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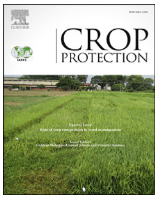

Baseline susceptibility and cross-resistance of cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, in *Bemisia tabaci* MED from China

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ABSTRACT

The tobacco whitefly *Bemisia tabaci* (Gennadius) is an important crop pest which threatens agriculture worldwide. It has evolved resistance to various classes of insecticides. Cycloxaprid is a new-generation neonicotinoid which is a (nitromethylene) imidazole analogue of imidacloprid which is highly efficient for the control of various Hemipteran pests in China. Studies were conducted to determine the baseline susceptibility to cycloxaprid of 18 field samples of *B. tabaci* collected from 9 geographical locations in China and the possibility of cross-resistance between cycloxaprid and other important neonicotinoids of one laboratory-selected resistant strain. The 50% lethal concentrations (LC_{50}) of cycloxaprid to these 18 samples ranged from 0.84 to 12.17 mg/L. Furthermore, compared with the susceptible laboratory strain, the imidacloprid-resistant strain exhibited a 27.9-fold resistance to imidacloprid and lower level of cross-resistance to acetamiprid (16.3-fold), thiacloprid (13.7-fold) and nitenpyram (16.6-fold), but no cross-resistance to cycloxaprid (1.9-fold). These results demonstrate that cycloxaprid could be one effective alternative insecticide for whitefly management that could reduce imidacloprid selection pressure.

1. Introduction

The tobacco whitefly *Bemisia tabaci* (Gennadius) cryptic species complex is one of the most notorious agricultural insect pests worldwide. It is a primary target of many chemical classes of insecticides such as neonicotinoids, pyrethroids and the juvenile hormone analog pyriproxyfen (Horowitz and Ishaaya, 2014; Bass et al., 2015). Among the *B. tabaci* species complex reported to date, the Mediterranean (MED or biotype Q) is highly invasive and caused substantial economic damage to crops in China (Chu et al., 2006; Pan et al., 2011, 2015). Currently, it has displaced Middle East-Minor Asia 1 (MEAM1 or biotype B) and became the dominant species in many areas of China (Zheng et al., 2017). The efficient control of *B. tabaci* populations by chemical insecticides has been challenged by the presence of multiple insecticide resistance in China (Luo et al., 2010; Zheng et al., 2017). Moreover, a globally significant reduction in the efficacy of conventional insecticides has been observed in some populations of *B. tabaci* as the result of the rapid development of insecticide resistance (Ahmad et al., 2002; Horowitz et al., 2005; Castle and Prabhaker, 2013; Shadmany et al., 2015). Moreover, a relatively high toxicity of these insecticides has been reported to non-target organisms, including arthropods and even humans (Zeng et al., 2013; Cimino et al., 2016). Therefore, it is urgent to replace these insecticides with an alternative which gives lower residues for modern crop protection strategies.

Neonicotinoids target the nicotinic acetylcholine receptor of the insect nervous system. They are one of the world's most important chemicals groups owing to their high toxicity against an extensive range of arthropods (Nauen et al., 2008; Jeschke et al., 2011). Particularly well suited to the control of Hemipteran sucking pests,

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they became useful insecticides against *B. tabaci* (Palumbo et al., 2001; Bass et al., 2015). They exhibited diverse applications and were primarily applied in crop protection by treating foliage, seeds and soil (Elbert et al., 2008). However, following their extensive employment, *B. tabaci* developed high levels of resistance to several commercialized neonicotinoids in many countries such as Spain, Cyprus and Israel (Elbert and Nauen, 2000; Nauen et al., 2002; Horowitz et al., 2004, 2005; Vassiliou et al., 2011). Similarly, *B. tabaci* in China also exhibited high levels of resistance to neonicotinoid insecticides after several years of application in the field (Yuan et al., 2012; Zheng et al., 2017).

Cycloxaprid is a neonicotinoid with a completely novel structure (Pan et al., 2014) with the NO_2 group in cis-configuration, while the NO_2 group in almost all commercialized neonicotinoids is in trans-configuration (Shao et al., 2011). Contrary to conventional neonicotinoids, which act as agonists of the arthropod nicotinic acetylcholine receptor (nAChR) (Tomizawa and Casida, 2003), the novel cis-configuration pharmacophore may involve a differential interaction with nAChRs (Shao et al., 2011). Lately, the nAChR binding site and metabolism of cycloxaprid insecticide have been illustrated (Shao et al., 2013; Liu et al., 2013), and it revealed cycloxaprid to be effective against imidacloprid-resistant pests, including the brown planthopper. It appears to activate a different site compared to imidacloprid on the nAChR (Shao et al., 2010; Cui et al., 2012). Cycloxaprid also exhibited high efficacy against different sucking pest such as the planthoppers *Nilaparvata lugens* (Stål) (Shao et al., 2010), the whitebacked planthopper *Sogatella furcifera* (Horváth) (Chang et al., 2015), the wheat aphid *Sitobion avenae* (Fabricius) (Cui et al., 2012), the mirid bug *Apolygus lucorum* (Meyer-Dür) (Pan et al., 2014), the cotton aphid *Aphis gossypii* (Cui et al., 2016) and the tobacco medfly *B. tabaci* (Wang et al., 2016). Therefore, it is a good candidate for controlling various agricultural pests in the field.

