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Lethal and sublethal effects of cyantraniliprole, a new anthranilic diamide insecticide, on *Bemisia tabaci* (Hemiptera: Aleyrodidae) MED

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ABSTRACT

The *Bemisia tabaci* (Gennadius) cryptic species complex comprises important insect pests that cause devastating damage to agricultural crops worldwide. In China, the *B. tabaci* MED species, formerly known as biotype 'Q', has surpassed the MEAM1 species, formerly known as biotype 'B', which is threatening agricultural production all over the country as an increasing number of resistance cases have been reported. This situation highlights the need for alternative pest control measures. In this study, the lethal effects of six neonicotinoid and two anthranilic diamide insecticides including the novel compound cyantraniliprole on *B. tabaci* MED were examined. The sublethal effects of cyantraniliprole on the physiology and behavior of *B. tabaci* MED were also assessed. Among eight insecticides tested, cyantraniliprole was the most toxic to *B. tabaci* MED with a LC_{50} of 2.05 mg/L. The sublethal effects of cyantraniliprole to adult *B. tabaci* MED were observed at LC_{10} (0.22 mg/L) and LC_{25} (0.63 mg/L) concentrations. At these concentrations, cyantraniliprole prolonged the developmental duration and decreased the survival rate of nymph stages, pseudopupae and adults. The oviposition duration and fecundity of females were also reduced significantly. The hatching rate of eggs laid by females exposed to LC_{10} and LC_{25} concentrations was also reduced. Altogether, these results demonstrate that cyantraniliprole could be an alternative insecticide for efficient control of *B. tabaci* MED populations in China.

1. Introduction

The whitefly, *Bemisia tabaci* (Gennadius), is a devastating agricultural insect pest that exhibits a high genetic diversity and is distributed worldwide. It has been known to infest more than 600 host plant species (De Barro et al., 2011; Li et al., 2011) primarily feeding on their phloem. *B. tabaci* not only damages plants directly but also indirectly by transmitting more than 100 different plant viruses during feeding (Hogenhout et al., 2008). According to the terminology, the word "biotypes" to refer individuals indicating the diverse genetic populations (Liu et al., 2007) and after that, the phylogenetic analyses propose that *B. tabaci* is a cryptic species complex and there are more than 20 morphologically indistinguishable species (Xu et al., 2010). Within the *B. tabaci* species complex, the Mediterranean (MED or biotype Q) and Middle East-Asia Minor 1 (MEAM1 or biotype B) species are highly invasive and have caused substantial economic damage to crops in China (Luo et al., 2002; Chu et al., 2006). Currently, *B. tabaci* is controlled through the application of insecticides such as neonicotinoids, pyrethroids and the juvenile hormone analog pyriproxyfen. However, a strong reduction in the efficacy of these insecticides has been reported in some populations of *B. tabaci* due to the rapid evolution of insecticide resistance (Ahmad et al., 2002; Horowitz et al., 2005; Castle and Prabhaker, 2013; Horowitz and Ishaaya, 2014; Shadmany et al., 2015). Additionally, a relatively high toxicity of these insecticides has been observed to non-target organisms, including arthropods and even humans (Zeng et al., 2013; Cimino et al., 2016). As a result, extensive employment of these insecticides is not an appropriate choice for producing agricultural products with low pesticide residues.

Anthranilic diamides have been shown to control a large number of insect pest species from different orders effectively (Knight and Flexner, 2007; Yeoh and Lee, 2007; Koppenhöfer and Fuzy, 2008; Peck et al., 2008; Jacobson and Kennedy, 2011). In addition

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to directly killing target insect pests at high doses, anthranilic diamides are usually used at low does compared to other insecticides and have also been demonstrated to cause various physiological and behavioral alterations in many insects at sublethal concentrations (Guo et al., 2013; Zhang et al., 2013, 2015; Teixeira et al., 2009). After the relative success of chlorantraniliprole, cyantraniliprole, which is the second systemic insecticide in this class, has been used to control hemipteran pests (Sattelle et al., 2008). It was proven to be powerful for the management of both adult and immature stages of whitefly and for the reduction of transmission of some plant viruses (Portillo et al., 2009; Schuster et al., 2009; Stansly et al., 2010). It was also suggested that cyantraniliprole could be effective to mitigate resistance to other insecticides in *B. tabaci* because it does not provide cross-resistance to the commonly used insecticides for whitefly control (Grávalos et al., 2015). Similarly to chlorantraniliprole, cyantraniliprole molecules bind to ryanodine receptors resulting in uncontrolled delivery and depletion of internal calcium, inhibiting muscle contraction. The toxicity levels of cyantraniliprole have been shown to be minimal in mammals while being toxic for several insects, which is a desirable toxicological profile (Lahm et al., 2005; Cordova et al., 2006; Sattelle et al., 2008). However, to understand the potential of cyantraniliprole as an effective control agent of *B. tabaci* populations, it is important to determine the lethal and sublethal effects of this insecticide on whiteflies.

