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The efficacy of novel diamide insecticides in *Grapholita molesta* suppression and their residues in peach fruits

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Abstract: The oriental fruit moth [(*Grapholita molesta* (Busck, 1916)] represents one of the most significant and destructive pests of peaches in Serbia and worldwide. Its susceptibility to the novel diamide insecticides, cyantraniliprole and chlorantraniliprole, was assessed in this article. The dissipation dynamics and behaviour of these insecticides in the peach fruit were also determined. Field trials were carried out at two localities in the Republic of Serbia (Čerević, Mala Remeta), according to standard EPPO methods. The cyantraniliprole (100 g a. i./l, SE) and chlorantraniliprole (200 g a. i./l, SC) based products were foliar applied at rate of 0.6 and 0.2 l/ha, respectively. The efficacy of the applied products was evaluated by counting the number of fruits damaged by the *G. molesta* larvae. The high efficacy of cyantraniliprole (89.5–94.1%) and chlorantraniliprole (93.5–95.6%) was achieved at both localities. Right after the drying of the deposit, the concentration of cyantraniliprole in the peach fruits was at the EU maximum residue level (MRL) of 1.5 mg/kg, while the MRL level of 1 mg/kg was achieved after seven days (0.95 mg/kg) for chlorantraniliprole. The cyantraniliprole and chlorantraniliprole half-life dissipation in the peach fruit were 2.50 and 3.15 days. It can be concluded that the high efficacy of the researched insecticides is a good indicator of *G. molesta* susceptibility in peach orchards.

Keywords: *Cydia molesta*, *Prunus persica*; cyantraniliprole; chlorantraniliprole; HPLC

The oriental fruit moth, *Grapholita molesta* (Busck, 1916) represents one of the most significant and destructive pests of peaches in Serbia and worldwide. It is regularly found in peach and quince orchards in Serbia and it requires constant monitoring and use of protective measures (Stamenković 2005). Damage is caused by larvae that feed by boring into the growing shoots or the fruits. Besides these types of direct damage, the indirect damage caused by *G. molesta* is also very significant. Fruit infestations reduce its quality and, thus, create economic losses, as well as increasing costs for sorting. Also, it can limit the export, because this pest belongs to a quarantine species in some importing countries, so the infested fruit must be discarded. The suppression of these insects largely relies on chemical protection with insecticides (Knight & Light 2013), but this control measure is threatened by its widespread resistance development (Knight 2010; Rodríguez *et al.* 2011). Thus, due to the necessary application of insecticides on peaches against *G. molesta*, good agricultural practices require the utilisation of products with a shorter pre-harvest interval (PHI) and more convenient ecotoxicological properties (Lazić *et al.* 2012), such as those from the

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diamide insecticide class. Cyantraniliprole and chlorantraniliprole represent second-generation anthranilic diamide insecticides which act on insect ryanodine receptors causing mortality from the uncontrolled release of the calcium ion stores in the muscle cells. In Serbia, cyantraniliprole and chlorantraniliprole based insecticides are registered for the control of *G. molesta* in peaches and nectarines. In some countries, these insecticides are recommended for protection in plum and apricot orchards against this pest. These insecticides provide many obvious benefits in agriculture, but their inappropriate use can result in unacceptably high levels of these compounds in the fruits. Even in cases when applied in compliance with good agricultural practices, the pesticides may leave residues (Lazić *et al.* 2012a). Our goal was to evaluate the efficacy of cyantraniliprole and chlorantraniliprole on the *G. molesta* susceptibility in peach orchards and to determine the residual behaviour, the dissipation dynamics and their PHI in peach fruits.

