \) of *P. marginatus* after different treatments are shown in Figure 2. The 50% survival rate of *P. marginatus* in the chlorfenapyr treatment groups occurred at ages 26, 24, and 32 d in the *F*₀, *F*₁, and *F*₃ generations, respectively, and at ages 26, 30, and 27 d in *F*₀, *F*₁, and *F*₃ of the control. Higher age-specific fecundity \( (m_x) \) and net maternity \( (l_xm_x) \) curves were observed in the *F*₁ of the control. Although the \( m_x \) had a higher peak in the control (53.46 at 35 d) in the *F*₃, the \( l_x \) and \( l_xm_x \) values were lower than those in the chlorfenapyr treatment.

![Survival rate plot](image)

Figure 2. Survival rate \( (l_x) \), fecundity \( (m_x) \), and net maternity \( (l_xm_x) \) of *P. marginatus* (*F₀*, *F₁*, and *F₃*) exposed to LC₃₀ dose of chlorfenapyr. CK represents the control.

The age-stage-specific reproductive value \( (v_{xj}) \) represents the contribution to future offspring of an individual from age *x* to stage *j* (Figure 3). The \( v_{xj} \) value of the *F₀*, *F₁*, and *F₃* generations at age zero is a finite rate of increase \( (\lambda) \), i.e., 1.178, 1.114, and 1.157 in the chlorfenapyr treatment and 1.193, 1.202, and 1.140, respectively, in the control. The \( v_{xj} \) value increased with age, with the \( v_{xj} \) curve significantly increasing when the female adults emerged. In general, the maximum \( v_{xj} \) values in different stages in the control were higher than those in the chlorfenapyr-treated group. The \( v_{xj} \) peaks were close to the TPOP.

The age-stage-specific life expectancy \( (e_{xj}) \) is the length of time that an individual of age *x* and stage *j* is expected to survive starting at age *x* (Figure 4). As expected, the highest \( e_{xj} \) values for all tested generations occurred at the egg stage. The life expectancy of a newly laid egg of *P. marginatus* control females in generations *F₀*, *F₁*, and *F₃* was 28.65, 30.26, and 26.86 d, respectively, and 27.45, 23.08, and 32.62 d, respectively, in the chlorfenapyr treatment. Thus, the life expectancy was similar between the control and chlorfenapyr treatments in the *F₀* generation but significantly different in the *F₁* and *F₃* generations. The \( e_{xj} \) values were higher in the female than in the male adults in all of the generations.

3.5. Population Prediction

The population growth of *P. marginatus* treated with sublethal chlorfenapyr concentrations was simulated using the TIMING-MSC hart program (Figure 5). Starting with an initial population of 10 newborn eggs, the *F₀*, *F₁*, and *F₃* populations of *P. marginatus* all developed to the fourth generation within 100 d in both the control and chlorfenapyr treatments. On the 100th d, the population with the largest number of individuals was the *F₁* of the control population (86,006,272), while the *F₁* in the chlorfenapyr treatment had the fewest number of individuals (262,695). Life tables from the 2.5th and 97.5th percentiles of \( R_0 \) and \( \lambda \) can be projected to describe the variability of population growth. When the
life tables of the 2.5th and 97.5th percentiles of $R_0$ were used to predict the variability in population growth, the population size of *P. marginatus* in the F1 chlorfenapyr treatment was 7237 and 1,808,058, respectively. However, when the life tables of the 2.5th and 97.5th percentiles of $\lambda$ were used, the population size of *P. marginatus* in the F1 chlorfenapyr treatment was 8659 and 1,633,191, respectively (Figure 6).
3.6. Detoxification Enzyme and Antioxidative Enzyme Activities

Compared with $F_0$-CK, there were no significant differences of CarE and AchE activities in $F_0$-Chlorfenapyr treated with LC$_{30}$ (Figure 7A). The activities of three antioxidative enzymes, i.e., SOD, CAT, and POD, differed significantly between $F_0$-Chlorfenapyr group and $F_0$-CK group. The SOD activities in $F_0$-Chlorfenapyr were significantly higher than that in $F_0$-CK ($t = 7.989$, $df = 4$, $p = 0.001$), but the CAT and POD activities in $F_0$-Chlorfenapyr
were significantly lower than those in F0-CK (CAT: $t = 5.642$, $df = 4$, $p = 0.005$; POD: $t = 5.220$, $df = 4$, $p = 0.006$) (Figure 7B).

