Physiological and Biochemical Responses to Sublethal Concentrations of the Novel Pyropene Insecticide, Afidopyropen, in Whitefly *Bemisia tabaci* MED (Q Biotype)

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Abstract: *Bemisia tabaci* is a devastating agricultural insect pest worldwide, and *B. tabaci* MED (formerly biotype ‘Q’) threatens the production of horticultural and economic crops in China as a growing number of cases of insecticide resistance have issued, highlighting the requirement for alternative methods and measures of pest management. In the present work, the toxicities of eight popular chemical agents, including the novel pyropene insecticide afidopyropen, on adults of *B. tabaci* MED were determined, and then physiological and biochemical responses to sublethal concentrations were confirmed. Among all tested chemical agents, afidopyropen exhibited the highest toxicity to adult whiteflies (LC$_{50}$: 7.38 mg/L). The sublethal effects of afidopyropen were studied at two sublethal concentrations, LC$_{10}$ (0.53 mg/L) and LC$_{25}$ (1.84 mg/L), and LC$_{25}$ treatment extended the duration of growth stages and reduced viabilities in the stages of nymphal, pseudopupae, and adults. The egg-laying days and eggs laid per female were also decreased significantly, as was hatchability in the LC$_{25}$ treatment. Metabolic enzyme assays suggested that the sublethal effects of LC$_{25}$ treatment could be ascribable to enhanced detoxification mediated by glutathione S-transferase. In summary, our findings indicate that afidopyropen can be used as a chemical agent for the management of *B. tabaci* MED whiteflies.

Keywords: sucking insect; pest control; toxicity; sublethal effects; metabolic enzymes

1. Introduction

The tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a notorious insect pest, which is harmful to horticultural and economic crops and highly invasive across the world, and it has been found to infest over 700 species of plants, primarily feeding in the phloem [1,2]. The whiteflies not only damage their hosts directly but transmit hundreds of plant viruses during sucking as well [3]. *B. tabaci* is considered a cryptic species complex, which includes the MED cryptic species (also named as biotype Q) and the MEAM1 cryptic species (also named as biotype B) historically. The MEAM1 and MED species complex, which includes the MED cryptic species (also named as biotype Q) and the MEAM1 cryptic species (also named as biotype B) historically. The MEAM1 and MED species complex, which includes the MED cryptic species (also named as biotype Q) and the MEAM1 cryptic species (also named as biotype B) historically.

More importantly, while *B. tabaci* MED has been controlled in China through the application of insecticides such as neonicotinoids, antranilic diamides, and butenolide insecticides for several years, a significant drop in the susceptibility to these popular chemical agents in whiteflies has been detected in different parts of China, owing to the development of
resistance [7–10]. Consequently, it is highly possible that extensive applications of such common chemical agents could not be an appropriate or effective step for controlling whiteflies without high insecticide residues in China.

Afidopyropen, a novel pyropene insecticide, targets the transient receptor potential vanilloid (TRPV) of insects and acts potently against sucking insects by negatively affecting feeding, which result in starvation, desiccation, and mortality, thereby reducing virus transmission [11,12]. It has been reported that afidopyropen can be used as an effective measure for controlling notorious sucking and piercing insect pests, such as *Aphis glycines* Matsumura (Hemiptera: Aphididae), *Monelliaopsis pecanis* Bissell (Hemiptera: Aphididae), *B. tabaci*, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and *Stephanitis pyrioides* Scott (Hemiptera: Tingidae) [13–17]. the relative lack of toxicity of afidopyropen to natural enemies means, that along with other selective chemical agents, afidopyropen can be beneficial to integrated biological and chemical pest management; for example, afidopyropen was not toxic to *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) and *Orius insidiosus* Say (Hemiptera: Anthocoridae), which are important natural enemies of *A. glycines* [18,19]. Additionally, afidopyropen provides an alternative chemical control option from a novel insecticide group, beyond the three that are currently heavily relied upon and alternated, that could be employed to aid in insecticide resistance avoidance and management programs, and considering that afidopyropen displays a new mechanism of killing insect pests, the application of afidopyropen is one hopeful supplement to common insecticides.