In the 1990s, a number of important insecticides such as imidacloprid, nitenpyram, acetamiprid, thiamethoxam, clothianidin and dinotefuran have been developed and used worldwide to control insect pests that damage host plants by sap-sucking (Bass et al., 2015). Despite these efforts, the *B. tabaci* MED species has outnumbered the existing MEAM1 species in China, becoming a dominant species and creating a new threat to agricultural crops throughout the country. More importantly, the MED species has developed resistance to several different insecticides, especially neonicotinoids (Luo et al., 2010; Pan et al., 2011). The use of cyantraniliprole is a promising alternative to existing insecticides, as it exhibits novel modes of action and acute toxicological profiles against insect pests. In the present study, we determined the lethal effects of cyantraniliprole on adult *B. tabaci* MED and compared its toxicity to that of six commercial neonicotinoids and one first-generation anthranilic diamide, chlorantraniliprole. Apart from investigating the effect of cyantraniliprole on the mortality of *B. tabaci*, sublethal effects on the insects' physiology and behavior must be considered for a comprehensive analysis (Desneux et al., 2007). Therefore, we also evaluated the sublethal effects of cyantraniliprole on the various developmental stages, on the fecundity and egg-laying duration of *B. tabaci* MED females and on egg hatchability.

2. Materials and methods

2.1. Insects

Bemisia tabaci MED populations were originally collected from damaged poinsettia (*Euphorbia pulcherrima* Wild. ex Klotz.) in Beijing, China in 2009 (Pan et al., 2011). The insects were fed on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') free of any insecticides under a 16L: 8D photoperiod at 27 ± 1 °C and 60 ± 10% RH. For bioassays, adults less than 7-days-old were randomly collected and used at an approximate 1:1 ratio of males and females.

2.2. Insecticides and chemicals

All neonicotinoid and anthranilic diamide insecticides used were technical grade formulations. Cyantraniliprole (95%) and chlorantraniliprole (95%) were purchased from DuPont Agricultural Chemicals Ltd., Shanghai, China. Acetamiprid (97%), imidacloprid (96%), clothianidin (97%), thiamethoxam (95%), dinotefuran (95%) and nitenpyram (98%) were purchased from Zhejiang Hetian Chemical Corporation Ltd., Zhejiang, China. Dimethyl sulfoxide (DMSO) was purchased from Shenzhen Baocheng Chemical Industry Co. Ltd, Shenzhen, China.

2.3. Lethal effects of the various anthranilic diamide and neonicotinoid insecticides on B. tabaci

The lethal effects of cyantraniliprole, chlorantraniliprole, acetamiprid, imidacloprid, clothianidin, thiamethoxam, dinotefuran and nitenpyram on adult *B. tabaci* were tested by the leaf-disc dipping bioassay method (http://www.sciencedirect.com/science/article/pii/S026121940900249XYang et al., 2013). Briefly, stock solutions (1000 mg/L) of all insecticides tested were prepared in Dimethyl sulfoxide (DMSO). Then, different concentrations of the working solutions were prepared by diluting the stock solution in 0.1‰ Triton X-100 in distilled water. In total, eight different concentrations (0.39 mg/L, 0.78 mg/L, 1.56 mg/L, 3.13 mg/L, 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L) were prepared for each insecticide. Cotton leaf discs (20 mm diameter) were prepared and dipped for 20 s in one of the eight concentrations of each insecticide solution with the adaxial surface facing down. Then, the leaf discs were dried by placing them in a flat-bottomed glass tube (76 mm long) containing agar (2 mL of 15 g/L). Control leaf discs were dipped in 0.1‰ Triton X-100 as described above and four replicates were made for each concentration. Adult insects maintained on insecticide-free cotton plant leaves in the greenhouse were collected in the tubes containing the leaf discs by inverting the tubes over the insects that allowed the adults to fly into the tubes. About 20-40 adults were randomly collected per tube and the sex ratio of insects was maintained to approximately 1:1. After insect collection, the open end of each tube was sealed with a cotton plug and maintained at 27 ± 1 °C, 60 ± 10% RH and a 16L: 8D photoperiod. After 48 h, the mortality was monitored under a microscope and immobile adults were considered dead.

2.4. Sublethal effects of cyantraniliprole on B. tabaci

Based on the bioassay performed with eight cyantraniliprole concentrations on *B. tabaci* described above, LC_{10} and LC_{25} values were calculated (see "Results") and used for sublethal exposure of *B. tabaci* adults following the leaf-dip protocol described above. Then, several fitness parameters were monitored; the developmental duration and survival rate of several stages in the next generation, the oviposition duration and the fecundity and the egg hatchability. Briefly, 15 insect-free cotton plants were placed in three separate insect-proof cages (two experimental cages and one control cage) with five plants in each cage. Plants in the two experimental cages (LC_{10} cage and LC_{25} cage) received treatment with cyantraniliprole at LC_{10} and LC_{25} concentrations, respectively, while the third cage contained untreated plants and served as the control. Then, 100 *B. tabaci* adults that were previously maintained on cyantraniliprole treated (LC_{10} or LC_{25}) leaf discs by the treatment protocol (Wang et al., 2016) were introduced into each experimental cage for measuring egg laying. The same numbers of untreated *B. tabaci* adults were introduced into the control cage. After 12 h of oviposition, the plants were removed from the cages and 10 leaves were randomly selected from plants of each of the three cages. Twenty eggs were left on each selected leaf using a microscope and contained in a leaf clip-cage (2.5 cm diameter, two clip cages per plants). The position of the eggs on these leaves was