4. Discussion

At present, there is no effective pesticide for controlling *P. marginatus*. This study was conducted to provide needed information on chlorfenapyr use on *P. marginatus*. No phytotoxicity has been observed when chlorfenapyr was used at recommended doses in the field (British Crop Production Council Pesticide Manual). In this study, the LC$_{90}$ of chlorfenapyr on 2nd-instar nymphs was 71.17 mg·L$^{-1}$ after 48 h of exposure. Approximately 60% of the field-recommended minimum dose (90 g a.i.·ha$^{-1}$) can achieve 90% control of *P. marginatus*, requiring a much lower dose than the commonly used field-recommended dose. The findings presented here suggest that chlorfenapyr can be an effective pesticide for control of *P. marginatus*.

In addition, insect populations are frequently exposed to low and sublethal concentrations of insecticides in the field because of heterogeneous spatial coverage on plants or environmental degradation of pesticide [33]. Sublethal effects, such as prolonged development and reduced longevity and fecundity, are usually observed in many pests after exposure to sublethal concentrations of insecticides [19,20]. In our study, chlorfenapyr treatments prolonged the durations of the nymphal instars and adult longevity in the F$_0$ generation. The insecticide-induced effects can be transgenerationally inherited [34]. In our study, the durations of the egg and young nymphal stages were increased in the F$_1$ generation, but the duration of older nymphal stages was reduced. In the subsequent F$_3$ generation, after chlorfenapyr treatment, the duration of the 1st instar was shortened, and APOP was lengthened. Sublethal and transgenerational effects of several insecticides have been reported in other insects. For example, flupyradifurone affected the duration and survival rate of the F$_1$ and F$_2$ generations of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) [35], while the LC$_{30}$ of six different insecticides had significant negative impacts on the life-history parameters of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) that led to reduced adult longevity and fecundity in the F$_0$ generation and reduced fertility and survival in the F$_3$ [36]. Our results provide evidence that the sublethal concentrations of chlorfenapyr do have significant sublethal and transgenerational effects on *P. marginatus*, and the negative sublethal effects may increase the biological fitness cost.
The indicators \(r\), \(R_0\), and \(\lambda\) are important for evaluating insect populations [18,37]. The results in this study showed that treatments with sublethal doses of chlorfenapyr resulted in significantly decreased \(r\), \(R_0\), and \(\lambda\) values in the \(F_1\) although no differences were observed in these parameters in the \(F_0\). Similarly, these demographic parameters were also significantly decreased following treatments with an LC\(_{50}\) dosage of triflumizopyrim in the \(F_3\) generation of \(Laodelphax striatellus\) Fallén (Hemiptera: Delphacidae) [38] and treatment with LC\(_5\) and LC\(_{10}\) dosages of chlorfenapyr in \(Bradyssia odoriphaga\) Yang and Zhang (Diptera: Sciaridae) [39].

Insecticide-induced hormesis that inhibits or increases the fecundity of insects has been reported in many studies [40,41]. In this study, we found that the LC\(_{30}\) concentration of chlorfenapyr effectively increased fecundity in the \(F_0\) but inhibited the population recovery of \(P.\ marginatus\) in the subsequent \(F_1\) and \(F_3\) generations. This effect was likely related to the ratio of female adults to total individuals (\(N_f/N\)) and the ratio of reproductive females (\(N_{fr}/N\)) in the treatment group being significantly higher than that in the control in the \(F_1\) and \(F_3\) generations (Table 1). Females play a decisive role in the growth of insect populations; therefore, a significant decrease in the proportion of females will result in a significant decrease in the reproductive rate of \(P.\ marginatus\).

Sublethal doses not only affect the growth, development, and reproduction of pests but also induce the changes of enzyme activity in the insect body, which is conducive to the accumulation and development of resistance [42]. The results of the present study showed that the activities of \(AChE\) and \(CarE\) were not affected by the LC\(_{30}\) of chlorfenapyr, but the activities of \(SOD\) were significantly activated, while \(CAT\) and \(POD\) were significantly decreased. The antioxidative enzymes \(SOD\), \(CAT\), and \(POD\) protect the cells from injury in organisms [20]. \(SOD\) can convert free superoxide anion radicals into hydrogen peroxide, and \(CAT\) and \(POD\) break the hydrogen peroxide into water and oxygen [43]. This finding suggested that \(SOD\), \(CAT\), and \(POD\) play an important role in the self-protection of \(P.\ marginatus\) to protect against chlorfenapyr.