Along with the lethal concentration for killing sucking pests directly, biological and physiological responses to sublethal concentrations of afidopyropen have been demonstrated in *D. citri* and *Aphis gossypii* Glover (Hemiptera: Aphididae) [16,20]. Specifically, sublethal concentrations could cause various physiological and behavioral alterations and exert negative impacts on a series of fitness components of arthropods, such as prolonging the growth time and reducing the fecundity of their F1 offspring. It has been shown that several popular commercialized chemical agents such as clothianidin, cyantraniliprole, cycloxaprid, and dinofuran could induce effects of sublethal concentrations on *B. tabaci* [21–24]. Hence, to better realize the potential use of afidopyropen as one promising insecticide against field populations of whiteflies, it is essential to confirm the effects of sublethal concentrations of afidopyropen on *B. tabaci*.

Currently, it has been shown that afidopyropen displays excellent toxic properties for killing a variety of sucking insect pests, and this compound shows little toxic potential to the environment and to humans [18]. However, sublethal effects of afidopyropen on whiteflies have not yet been demonstrated. In the current work, the toxicity of afidopyropen on adults of *B. tabaci* MED was determined in comparison with the toxicities of seven other commonly used insecticides. Beyond investigating the effects of afidopyropen on the mortality of the adults, the sublethal effects on the target insect’s biology and biochemistry must be considered for a comprehensive assessment [25]. As such, the sublethal effects of afidopyropen on different growth stages, oviposition time, fertility of females, and egg hatch rates were evaluated, respectively. Then, to determine the biochemical responses to sublethal concentrations of afidopyropen, the activities of three main detoxifying enzymes, cytochrome P450 monooxygenases (P450s), glutathione S-transferase (GST), and esterases (EST), were measured.

2. Materials and Methods

2.1. Insects

The population of *Bemisia tabaci* MED was obtained from Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China [6], with collection beginning in 2015. Until now, the population has not been exposed to insecticides over ten years and has been reared on the plants of cotton *Gossypium hirsutum* with the photoperiod as 16:8 h light/dark at the temperature of 27 ± 1 °C and the relative humidity of 60 ± 10%. In the method of insecticide bioassay, adults of *B. tabaci* MED which emerged within
seven days were sampled at random, and sex ratio was considered at about 1:1 of females and males.

2.2. Insecticides and Chemicals

All insecticides used were analytically standardized. Afidopyropen (Dr. Ehrenstorfer, CAS# 915972-17-7, catalog# DRE-C10047000) and sulfoxaflor (Dr. Ehrenstorfer, CAS# 946578-00-3, catalog# DRE-C17015000) were purchased from Dr. Ehrenstorfer, Germany. Acetamiprid (Sigma Aldrich, CAS# 160430-64-8, catalog# 33674-100MG-R), flonicamid (Sigma Aldrich, CAS# 158062-67-0, catalog# 32509-25MG), flupyradifurone (Sigma Aldrich, CAS# 951659-40-8, catalog# 37050-100MG), pymetrozine (Sigma Aldrich, CAS# 123312-89-0, catalog# 46119-250MG-R), imidacloprid (Sigma Aldrich, CAS# 138261-41-3, catalog# 37894-100MG), thiamethoxam (Sigma Aldrich, CAS# 153719-23-4, catalog# 37924-100MG-R), dimethyl sulfoxide (Sigma Aldrich, CAS# 67-68-5, catalog# D8418-500ML) and Triton X-100 (Sigma Aldrich, CAS# 9002-93-1, catalog# 93443-100ML) were purchased from Sigma Aldrich, Shanghai, China.

