Crop Protection 91 (2017) 82–88

Contents lists available at ScienceDirect

Crop Protection

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

Determination of resistance and resistance mechanisms to thiacloprid in *Cydia pomonella* L. (Lepidoptera: Tortricidae) populations collected from apple orchards in Isparta Province, Turkey

Mesut İşci [a], Recep Ay [b, *]

[a] Fruit Research Institute, Department of Plant Protection, Isparta, Turkey
[b] Suleyman Demirel University, Faculty of Agriculture, Department of Plant Protection, 32260, Isparta, Turkey

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form
21 September 2016
Accepted 23 September 2016

Keywords:
Cydia pomonella
Resistance
Thiacloprid
Chlorpyrifos
Detoxifying enzymes
Negative cross-resistance

ABSTRACT

The codling moth, *Cydia pomenella* is considered as the most important pest of apple worldwide and it causes significant economic losses yearly in orchards where it is not controlled effectively. The purpose of this study was to determine the resistance ratios to thiacloprid and the detoxification enzymes of *Cydia pomonella* from apple orchards in Isparta, Turkey. Populations of codling moth were collected from six orchards in the region and the diapausing larvae were treated with thiacloprid and chlorpyrifos by topical application.

The LD_{50} values of field and a susceptible population were used to determine the resistance ratios to thiacloprid and chlorpyrifos. The corresponding LD_{50} values of *C. pomonella* populations showed a low (5.5–6.7 fold) or medium resistance (11.2–16.5 fold) against thiacloprid but were susceptible to chlorpyrifos. In studies conducted with synergists, piperonyl butoxide (PBO) and S,S,S, tributyl phosphorotrithioate (DEF) had a significant synergistic effect on two populations (from Gelendost and Senirkent) that medium resistance to thiacloprid. The levels of detoxifying enzymes [esterase, glutathion –S– transferase (GST) and cytochrome P450 monooxygenase (P450)] were investigated using biochemical methods and differed depending on the population.

Based on the results of the enzyme analyses, the P450 and esterase enzymes may play a role in the resistance in codling moths to thiacloprid.

1. Introduction

The codling moth (*Cydia pomonella* L.) is an important pest of apples, pears, quince and walnuts that is found in all countries, with the exception of Japan, Taiwan, Korea, parts of eastern China and Western Australia and possibly Brazil. Larvae feeding in the fruits cause two types of damage. First, the larvae create a deep entrance hole as they bore toward the pip of the fruit and the second type occurs from shallow holes in the fruit (Barnes, 1991; Beers et al., 1993, 2003; Pedigo and Rice, 2009). Turkey is the third largest apple producer behind China and the USA (Anonymous, 2015a). In Isparta, apple production has reached 634,000 tons produced on 21,761 ha which represents 22% of the countrywide production and it contributes annually to the economy of Isparta by approximately 231 million dollars (Anonymous, 2015b). In the region of Isparta that is located at south western of Turkey, the codling moth has two generations per year.

Producers prefer to use chemicals for the control of the pest. When pesticides fail, the producer increases the dose or the frequency of insecticide application. Unfortunately, the use of high doses to manage pests causes the occurrence of resistance (FAO, 2012). The resistance to insecticides in the codling moth first developed in two different populations to lead arsenate (Hough, 1928). The insecticide DDT began to replace arsenate at the end of the 1940s and the first resistance to DDT was registered in 1951, with widespread resistance to DDT emerging within the next 10 years. Because of the resistance to DDT, the widespread use of organophosphorus insecticides began in the mid - 1950s, providing effective protection against the codling moth (Dunley and Welter, 2000). The resistance to organophosphorus insecticides has since been reported in different countries by many researchers, including the USA (Welter et al., 1991; Bush et al., 1993; Knight et al., 1994),

* Corresponding author.
E-mail address: recepay@sdu.edu.tr (R. Ay).

http://dx.doi.org/10.1016/j.cropro.2016.09.015
0261-2194/

North Africa (Blomefield, 1994) and France (Sauphanor et al., 1998).

For the insecticide growth regulator diflubenzuron, resistance was identified for the first time in Europe (Waldner, 1993), in Italy (Riedl and Zelger, 1994) and in the southwestern France (Sauphanor et al., 1994). In the following years, the organophosphate group of insecticides was examined for the development of resistance by Sauphanor et al. (2000); Knight et al. (2001), Reyes et al. (2007), Stara and Kocourek (2007) and Voudouris et al. (2011).

Reyes et al. (2007), Voudouris et al. (2011), Cichon et al. (2013) and İşci and Ay (2013, 2014) studied the development of resistance to the neonicotinoid group of insecticides, including thiacloprid. The resistance of the codling moth to other groups of insecticides has also been examined (Knight et al., 2001; Reyes et al., 2007; Sauphanor et al., 1997; Ioriatti et al., 2007; Sanchez et al., 2008).

Thiacloprid is a neonicotinoid insecticide and is in the N-cyanoamidines group, the classification of which is based on the molecular properties of the group (Elbert et al., 2008). For the neonicotinoid insecticides, the mode of action is connected to the nicotinic ACh receptors, with the insecticide behaving as ACh (acetylcholine) affecting the insect's central nervous system of the insect (Elbert et al., 2008; Millar and Denholm, 2007; Jeschke and Nauen, 2008). Thiacloprid is a broad-spectrum, systemic and traslaminar neonicotinoid insecticide. Thiacloprid has been registered since 2006 and used in Turkey against *Cydia pomonella* and *Aphis pomi* Deg. attacking apple (Anonymous, 2016). Chlorpyrifos is abroad-spectrum and contact-acting organophosphate insecticide and acaricide. Since 1981, it is has been registered and used in Turkey on agricultural crops against many pests including *C. pomonella* (Anonymous, 2016).