In summary, the sublethal exposure to parental papaya mealybugs led to a significant increase in the durations of the egg and nymph stages and reduced the longevity, APOP, and population parameters \(r\), \(R_0\), and \(\lambda\) in the \(F_1\) generation, but recovery to the control level occurred in the \(F_3\) generation. That is, lower concentrations of chlorfenapyr can potentially be used to control papaya mealybugs in the field at less than the recommended field dose. While our study may indicate that lower concentrations are possible, other aspects, such as the efficacy of the application, location of the pest on the plant, residue, etc., should be considered and evaluated when determining the optimal dosage of pesticide. Our study demonstrated that chlorfenapyr-mediated sublethal effects occur in at least two successive generations of \(P.\ marginatus\). Therefore, when reapplying chlorfenapyr at an appropriate time, it is necessary to comprehensively consider this result, pest damage, natural enemies, and economic threshold, etc., in order to suppress \(P.\ marginatus\) population and prevent its outbreak. Low doses and prolonged application intervals will help to delay the development of insecticide resistance and reduce the adverse effects of pesticides on the environment and the human population.

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**References**

1. Finch, E.A.; Beale, T.; Chellappan, M.; Goergen, G.; Gadratagi, B.G.; Khan, M.A.M.; Rehman, A.; Rwomushana, I.; Sarma, A.K.; Wyckhuys, K.A. The potential global distribution of the papaya mealybug, *Paracoccus marginatus*, a polyphagous pest. *Pest Manag. Sci.*** 2021, *77*, 1361–1370. [CrossRef][PubMed]

2. Miller, D.R.; Miller, G.L.; Watson, G.W. Invasive species of mealybugs (Hemiptera: Pseudococcidae). *Proc. Entomol. Soc. Wash.* 2002, *104*, 825–836.

3. Zhang, J.T.; Wu, S.A. A new invasive mealybug, *Paracoccus marginatus* (Hemiptera: Coccoidea: Pseudococcidae), in mainland China. *Environ. Entomol.* 2015, *37*, 441–447.

4. Miller, D.R.; Miller, G.L. Redescription of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae), including descriptions of the immature stages and adult male. *Proc. Entomol. Soc. Wash.* 2002, *104*, 1–23.

5. Krishnan, J.U.; George, M.; Ajesh, G.; Jithine, J.; Lekshmi, N.; Deepasree, M. A review on *Paracoccus marginatus* Williams, papaya mealybug (Hemiptera: Pseudococcidae). *J. Entomol. Zool. Study* 2016, *4*, 528–533.

6. Galanihe, I.D.; Jayasundera, M.U.P.; Vithana, A.; Asselaarachchi, N.; Watson, G.W. Occurrence, distribution and control of papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), an invasive pest in Sri Lanka. *Trop. Agric. Res. Ext.* 2011, *13*, 81–86. [CrossRef]

7. Seni, A.; Sahoo, A.K. Efficacy of certain insecticides on papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae). *J. Entomol. Zool. Study* 2015, *3*, 14–17.8.

8. Li, J.Y.; Shi, M.Z.; Wang, Q.Y.; Luo, Y.Y.; Zheng, L.Z.; Fu, J.W. Screening and sensitivity of pesticides for controlling new invasive pest *Paracoccus marginatus* on papaya plants. *Fujian J. Agric. Sci.* 2020, *35*, 74–79.

9. Black, B.C.; Hollingworth, R.M.; Ahammadshahib, K.L.; Kukel, C.D.; Donovan, S. Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles. *Pestic. Biochem. Physiol.* 1994, *50*, 115–128. [CrossRef]

10. Gao, Y.; Kim, M.J.; Kim, J.H.; Jeong, I.H.; Clark, J.M.; Lee, S.H. Transcriptomic identification and characterization of genes responding to sublethal doses of three different insecticides in the western flower thrips, *Frankliniella occidentalis*. *Pestic. Biochem. Physiol.* 2020, *167*, 104596. [CrossRef]

11. Darabian, K.; Yarahmadi, F. Field efficacy of azadirachtin, chlorfenapyr, and *Bacillus thuringiensis* against *Spodoptera exigua* (Lepidoptera: Noctuidae) on sugar beet crop. *J. Entomol. Res. Soc.* 2017, *19*, 45–52.