2.3. Toxicities of the Eight Insecticides to B. tabaci

The toxicities of afidopyropen, acetamiprid, flonicamid, flupyradifurone, imidacloprid, pymetrozine, sulfoxaflor, and thiamethoxam on whitefly adults were measured using the method of bioassay as described [10]. In brief, stock solutions of the eight tested chemical agents were made in dimethyl sulfoxide. After this, working solutions of differing concentrations were made by diluting the stock solution in distilled water with 0.1% Triton X-100, and six working concentrations were diluted for the bioassay of each chemical agent. Twenty-millimeter diameter discs of cotton leaf were cut and dipped, with the adaxial surface facing downwards, in each replica of every working concentration for twenty seconds. The discs of cotton leaves were air-dried, then placed into 2 mL agar (15 g/L) in one 76 mm long glass tube, and four replicates were used for every working concentration of tested insecticide. In each tube, 20–40 whitefly adults were introduced, then placed in the rearing chamber with the photoperiod as 16:8 h light/dark at the temperature of 27±1°C and the relative humidity of 60±10%. The mortality of each bioassay was checked after 48 h, and motionless adults were regarded as dead.

2.4. Sublethal Effects of Afidopyropen on B. tabaci

On the basis of the above six-concentration bioassay of afidopyropen on the whitefly adults, values of LC$_{25}$ and LC$_{10}$ were obtained and set as the two sublethal concentrations for the study of sublethal effects of afidopyropen. A series of fitness components were checked, including the growth time and survivability of every developmental stage of F$_1$ offspring, days of egg laying, fecundity, and hatch rate of eggs. In brief, three insect-rearing cages were prepared and five plants of whitefly-free cotton were put into each cage. Among the three cages, two of them were set as treatment cages (LC$_{25}$ cage and LC$_{10}$ cage) and the rest were pointed as the control cages. One hundred adults of B. tabaci, previously maintained on leaf discs treated with afidopyropen (LC$_{10}$ or LC$_{25}$), according to the referenced protocol [22], were introduced into each experimental cage, and egg laying was measured. An equal number of B. tabaci adults without treatment were put into the control cage. After a 12 h egg-laying period, the cotton plants were moved out of the cages, respectively, and ten leaves were sampled at random in each plant. Using a microscope, twenty eggs were left on each sampled leaf and contained in one 2.5 cm-diameter leaf clip-cage, and two clip cages were set in each plant. On the leaves, positions of eggs were marked with pen, which helped us to check the development of the eggs until emergence of adults. Leaves/eggs were kept in an isolated climatic chamber at 27±1°C, 60±10% relative humidity, and a 16:8 h light/dark photoperiod (L: D). To determine the fecundity of treated insects, newly emerged whitefly adults were move onto new leaves, and kept in clip cages until they were all dead, after which hatchability was checked.
2.5. Metabolic Enzyme Assays

Activities of esterases, glutathione S-transferases and P450 monooxygenases were measured based on our previously published method [26]. According to the method, to measure P450 monooxygenases activity, one hundred randomly selected whitefly adults with mixed sex were used for each sample, and the enzyme activity was confirmed with the use of 7-ethoxycoumarin O-deethylase (ECOD) as the substrate. In the test of esterase, twenty whitefly adults were collected at random and used as each replicate for the test, and the activity was determined with the use of α-naphthyl acetate (α-NA) as the substrate. In the test of glutathione S-transferases, fifty whitefly adults of mixed sex were used for each sample and the activity was confirmed with the use of 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. In all tests of the three enzymes, three replicates were set up in LC25, LC10, and the control group, respectively.

2.6. Statistical Analysis

Analysis of insecticide bioassays was performed to confirm statistically significant differences in death rate by PoloPlus [27]. To analyze the significance of statistics in the survival rates and growth time of whiteflies among LC25, LC10, and the control group, along with fecundity, egg-laying days per female, and hatch rate of eggs, a one-way analysis of variance (ANOVA) and Tukey’s honest significance difference (HSD) test for multiple comparisons were conducted. Activities of the three tested metabolic enzymes were compared among LC25, LC10, and the control group by the use of a one-way ANOVA, followed by Tukey’s HSD test. All analyses of statistics were carried out using SPSS software [28].