MATERIALS AND METHODS

Field trials. In 2017, field trials were conducted in the Republic of Serbia at the localities Čerević (45°11'51.1"N, 19°40'10.8"E, 161 m a.s.l.) and Mala Remeta (45°05'46.6", 19°44'58.8"E, 228 m a.s.l.). Over the course of the trial, the average temperatures (17–23°C) and precipitation (2.2–2.7 mm) were recorded on a daily basis. The experiments were carried out according to the standard of the European and Mediterranean Plant Protection Organization (EPPO) (2014, 2015) methods for the experimental design, data analysis, insecticide efficiency and phytotoxicity. Products based on cyantraniliprole (100 g a. i./l, SE) and chlorantraniliprole (200 g a. i./l, SC) were foliar applied at rate of 0.6 and 0.2 l/ha, respectively, using a Solo 423 Port mistblower (Solo, Germany) with a wide spray diffuser and water consumption of 1000 l/ha. The orchards were 5 years old with the represented peach cultivar Royal gem, with a planting distance of 4.5 × 2 m. The treatment was conducted on the 27th of May 2017 at Čerević, while at Mala Remeta, the treatment was accomplished on the 29th of May 2017 (for controlling the first generation of the *G. molesta*). At the time of the treatment, the peaches were in the BBCH 74 phenophase. The experiment was set up in four replications, with the layout of the basic plots according to a randomised block system. The basic plot consisted of four peach trees. Before setting the trial at both localities, the oriental fruit moth was monitored and its number was determined by counting the moths caught in pheromone RAG traps (Csalomon, Hungary). The efficacy of the tested insecticides was evaluated on the basis of the number of damaged fruits by the *G. molesta* larvae, i.e., 100 fruits per repetition. The efficacy evaluations were carried out 2 weeks after the treatment and shortly before harvesting.

Cyantraniliprole and chlorantraniliprole residue determination. In order to evaluate the cyantraniliprole and chlorantraniliprole residues in the peach fruits, around 1.0 kg of fruit samples were randomly picked from each repetition and delivered to the laboratory for Biological Research and Pesticides, Department for Plant and Environmental Protection, Agricultural Faculty Novi Sad. The fruit samples were taken 1 h after the pesticide application and, afterwards, during 1 to 7 days after the application for the analysis of the cyantraniliprole residues and 1 h after the application and, afterwards, 1, 3, 5, 7, 9, 11, 13 and 15 days after the chlorantraniliprole application. The untreated samples were also collected and used as a control. The extraction procedure involved a widely used QuEChERS-based method (EN 15662) (Anastassiades *et al.* 2003), while the cyantraniliprole residues were analysed using an high-performance liquid chromatography (HPLC) with a diode-array detector (DAD), series 1100 (Agilent Technologies, USA). The separation was conducted on a Zorbax Eclipse XDB-C18 (Agilent Technologies, USA) column (50 × 4.6 mm, 1.8 μm) under an isocratic working regime based on the mobile phase acetonitrile/water (1.5% acetic acid) with a ratio of 30 : 70, w/v, a flow rate of 1.0 ml/min, a column temperature of 25°C and a detection wavelength 254 nm. Each analytical sample was triply considered. The extraction of chlorantraniliprole from the fruits was conducted using the same method, while the HPLC/DAD conditions for the determination were: the acetonitrile/water acidified with H_3PO_4 (pH 2.5) at a ratio of 75 : 25, at the ambient temperature; a flow rate of 0.75 ml/min; and a detection wavelength of 230 nm. Evaluation of the chromatographic conditions was carried out by the linearity of the detector response, accuracy and precision of the method, and the matrix effect, as well as determination of the limit of quantification (LOQ). The linearity was evaluated at five concentrations ranging from 0.03–3.0 mg/kg for cyantraniliprole and 0.1–5.0 mg/kg for chlorantraniliprole. In order to evaluate the accuracy, the control peach fruit samples were spiked with a known amount of the cyantraniliprole

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(0.03, 0.3 and 0.45 mg/kg) and the chlorantraniliprole (0.1, 1.0 and 5.0 mg/kg) solution. The precision of the method was evaluated through repeatability (0.3 and 1.0 mg/kg) and expressed as the RSD of the peak areas. The matrix effects were examined comparing the slopes of the calibration curve of the matrix-matched standards (MMC) and solvent-based standards (SC). The LOQ was estimated as the lowest concentration at which the recovery value was acceptable.