12. Wang, X.L.; Wang, J.; Cao, X.W.; Wang, F.L.; Yang, Y.H.; Wu, S.W.; Wu, Y.D. Long-term monitoring and characterization of resistance to chlorfenapyr in *Plutella xylostella* (Lepidoptera: Plutellidae) from China. *Pest Manag. Sci.* 2019, *75*, 591–597. [CrossRef][PubMed]

13. Cheng, S.H.; Lin, R.H.; You, Y.; Lin, T.; Zeng, Z.H.; Yu, C.H. Comparative sensitivity of *Neoseiulus cucumeris* and its prey *Tetranychus cinnabarinus*, after exposed to nineteen pesticides. *Ecotox. Environ. Safe* 2021, *217*, 112234. [CrossRef][PubMed]

14. British Crop Production Council Pesticide Manual (The e-Pesticide Manual). In *British Crop Production Council: Cambridge, UK, 2012.*

15. Havasi, M.; Kheradmand, K.; Mosallanejad, H.; Fathipour, Y. Sublethal effects of diflovidazin on life table parameters of two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Int. J. Acarol.* 2016, *44*, 115–120. [CrossRef]

16. Zhao, Y.H.; Wang, Q.H.; Ding, J.F.; Wang, Y.; Zhang, Z.Q.; Liu, F.; Mu, W. Sublethal effects of chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of *Bradysia odoriphaga* (Diptera: Sciaridae). *Pestic. Biochem. Physiol.* 2018, *148*, 93–102. [CrossRef]

17. Chi, H.; You, M.S.; Atlihan, R.; Smith, C.L.; Kavousi, A.; Özgökçe, M.S.; Güncan, A.; Tuan, S.J.; Fu, J.W.; Xu, Y.Y. Age-stage, two-sex life table: An introduction to theory, data analysis, and application, *Entomol. Gen.* 2020, *40*, 103–124. [CrossRef]

18. Mahmoodi, L.; Mehrkhou, F.; Guz, N.; Forouzan, M.; Atlihan, R. Sublethal effects of three insecticides on fitness parameters and population projection of *Brevicoryne brassicae* (Hemiptera: Aphididae). *J. Econ. Entomol.* 2020, *113*, 2713–2722. [CrossRef]

19. Chen, L.; Wang, X.G.; Zhang, Y.Z.; Yang, R.; Zhang, S.R.; Xu, X.; Zhu, M.J.; Gong, C.W.; Hasnain, A.; Shen, L.T. The population growth, development and metabolic enzymes of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae) under the sublethal dose of triflumizopyr. *Chemosphere* 2020, *247*, 125865. [CrossRef]

20. Shan, Y.X.; Zhu, Y.; Li, J.J.; Wang, N.M.; Yu, Q.T.; Xue, C.B. Acute lethal and sublethal effects of four insecticides on the lacewing (*Chrysoperla sinica* Tjeder). *Chemosphere* 2020, *250*, 126321. [CrossRef]

21. Su, J.Y.; Lai, T.C.; Li, J. Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. *Crop. Prot.* 2012, *42*, 217–222. [CrossRef]

22. Zou, C.S.; Lv, C.H.; Wang, Y.J.; Cao, C.W.; Zhang, G.C. Larvicidal activity and insecticidal mechanism of *Chelidonium majus* on *Lymnantria dispar*. *Pestic. Biochem. Physiol.* 2017, *142*, 123–132. [CrossRef][PubMed]
23. Chen, Y.Z.; Zhang, B.W.; Yang, J.; Zou, C.S.; Li, T.; Zhang, G.C.; Chen, G.S. Detoxification, antioxidant, and digestive enzyme activities and gene expression analysis of *Lymandria dispar* larvae under carvacrol. *J. Asia-Pac. Entomol.* 2021, 24, 208–216. [CrossRef]

24. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 2004, 48, 909–930. [CrossRef] [PubMed]

25. Rumpf, S.; Hetzel, F.; Frampton, C. Lacewings (Neuroptera: Hemerobiidae and Chrysopidae) and integrated pest management: Enzyme activity as biomarker of sublethal insecticide exposure. *J. Econ. Entomol.* 1997, 90, 102–108. [CrossRef]

26. Ding, Q.; Xu, X.; Wang, X.; Ullah, F.; Gao, X.W.; Song, D.L. Characterization and functional analysis of two acetylcholinesterase genes in *Bradyisia odoriphaga* Yang et Zhang (Diptera: Sciaridae). *Pestic. Biochem. Physiol.* 2021, 174, 104807. [CrossRef]

27. Ullah, F.; Gul, H.; Desneux, N.; Said, F.; Gao, X.W.; Song, D.L. Fitness costs in chlorfenapyr-resistant populations of the chive maggot, *Bradyisia odoriphaga*. *Ecotoxicology* 2020, 29, 407–416. [CrossRef] [PubMed]

28. Chi, H.; Liu, H. Two new methods for the study of insect population ecology. *Bull. Inst. Zool. Acad. Sin.* 1985, 24, 225–240.