3. Results

3.1. Toxicities of Eight Common Chemical Agents

The values of LC50 of all the tested chemical agents administered to whitefly adults by feeding are indicated in Table 1, and the death rate of the control in each bioassay was no more than 5%. Among the eight tested chemical agents, the most toxic to adults of B. tabaci MED was afidopyropen (LC50 = 7.38 mg/L), followed by acetamiprid (LC50 = 9.85 mg/L), sulfoxaflor (LC50 = 11.73 mg/L), flonicamid (LC50 = 13.28 mg/L), flupyradifurone (LC50 = 18.74 mg/L), thiamethoxam (LC50 = 19.29 mg/L), imidacloprid (LC50 = 26.37 mg/L), and pymetrozine (LC50 = 116.37 mg/L), which were 1.33, 1.59, 1.80, 2.54, 2.61, 3.57, and 15.77 times less toxic than afidopyropen, respectively.

| Insecticides    | Number | Slope ± SE | LC50 (95% Fiducial Limits) (mg/L) | X² (df) | p Values |
|-----------------|--------|------------|-----------------------------------|--------|----------|
| Afidopyropen    | 722    | 1.12 ± 0.10| 7.38 (6.02–9.25)                  | 2.96 (4)| 0.59     |
| Acetamiprid     | 768    | 1.43 ± 0.15| 9.85 (7.67–12.15)                 | 2.44 (4)| 0.73     |
| Flonicamid      | 740    | 1.19 ± 0.06| 13.28 (1.72–2.42)                 | 2.05 (4)| 0.76     |
| Flupyradifurone | 755    | 1.27 ± 0.08| 18.74 (15.89–21.98)               | 3.29 (4)| 0.55     |
| Imidacloprid    | 761    | 1.57 ± 0.11| 26.37 (21.15–32.85)               | 1.13 (4)| 0.88     |
| Pymetrozine     | 736    | 1.17 ± 0.07| 116.37 (88.43–161.56)             | 0.97 (4)| 0.91     |
| Sulfoxaflor     | 749    | 1.25 ± 0.09| 11.73 (9.88–14.65)                | 3.68 (4)| 0.47     |
| Thiamethoxam    | 767    | 1.09 ± 0.06| 19.29 (16.39–24.42)               | 3.29 (4)| 0.49     |

3.2. Sublethal Effects of Afidopyropen on B. tabaci Biology

Considering that most chemical agents could degrade rapidly or gradually after the first-time field applications [26], turning lethal concentrations into sublethal concentrations, we evaluated the sublethal effects of LC25 and LC10 of afidopyropen. The bioassays of afidopyropen toxicity in whitefly adults determined the LC25 to be 1.84 mg/L and the LC10 to be 0.53 mg/L. Figure 1 shows that the LC25 treatment of afidopyropen delayed the time of each growth period in comparison with the control group; the LC10 concentration of
afidopyropen only prolonged the pseudopupae and adult stages. Figure 2 shows that the survival rates of the second and third instar nymphs, pseudopupae, and adults of *B. tabaci* were significantly reduced with LC$_{25}$ treatment in comparison with the unexposed whitefly adults and the LC$_{10}$ treatment ones. Furthermore, exposure to the LC$_{25}$ treatment significantly reduced reproductive parameters such as fecundity (119.40 ± 9.92 eggs/female) (Figure 3A) and oviposition duration (9.64 ± 1.04 days) (Figure 3B) as compared with the control (146.87 ± 8.12 eggs/female and 12.79 ± 1.11 days, respectively). In addition, the egg hatch rate of the LC$_{25}$-treated group (82.45 ± 2.26%) (Figure 3C) was significantly lower than the control (91.44 ± 1.96%) and LC$_{10}$ treated groups (90.19 ± 2.17%), respectively.

