Crop Protection 83 (2016) 15–19

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Lethal and sublethal effects of a novel cis-nitromethylene neonicotinoid insecticide, cycloxaprid, on *Bemisia tabaci*

Ran Wang [a], Huixin Zheng [b], Cheng Qu [a], Zehua Wang [a], Zhiqiang Kong [c], Chen Luo [a, *]

[a] Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
[b] Department of Pesticide Science, Hunan Agricultural University, Changsha 410128, China
[c] Institute of Agro-Products Processing Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form
25 January 2016
Accepted 27 January 2016
Available online 4 February 2016

Keywords:
Bemisia tabaci
Cycloxaprid
Neonicotinoids
Sublethal effect
Toxicity

ABSTRACT

The tobacco whitefly, *Bemisia tabaci* (Gennadius), is an important pest because of its potential to threaten agricultural crops worldwide. Currently, this pest is controlled by the application of chemical insecticides. In our pursuit to identify better insecticides for an effective control of this insect pest, we investigated the lethal effects of five neonicotinoid insecticides including four commercial neonicotinoids and a novel neonicotinoid (cycloxaprid) on *B. tabaci* MED and MEAM1 cryptic species. In addition, we assessed the sublethal effects of cycloxaprid on *B. tabaci* MED. Lethal effects of the insecticides were determined using the leaf-dip bioassay, and the results showed that among the tested insecticides cycloxaprid was more toxic to *B. tabaci* MED and MEAM1 than others, with LC_{50} values of 0.70 mg/L and 0.59 mg/L, respectively. Cycloxaprid at LC_{25} (0.16 mg/L) induced sublethal effects in adult MED by prolonging the developmental periods and decreasing the survival rates of all larval instars, pseudopupal and adult stages. Moreover, it significantly shortened the oviposition period of females and decreased their fecundity. Hatching rate of eggs laid by females exposed to LC_{25} was also markedly reduced. These results indicate that cycloxaprid is a novel alternate insecticide that may effectively control *B. tabaci* populations.

1. Introduction

The tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most devastating agricultural pests that destroys a variety of crops including vegetable, ornamental and field crops (Byrne and Bellows, 1991; Oliveira et al., 2001). It is mainly distributed in the tropical and subtropical areas, and is considered to be a highly cryptic species complex with more than 36 described species (Dinsdale et al., 2010; De Barro et al., 2011). In China, damage to agricultural crops is caused by two of the most prevalent cryptic species of *B. tabaci*, the Middle East-Asia Minor1 (MEAM1, formerly known as biotype 'B') and the Mediterranean (MED, formerly known as biotype 'Q') (Luo et al., 2002; Chu et al., 2006). Currently, the application of insecticides is the primary strategy to control *B. tabaci* in many cropping system worldwide. Among the widely used insecticides are the broad-spectrum insecticides such as organophosphates and pyrethroids. However, the evolution of insecticide resistant *B. tabaci* strains has deterred the use of conventional insecticides (Ahmad et al., 2002). In addition, these insecticides are highly toxic to non-target insects, beneficial arthropods, wild animals and even humans. Therefore, extensive application of these insecticides is not suitable to obtain commercial crops with low insecticide residue.

Neonicotinoids are one of the most important chemical insecticides used worldwide due to their high insecticidal activities against a broad range of insect pests (Nauen et al., 2008; Jeschke et al., 2011). They have versatile applications and are mainly used in crop protection and animal health care, particularly against coleopteran, dipteran, and lepidopteran insect pests, by treating foliage, soil and seeds (Elbert et al., 2008). Apart from directly killing target arthropods, the neonicotinoids have been reported to induce a series of physiological and behavioral sublethal effects in various non-target insects (Cutler et al., 2009; Shi et al., 2011; Fogel et al., 2013). Cycloxaprid is a novel neonicotinoid (Pan et al., 2014) with the NO_2 group in cis-configuration, whereas the NO_2 group in nearly all commercial neonicotinoid insecticides are in trans-configuration (Shao et al., 2011). Unlike conventional neonicotinoid insecticides, which act as agonists of the insect nicotinic

* Corresponding author.
E-mail address: luochen1010@126.com (C. Luo).

http://dx.doi.org/10.1016/j.cropro.2016.01.015
0261-2194/

acetylcholine receptor (nAChR) (Tomizawa and Casida, 2003), the unique cis-configuration pharmacophore may involve a differential interaction with nAChRs (Shao et al., 2011). Recently, the nicotinic acetylcholine receptor binding site and metabolism of cycloxaprid insecticide have been demonstrated (Shao et al., 2013; Liu et al., 2013). Compared to imidacloprid, thiamethoxam and nitenpyram, cycloxaprid exhibited remarkably high efficacy to control the planthoppers, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horváth) (Liu et al., 2013). Cycloxaprid also showed high insecticidal activity against other sucking and biting insect pests, such as the wheat aphid, *Sitobion avenae* (Fabricius) and the mirid bug, *Apolygus lucorum* (Meyer-Dür) (Cui et al., 2012; Pan et al., 2014). However, the lethal and sublethal effects of cycloxaprid on *B. tabaci* are not known.