29. Chi, H. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 1988, 17, 26–34. [CrossRef]

30. Chi, H. TWOSEX-MSChart: A Computer Program for the Age-Stage, Two-Sex Life Table Analysis; National Chung Hsing University: Taichung, Taiwan, 2022; Available online: http://140.120.197.173/Ecology/prod02.htm (accessed on 1 August 2022).

31. Wei, M.F.; Chi, H.; Guo, Y.F.; Li, X.W.; Zhao, L.L.; Ma, R.Y. Demography of *Taichung*, Taiwan, 2022; Available online: http://140.120.197.173/Ecology/prod02.htm (accessed on 8 August 2022).

32. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 2007, 52, 81–106. [CrossRef]

33. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 2007, 52, 81–106. [CrossRef]

34. Brevik, K.; Lindström, M.; McKay, S.D.; Chen, Y.H. Transgenerational effects of insecticides—Implications for rapid pest evolution in agroecosystems. *Curr. Opin. Insect Sci.* 2018, 26, 34–40. [CrossRef]

35. Li, J.Y.; Liu, J.; Chi, B.J.; Chen, P.; Liu, Y.J. Sublethal and transgenerational effects of six insecticides on *Bradyisia odoriphaga* (Diptera: Tephritidae). *J. Asia-Pac. Entomol.* 2021, 24, 14–23. [CrossRef]

36. Nozad-Bonab, Z.; Hejazi, M.J.; Iranipour, S.; Arzanlou, M.; Biondi, A. Lethal and sublethal effects of synthetic and bio-insecticides on *Bradyisia odoriphaga* (Diptera: Sciaridae) reared on four cultivars of *Pyrus bretschneideri* (Rosales: Rosaceae) and *P. communis* pears with estimations of confidence intervals of specific life table statistics. *J. Econ. Entomol.* 2020, 113, 2343–2353. [CrossRef]

37. Tang, Q.L.; Ma, K.S.; Chi, H.; Hou, Y.M.; Gao, X.W. Transgenerational hermetic effects of sublethal dose of flupyradifurone on the green peach aphid, *Myzus persiceae* (Sulzer) (Hemiptera: Aphididae). *PLoS ONE* 2019, 14, e0208058. [CrossRef] [PubMed]

38. Liu, J.Y.; Liu, J.; Chi, B.J.; Chen, P.; Liu, Y.J. Sublethal and transgenerational effects of six insecticides on *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *J. Asia-Pac. Entomol.* 2021, 24, 14–23. [CrossRef]

39. Nozad-Bonab, Z.; Hejazi, M.J.; Iranipour, S.; Arzanlou, M.; Biondi, A. Lethal and sublethal effects of synthetic and bio-insecticides on *Trichogramma brassicae* parasitizing *Tuta absoluta*. *PLoS ONE* 2021, 16, e024334. [CrossRef] [PubMed]

40. Zhang, S.R.; Wang, X.; Gu, F.C.; Gong, C.W.; Chen, L.; Zhang, Y.M.; Hasnain, A.; Shen, L.T.; Jiang, C.X. Sublethal effects of triflumezopyrim on biological traits and detoxification enzyme activities in the small brown planthopper *Laodelphax striatellus* (Hemiptera: Delphacidae). *Front. Physiol.* 2020, 11, 261. [CrossRef] [PubMed]

41. Gu, H.; Ullah, F.; Haæmez, M.; Tariq, K.; Desneux, N.; Gao, X.W.; Song, D.L. Sublethal concentrations of clotiamadin affect fecundity and key demographic parameters of the chive maggot, *Bradyisia odoriphaga*. *Ecotoxicology* 2021, 30, 1150–1160. [CrossRef]

42. Wang, L.; Zhang, Y.J.; Xie, W.; Wu, Q.J.; Wang, S.L. Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Pestic. Biochem. Physiol.* 2016, 132, 102–107. [CrossRef]

43. Dong, J.F.; Wang, K.; Li, Y.; Wang, S.L. Lethal and sublethal effects of cyraniliprole on *Helicoverpa assulta* (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* 2017, 136, 58–63. [CrossRef]

44. Guedes, R.; Smagghe, G.; Stark, J.; Desneux, N. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annu. Rev. Entomol.* 2016, 61, 3.1–3.20. [CrossRef]

45. Filipović, A.; Mrdaković, M.; Ilijin, L.; Vlahović, M.; Todorović, D.; Grčić, A.; Perić-Mataruga, V. Effect of fluoranthene on antioxidative defense in different tissues of *Lymandria dispar* and *Euproctis chrysorrhoea* larvae. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 2019, 224, 108565. [CrossRef]