**Figure 1.** Sublethal effects of afidopyropen (LC$_{10}$ and LC$_{25}$) on development time in specific life stages of *B. tabaci* MED. CK denotes the control group, and diverse letters display significant differences (*p* < 0.05).

**Figure 2.** Sublethal effects of afidopyropen (LC$_{10}$ and LC$_{25}$) on survival of specific life stages of *B. tabaci* MED. CK denotes the control group.
3.3. Activities of Metabolic Enzymes

According to Table 2, compared to the CK group of *Bemisia tabaci* MED adults, GST activities assayed with CDN Bs were markedly higher in the LC25 group (elevated 1.31-fold) \( (p < 0.001) \), whereas a significant difference was not observed for the LC10 group (elevated 1.05-fold). Additionally, the activities of P450 and esterases were also measured in the LC25 and LC10 and CK groups, but no significant differences were observed among the LC25, LC10, and CK groups of *B. tabaci* MED.

Table 2. Activities of three metabolic enzyme in treatments of LC10 and LC25, and the CK.a.

| Strain | P450 Monooxygenase Activity pmol min\(^{-1}\) mg\(^{-1}\) Protein ± SE | Esterase Activity nmol min\(^{-1}\) mg\(^{-1}\) Protein ± SE | Ratio \(^{b}\) | Glutathione S-Transferase Activity nmol min\(^{-1}\) mg\(^{-1}\) Protein ± SE | Ratio \(^{b}\) |
|--------|---------------------------------------------------------------|---------------------------------------------------------------|-----------|---------------------------------------------------------------|-----------|
| CK     | 8.94 ± 1.36 a                                               | 26.45 ± 4.05 a                                               | 1.14 a    | 786.33 ± 45.52 a                                             | 1.05 b    |
| LC10   | 10.15 ± 1.94 a                                             | 27.92 ± 3.88 a                                               | 1.06      | 822.93 ± 58.13 a                                             | 1.31 b    |
| LC25   | 10.67 ± 2.33 a                                             | 25.84 ± 5.19 a                                               | 0.98      | 1026.74 ± 51.88 a                                            |           |

\(^{a}\) Diverse letters display significant differences \( p < 0.05 \) among the mean values of enzyme activities in each column. \(^{b}\) Ratio = activity of LC10 or LC25 / activity of CK.