Statistical analysis. The results of the field trials were presented as the absolute and mean values for the number of damaged fruits, the standard deviation from the average values (SD), the efficacy (E) according to Abbott (Wentzel 1963) and statistically analysed by an ANOVA and the Fisher LSD test for the confidence interval of 95%, in the statistical program R (version 3.2.2).

The cyantraniliprole and chlorantraniliprole dissipation in the peach fruits was studied by subjecting the data to the first-order kinetic Equation 1:

$$C_t = C_0 \times e^{-kt} \quad (1)$$

where C_t – the insecticide residue at time t; C_0 – the initial deposits of the insecticide, k – the constant of the dissipation rate in days

The half-life ($t_{1/2}$) was calculated from the k value using Equation 2:

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

where: ln – natural logarithm; k – the constant of the dissipation rate in days.

RESULTS

Monitoring of Grapholita molesta flight. Sex pheromone traps were used for the monitoring of the oriental fruit moth flight during the study. Two sticky rag traps were installed in the trial orchards. Both of them were baited with a standard orfamone OFM L2 capsule. The sex pheromone traps were installed in the centre of the trial orchard on the 10th of May 2017 (Čerević) and the 11th of May 2017 (Mala Remeta). All the traps were checked twice a week. The traps installed in the reference orchard Čerević and in Mala Remeta caught 16 moths and 8 moths in total (Figure 1), respectively, during the field trials.

Evaluation of the cyantraniliprole and chlorantraniliprole biological efficacy. The results of the efficacy studies of the insecticides based on cyantraniliprole and chlorantraniliprole in the peach orchards for the control of *G. molesta* are presented in Table 1. Fourteen days after the application of the insecticides for the control of the 1st generation of *G. molesta*, the number of damaged fruits at the locality of Čerević was significantly lower compared to the control, with an efficacy of 91.3% for cyantraniliprole and 95.6% for chlorantraniliprole. The efficacy of cyantraniliprole and for chlorantraniliprole, 24 days after use, was 90.3 and 93.5%, respectively. At the locality of Mala Remeta, fourteen days after the insecticide application for the control of the 1st generation of *G. molesta,* the number of damaged peach fruits was at a significantly lower level compared to the control, with an efficacy of 94.1% for cyantraniliprole and 94.7% for chlorantraniliprole. During the second evaluation, 24 days after the treatment, the number of damaged fruits was also at a significantly lower level in regard to the control, and the cyantraniliprole and the chlorantraniliprole efficacy was 89.5% and 94.1%, respectively.

Determination of the cyantraniliprole and chlorantraniliprole residues. The method for the determination of the cyantraniliprole residues in the peaches was developed and validated in accordance with the requirements of the method in the SANTE (11813/2017) standard, providing results for the linearity (r^2 = 0.9998), accuracy (86.3–93.9%), repeatability (0.57%) and limit of quantification (0.03 mg/kg). The matrix effect was not so high (114.3%), however, the MMC was used for the determination of the cyantraniliprole residues. After validation, the method was employed to determine the residues in the peach fruits, after application of the cyantraniliprole based plant protection product. The cyantraniliprole residue levels in the peach samples are presented in Table 2. Immediately after the drying of the deposit, the concentration of cyantraniliprole (1.46 mg/kg) in the peach fruits was at the EU MRL (Commission Regulation EU 2018/832) of 1.5 mg/kg. In this experiment, the initial cyantraniliprole residue concentration level dropped by half three days after the treatment. The cyantraniliprole dissipation rate in the peaches the followed first-order kinetics, $C = 1.46e^{-0.28t}$ (r^2 = 0.964) with a half-life (DT_{50}) of 2.5 days. According to the MRL and based on the obtained results, the calculated PHI of cyantraniliprole in the peach fruits was seen one day after the treatment. In addition, the results suggest that the half-life of cyantraniliprole in the peach fruits is three days. The results of the method validation for the determina-