In this study, we determined the lethal and sublethal effects of cycloxaprid on the adult MED and MEAM1 cryptic species of *B. tabaci*, and compared the insecticidal activities with four other commercial neonicotinoid insecticides under controlled conditions in the laboratory. Moreover, we assessed the sublethal effects of cycloxaprid on the developmental periods, fecundity, egg-laying duration of *B. tabaci* MED adults, and the potential impact on the egg hatchability.

2. Materials and methods

2.1. Insects

Laboratory susceptible strains of *B. tabaci* MED and MEAM1 cryptic species were obtained from the Institute of Vegetables and Flowers in the Chinese Academy of Agricultural Sciences, and established in the laboratory at the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, China which were described as Lab-Q and Lab-B by Guo et al. (2014). All strains were maintained on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') without exposure to insecticides, under a 16 h photoperiod at 27 ± 1 °C and 60 ± 10% humidity. All adults used in bioassays were less than 7 days old. Males and females were used in a 1:1 ratio.

2.2. Insecticides and chemicals

All neonicotinoid insecticides tested in this study were technical grade formulations. Imidacloprid (96%), acetamiprid (97%), thiamethoxam (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.

2.3. Lethal effects of the various neonicotinoid insecticides on *B. tabaci*

The toxicity of cycloxaprid, imidacloprid, nitenpyram, acetamiprid and thiamethoxam on adult *B. tabaci* was tested using a leaf-dip bioassay previously described by Yang et al. (2013). Cycloxaprid stock solution (1000 mg/L) was prepared by dissolving in DMSO while the remaining insecticides (stock solution 1000 mg/L) were dissolved in acetone. The working concentrations were then prepared by diluting the stock solution in distilled water containing Triton X-100 (0.1‰). Leaf discs (22 mm diameter) from cotton plants were dipped for 20s in the insecticide solution or in distilled water containing 0.1‰ triton X-100 (control). After drying, the leaf discs were placed in a flat-bottomed glass tube (78 mm long) containing agar (2 mL of 15 g/L) with their adaxial surface facing down. Adult whiteflies were collected in these tubes by inverting the tubes above the leaves on cotton plants maintained in the glasshouse. This allowed the adult flies (male female ratio 1:1) to fly into the tube. After collecting 25–45 adults per tube, the open end of the tube was sealed with a cotton plug, and maintained at 27 ± 1 °C, 60 ± 10% and a 16 h photoperiod. After 48 h, mortality was evaluated using a microscope. Adults showing no sign of movement were scored as dead. Bioassays consisted of four replicates for each concentration, with eight concentrations and the control for each bioassay.

2.4. Sublethal effects of cycloxaprid on *B. tabaci*

We first determined the concentration–mortality regression line using the data obtained from the lethal experiment involving eight cycloxaprid concentrations (from 0.1 mg/L, 0.2 mg/L, 0.4 mg/L, 0.8 mg/L, 1.6 mg/L, 3.2 mg/L, 6.4 mg/L, 12.8 mg/L). The LC_{25} value were then calculated from the regression lines (see "Results" section).

Adult *B. tabaci* were exposed to sublethal concentration of cycloxaprid and the following parameters were evaluated: developmental duration and survival rate of the larval instars, pseudopupae and adults, and fecundity, egg-laying duration of *B. tabaci* MED, and the hatching rate of eggs. Briefly, 10 insect-free host plants each were placed in two separate insect-proof cages equally. Plants in the experimental cage received LC_{25} treatment and the other served as the control and received no treatment. One hundred adult *B. tabaci* MED that previously fed on cycloxaprid treated (LC_{25}) cotton leaves by leaf dipping method (Yang et al., 2013) were then introduced into the experimental cage for egg laying. Untreated *B. tabaci* MED adults (n = 100) were introduced into the control cage. All adults used in bioassays were less than 7 days old. After 12 h, the plants were removed from the cages and two leaves per plant were marked. The eggs on the unselected leaves were removed by observation through a microscope. There were 20 eggs per selected leaf and the abaxial surface of the selected leaves was sketched and the position of each egg was marked. Ten selected leaves were respectively utilized for cycloxaprid treated group and the control group. These drawings allowed us to follow each whitefly egg until adult emergence. The plants were then placed in a separate climatic chamber at 27 ± 1 °C, 60 ± 10% RH and a 16L: 8D. When pseudopupae were observed on a specific leaf, the plant was moved to a cage with an insect-proof net. Nymphs and adults on each plant were counted and recorded every day. Newly emerged adults were individually transferred to a new leaf, and these leaves were cut from the stem to record the fecundity until the death of all individuals. Hatching rates of the eggs laid by the females were also recorded.

2.5. Statistical analysis

Probit analyses were performed to determine statistical significance of the concentration-dependent mortality using PoloPlus (LeOra software, 2002). The resistance factor (RF) calculated between the estimated LC_{50} of MED and MEAM1 populations through Robertson and Preisler (1992) method. All data were checked for normality using non-parametric Kolmogorov–Smirnov tests ($P < 0.05$). Data showing a normal distribution (number of eggs laid per female, oviposition period duration and hatchability of eggs) were compared using Student's t-test ($P < 0.05$). For data that were not normally distributed (developmental periods and survival rates), direct estimates were compared using the non-parametric Mann–Whitney U-test ($P < 0.05$) (SPSS, 2001). Comparisons were made between the LC_{25} treatment and the control.