4. Discussion

It has been reported that afidopyropen is a promising alternative compound that could be effectively used in crop protection against insect pests \[11,12\]. Understanding the effects of any pesticide is important for the implementation of appropriate resistance management strategies, to reduce pesticide treatment thresholds of pest insects, and to prevent the reduced efficacy of the control method. In the current study, we found that the toxicity of afidopyropen was the highest among the eight commonly used insecticides tested against *B. tabaci* MED, while imidacloprid, thiamethoxam, flupyradifurone, flonicamid, sulfoxaflor, and acetamiprid exhibited moderate to relatively high efficacy, and pymetrozine displayed low toxicity. Considering this, in China, increasing instances of insecticide resistance to several widely used insecticides such as flupyradifurone, cyrantraniliprole and spirotetramat, have recently been reported in whiteflies \[10,29,30\]; identifying new chemical agents with different mechanisms of action is important for avoiding selection for the identical mechanism(s) of resistance. The newly introduced insecticide afidopyropen displayed a high toxicity against *B. tabaci* and no cross-resistance was detected, indicating that afidopyropen could be utilized as an effective chemical agent in controlling *B. tabaci* in China, or that afidopyropen could be considered as an effective alternative insecticide to be used in insect resistance abatement programs for *B. tabaci* in China \[14\].
In addition to the direct, lethal toxic effects of chemical agents against insect pests, sublethal concentrations of various insecticides also exerted an impact on the most important components required for insect survival and growth, including the reduction in the oviposition period, fecundity, and hatchability of eggs, as demonstrated [25]. In the present work, the LC_{25} concentration of afidopyropen markedly delayed the growth time of the egg and nymphal stages, and also negatively affected fecundity and hatchability, in comparison with the LC_{10} and control groups. Similarly, in the cotton aphid *Aphis gossypii*, sublethal concentrations (LC_{10}) of afidopyropen significantly decreased the longevity, fecundity, and oviposition days of female adults and, moreover, the total per-adult survival of the F_{1} generation was also significantly reduced by 30% with the LC_{10} treatment of afidopyropen [20]. In whiteflies, it has been reported that sublethal concentrations of various types of insecticides such as clothianidin, cyantraniliprole, cycloxaprid, and dinotefuran significantly reduced the life spans of adults and decreased the rates of pupation and survival, respectively with the treatment of LC_{10} or LC_{25} [21–24]. On the contrary, sublethal effects of chemical agents sometimes could be beneficial for insects, with most cases showing that sublethal concentrations could produce a higher fecundity of females and increased insect population growth in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), respectively [31,32], although no similar cases for afidopyropen have been reported so far. Considering that sublethal effects of insecticides interact with a series of life-history traits involved in the development of reproduction of insect pests such as developmental rate, survival rate, foraging and fecundity, it is highly possible that the sublethal effects exert an impact on population reproduction of insects [25]. On this basis, specific strategies of pest management could be formulated for each target insect pest according to its traits. The sublethal effects of chemical agents on life span and fecundity of the insects resulted from biochemical responses, and it has been reported that the activities of various detoxifying enzymes could contribute to the underlying biochemistry of the observed sublethal effects [33,34]. Among them, decreased and increased activities of glutathione S-transferase (GST) are possible in chemically stressed animals, relying on the type of chemical, timing, and dosage exposure [35].

In the hard tick *Haemaphysalis longicornis* Neumann (Acari: Ixodidae), the upregulation of two GST genes was detected after treatment with sublethal doses of flumethrin and chlorpyrifos, respectively, and it has been demonstrated that GST genes can be essential for the metabolism of flumethrin in the larvae stage and in male adult ticks [36]. In the present work, we found that the increased activity of glutathione S-transferase may be contributing to the biochemical effects of the LC_{25} afidopyropen treatment in *B. tabaci*.

The two other types of detoxification enzymes examined, cytochrome P450 monoxygenases and esterases, were not found to be significantly involved. Similarly, in *Spodoptera littura* Fabricius (Lepidoptera: Noctuidae), GST genes induced by sublethal doses of chlorpyrifos were highly expressed, indicating that GSTs were involved directly or indirectly in the response to chlorpyrifos treatment [37]. Hence, GSTs possibly have a pivotal role in the biochemical response of arthropods to sublethal concentrations of insecticides. In general, in response to exposure to chemical agents, insects have developed various mechanisms of adaptation, including biological and physiological changes causing avoidance, changed penetration, metabolic detoxification, and mutation of target sites [38], and we will work further on the mechanisms of afidopyropen-induced effects in *B. tabaci*.

5. Conclusions

Our results suggest that the novel pyropene insecticide afidopyropen could be a good candidate for controlling *Bemisia tabaci* MED in the field as it exhibits the highest toxicity against *B. tabaci* amongst eight other commonly used insecticides. Our results also indicate that the LC_{25} treatment of afidopyropen could induce significant sublethal effects such as a prolonged growth time and reduced rates of survival in various life stages, egg-laying days, female fecundity, and the egg-hatching rate. The metabolic enzyme assays suggested that the sublethal effects of LC_{25} could be ascribable to increased detoxification
mediated by GST. Altogether, afidopyropen exhibited a great insecticidal potency against B. tabaci and showed significant negative effects on development and reproduction, which possibly affect the population growth of B. tabaci, indicating that it could be regarded as an efficacious chemical agent in the insecticide resistance management of B. tabaci.

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