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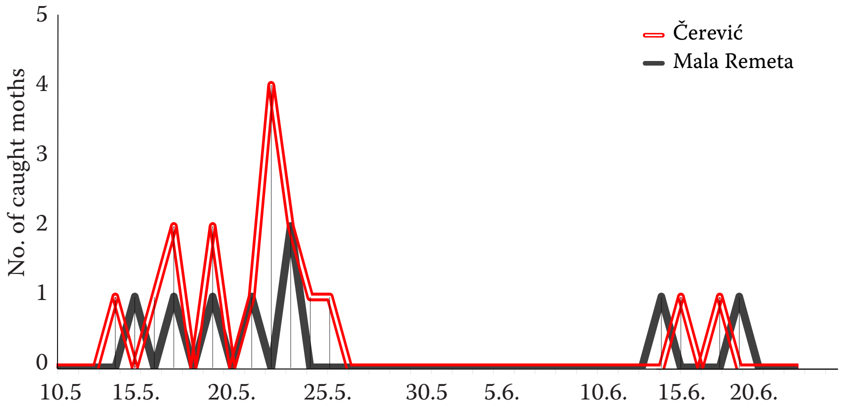

Figure 1. The flight dynamics of *Grapholita molesta* at the locality of Mala Remeta and Čerević (2017)

Table 1. The insecticide efficacy in the control of *Grapholita molesta* at Mala Remeta and Červić

	Dose (l/ha)	14 days after the treatment			24 days after the treatment		
		$\bar{x}$	SD ±	*E* (%)	$\bar{x}$	SD ±	*E* (%)
Mala Remeta							
Cyantraniliprole (100 g a.i./l)	0.6	0.25[b]	0.50	94.1	0.50[b]	0.58	89.5
Chlorantraniliprole (200 g a.i./l)	0.2	0.25[b]	0.50	94.7	0.25[b]	0.50	94.1
Control	–	4.25[a]	1.50	–	4.75[a]	0.96	–
LSD (0.05%)			1.43			1.19	
Čerević							
Cyantraniliprole (100 g a.i./l)	0.6	0.50[b]	1.00	91.3	0.75[b]	0.50	90.3
Chlorantraniliprole (200 g a.i./l)	0.2	0.25[b]	0.50	95.6	0.50[b]	0.50	93.5
Control	–	5.75[a]	0.95	–	7.75[a]	1.71	–
LSD (0.05%)			1.69			1.81	

$\bar{x}$ – average number of damaged fruits; SD – standard deviation; *E* – efficacy; different letters indicate significant differences between groups ($P < 0.05$)

tion of the chlorantraniliprole residues [(linearity (r^2 = 0.9999), accuracy (79.3–95.1%), repeatability (0.97%) and limit of quantification (0.05 mg/kg)] in the peach fruits were completely in accordance with criteria of SANTE (11813/2017). After the application of chlorantraniliprole, the maximum level of the residues in the peach fruits was determined after drying the deposit and it was 2.32 mg/kg. The average value of the chlorantraniliprole residues in the samples collected one day after treatment was 2.04 mg/kg, with a loss of 12.07%. The study established that the level of chlorantraniliprole was below the MRL of 1 mg/kg seven days after the application. With further research, it was found that the chlorantraniliprole content in the samples was progressively decreasing. The loss after the ninth day was 78.02%. After the pre-harvest period of 14 days, the level of chlorantraniliprole was 0.12 mg/kg, which is significantly below the allowed level. The DT_{50} of this substance was calculated based on the residue content. With the use of chlorantraniliprole in the amount of 0.2 l/ha, the half-life was 3.15 days (Table 3).

Table 2. The exponential model best fit experimental data for the decline curves of cyantraniliprole in the peach fruits (r^2 = 0.964).

Interval/days	Cyantraniliprole (mg/kg)	Persistence (%)	Loss (%)
Initial	1.46	100	0
1	1.32	90.40	9.60
2	1.20	82.30	17.70
3	0.65	44.58	55.42
4	0.46	31.82	68.18
5	0.39	26.75	73.25
6	0.24	16.60	83.40
7	0.19	13.03	86.97
8	0.13	8.64	91.36
MRL (mg/kg)		1.5 mg/ml	
DT_{50} (days)		2.5	

MRL – the maximum residue level; DT_{50} – the half-life

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Table 3. The residues of chlorantraniliprole in the peach fruits