However, characterization of the baseline susceptibility is required for new insecticides to be widely applied in case of cross-resistance conferred by insecticides already used in the field (Jutsum et al., 1998). Hence, the aim of our study was to determine the baseline susceptibility of cycloxaprid in 18 field samples of *B. tabaci* MED collected from China during 2015–2016. Furthermore, cross-resistance of a moderate imidacloprid-resistant strain to cycloxaprid was characterized to further understand cross-resistance profiles among neonicotinoid insecticide family.

2. Materials and methods

2.1. *B. tabaci strains*

Nine samples of *B. tabaci* in 2015 and nine samples in 2016 were collected separately from Beijing (BJ), Tianjing (TJ), Hebei (HB), Shanxi (SX), Shandong (SD), Zhejiang (ZJ), Hunan (HuN), Hubei (HuB) and Hainan (HaiN) provinces in China (Table 1). For each sample, at least 3000 adults were collected at random from the crop leaves for getting their F_1 progeny. On the other hand, approximately 200 individuals of each sample were collected randomly and placed in a 1.5-ml centrifuge tube with 95% ethanol, and stored at −20 °C for later cryptic determination. All samples were identified as MED cryptic species according to direct sequencing of mitochondrial cytochrome oxidase I (Luo et al., 2002).

Two additional laboratory strains were used in this study. MED-S is a susceptible stain originally collected from poinsettia plants in Beijing, China in 2009 and reared in laboratory without exposure to any insecticides. MED-R is an imidacloprid-resistant strain that has been collected from tomato plants in Xiangyang, Hubei province of China in 2013, and it was continually selected with imidacloprid for 40 generations in laboratory. The collection information for all field-collected samples was presented in Table 1. All individuals collected from field were maintained on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') without exposure to any insecticides under a 16L: 8D photoperiod at 27 ± 1 °C and 60± 10% relative humidity (RH). For bioassays, less than 7-days-old adults were randomly collected at an approximate 1:1 ratio of males and females.

2.2. *Insecticides*

All neonicotinoid insecticides tested in this study were technical grade formulations. Imidacloprid (96%), acetamiprid (97%), thiacloprid (95%) and nitenpyram (98%) were obtained from Jiangsu Longdeng Chemical Corporation. Cycloxaprid (97%) was provided by FMC Corporation (Shanghai). Dimethyl sulfoxide (DMSO) and acetone were purchased from Beijing Chemical Reagent Co. Ltd.

Table 1
Sampling dates, host crops, and developmental stages of *B. tabaci* MED.

Sample	Location	Site	Time of collection	Host plant	Developmental stage
MED-S	Laboratory				
BJ	Beijing	39.56N, 116.20E	Aug 2015	Cucumber	Adult
	Beijing	39.56N, 116.20E	Aug 2016	Cucumber	Adult
TJ	Tianjing	39.39N, 117.05E	Oct 2015	Tomato	Adult
	Tianjing	39.39N, 117.05E	Oct 2016	Tomato	Adult
HB	Shijiazhuang, Hebei	38.05N, 114.52E	Sept 2015	Tomato	Adult
	Shijiazhuang, Hebei	38.05N, 114.52E	Sept 2016	Tomato	Adult
SX	Taiyuan, Shanxi	37.88N, 112.56E	Oct 2015	Eggplant	Adult
	Taiyuan, Shanxi	37.88N, 112.56E	Oct 2016	Eggplant	Adult
SD	Jinan, Shandong	36.68 N, 116.99E	July 2015	Cotton	Adult
	Jinan, Shandong	36.68 N, 116.99E	July 2016	Cotton	Adult
ZJ	Hangzhou, Zhejiang	30.26N, 120.19E	Sept 2015	Pepper	Adult
	Hangzhou, Zhejiang	30.26N, 120.19E	Sept 2016	Pepper	Adult
HuN	Changsha, Hunan	28.23N, 112.95E	Sept 2015	Melon	Adult
	Changsha, Hunan	28.23N, 112.95E	Sept 2016	Melon	Adult
HuB	Wuhan, Hubei	30.59N, 114.31E	Sept 2015	Tomato	Adult
	Wuhan, Hubei	30.59N, 114.31E	Sept 2016	Tomato	Adult
HaiN	Haikou, Hainan	20.05N, 110.21E	Apr 2015	Melon	Adult
	Haikou, Hainan	20.05N, 110.21E	Apr 2016	Melon	Adult

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