In this study, populations of codling moth collected from apple orchards in Isparta were investigated for the ratios and the mechanisms of resistance to thiacloprid and chlorpyrifos.

2. Materials and methods

2.1. Insects

The INRA (AVIGNON)/French National Institute for Agricultural Research provided the susceptible population (Sv) that was used as the reference strain in this study. It was collected from orchards in the southwest regions of France and has been maintained in the laboratory since 1995 (Bouvier et al., 2001; Berling et al., 2009). Field populations were collected from the city and the districts of Isparta. The properties of these populations are represented in Table 1. Samples were collected from a few neighbourhood apple orchards at each site. Diapausing larvae of field populations were collected from apple orchards by using corrugated cardboard tree bands.

All populations were maintained with a 16:8 h L:D photoperiod at 25 ± 1 °C with 60± 5% relative humidity: the production of codling moths under the same conditions was conducted with artificial feed (Codling Moth Diet; Soutland Products Inc.) (Reuveny and Cohen, 2007; Stara and Kocourek, 2007). Bioassays were carried out on fifth-stage larvae from the first to third generation laboratory population.

Table 1
Collection site and date of *Cydia pomonella* populations tested for their response to thiacloprid and their detoxification enzyme.

Collection site	Collection date
INRA (France) (Susceptible Sv)	–
Isparta Central	13.07.2011
Senirkent	12.06.2012
Gelendost	11.06.2012
Tepeli-Eğirdir	30.06.2013
Serpil-Eğirdir	02.07.2013
Yalvaç	05.07.2013

2.2. Insecticide and synergist bioassays

2.2.1. Insecticide bioassays

The tests were based on the method described by Sauphanor et al. (1997) and a commercial formulation of the insecticide thiacloprid (Calypso OD 240 g/l; BayerCropscience) and chlorpyrifos (Dursban 4 EC 480 g/l; Dow Agrosciences) was used in this study. Accordingly, the LD_{50} values of the *C. pomonella* populations to thiacloprid were determined by topical application. First, the insecticide was diluted with distilled water to prepare the different treatment concentrations. The test treatments were 1 control +6 concentrations, with 5 replications of each. Each replication included five fifth-stage larvae. The insecticide treatments of 1.0 µl, were applied to the middle of the thorax on the dorsaline of the fifth-stage larvae with a micropipette. Distilled water was applied as the control. Following the application of thiacloprid, the larvae were transferred to an artificial diet in Petri dishes in groups of five, and after 72 h, the live and dead larvae were counted. Before each experiment, mortality tests were performed to determine the concentration range for approximately 10–90% mortality. Experiments in which control mortality exceeded 10% were repeated. The pooled data were analyzed using Probit analysis (POLO PC) computer program (LeOra Software, 1994) to estimate the LD_{50} and LD_{90} values with 95% CLs. The resistance ratio was calculated according to the formula: RR = LD_{50} value of the orchard population/LD_{50} value of the susceptible population. The resistance ratios were classified according to Koh et al. (2009): low resistance RR $\leq$ 10, medium resistance 10 < RR $\leq$ 40, high resistance 40 < RR $\leq$ 160 and very-high resistance RR > 160.

2.2.2. Synergist bioassays

Three synergists, piperonyl butoxide (PBO), S,S,S, tributyl phosphorotrithioate (DEF) and diethyl maleate (DEM), were used to determine whether enzymes played a role in the metabolism of resistance to the insecticide. The monooxygenase enzyme inhibitor PBO (2000 mg/l), the GST enzyme inhibitor DEM (1000 mg/l) and the esterase enzyme inhibitor DEF (1000 mg/l) were prepared for use (Van Leeuwen et al., 2004; Wang et al., 2009; Van Leeuwen and Tirry, 2007). All synergist were dissolved in acetone: distillated water (1:1; v: v) The synergist solutions were applied to the thorax dorsaline of the fifth instars larvae as 2.0 µl of PBO, 1.0 µl of DEF, and 1.0 µl of DEM with a micropipette one hour before the insecticide applications. The synergists were dissolved in acetone. One hour after the application of the synergists, the insecticide concentrations were applied to the larvae as described previously. The treatments for insecticide tests were 1 control +6 concentrations, with 5 replications. Only the synergist was applied to the control. The live and dead larvae were counted after 72 h. Experiments where control mortality exceeded 10% were repeated. The synergistic ratio (SR) was calculated using the following formula:

SR = LD_{50} of thiacloprid without a synergist/LD_{50} of thiacloprid with a synergist (Kim et al., 2004).

2.3. Biochemical studies

2.3.1. Enzyme extract-preparation

Some of the larvae used for bioassay studies were stored at −80 °C for biochemical studies in a deep freeze. Ten fifth instar codling moth larvae were homogenized in 2.0 ml of homogenization buffer (0.1 M sodium phosphate buffer, pH: 7.6, and 1 mM