Interval/days	Chlorantraniliprole (mg/kg)	Persistence (%)	Loss (%)
Initial	2.32	100	0
1	2.04	87.93	12.07
3	1.73	74.57	25.43
5	1.32	56.90	43.10
7	0.95	40.95	59.05
9	0.51	21.98	78.02
11	0.23	9.91	90.09
13	0.10	4.31	95.69
15	0.12	5.17	94.83
MRL (mg/kg)		1 mg/ml	
DT_{50} (days)		3.15	

MRL – the maximum residue level; DT_{50} – the half-life

DISCUSSION

Comparison of the number of damaged fruits, depending on the locality, showed a highly uniform presence of damaged fruits, evident at both of the localities. Tamaš *et al*. (2015) conducted field trials according to the standard EPPO method in order to evaluate the efficacy of cyantraniliprole and chlorantraniliprole in peach orchards for the *G. molesta* control in Serbia. The results indicated a high efficacy of cyantraniliprole (0.6 l/ha, 100 g a.i./l) ranging from 94.5 to 97.9% and 94.2–98.7% for chlorantraniliprole (0.2 l/ha, 200 g a.i./l), which is consistent with our results. Similar results were obtained in field trials carried out in Italy and Spain. According to Milanese *et al*. (2014), results of 19 trials conducted with a cyantraniliprole based product (100 g/l) showed the good control of *G. molesta* in peaches and nectarines. Due to its high efficacy, selectivity toward the crop and favourable toxicological and eco-toxicological profile, cyantraniliprole may be a valuable tool for insecticide resistance management (IRM) and integrated pest management (IPM) programmes (Bassi *et al*. 2009; Milanese *et al*. 2014).

In recent years, a number of studies dealt with the behaviour of cyantraniliprole and chlorantraniliprole in plants, but none of them were conducted on peach fruits. Hong-Mei *et al*. (2014) established that the half-life of cyantraniliprole in pepper ranged from 9.2–11.2 days. Also, Sun *et al*. (2012) showed that the half-life of cyantraniliprole ranged from 2.9–6.4 days in the pak choi. In tomatoes, the half-life of cyantraniliprole was 2.6 days, with a PHI of 3 days, after treatment at the recommended dose (Malhat *et al*. 2018). According to Hu *et al*. (2013), the DT_{50} values of cyantraniliprole were 1.1 and 2.7 days in watermelons. The cyantraniliprole half-lives determined in rice straw at three localities were 3.2, 4.4 and 6.3 days (Zhang *et al*. 2013). The chlorantraniliprole residues in vegetables, fruits, and cereals (corn, peaches, pears, rice, tomatoes, and zucchinis) were also determined by Pengjun *et al*. (2010). Schwarz *et al*. (2011) applied the QuEChERS method for determining the content of the chlorantraniliprole and cyantraniliprole residues in four types of the matrix (oil, aqueous, acidic, and dry). The quantification limit in all of the tested matrices was 0.01 mg/kg, with a recovery factor of 87–107%. Pan *et al.* (2015) developed and validated the method for the simultaneous determination of chlorantraniliprole and cyantraniliprole in apples, grapes, spaghetti, tomatoes, rice, and wheat. An ultra-performance liquid chromatography – tandem mass spectrometer was used to determine the residue content, while the extraction was performed with acetonitrile. At three levels of concentration, the yield extraction was 95.5–106.2%. Farag (2012) developed the method for determining the residues of chlorantraniliprole in grapes. The values of the limit of detection(LOD) and LOQ of 0.02 mg/kg and 0.06 mg/kg, respectively, as well as the high yield values of extraction from 95.11 to 102% confirmed the possibility of using this method on real samples.

According to the available literature, this is the first research of cyantraniliprole and chlorantraniliprole dissipation dynamics in peach fruits. Based on the presented results, it can be concluded that high efficacies of the investigated insecticides from the group of diamides (cyantraniliprole and chlorantraniliprole), are a good indication of *G. molesta* susceptibility. Also, the obtained results of the dissipation study showed that the cyantraniliprole and chlorantraniliprole in the peach fruits declined rapidly and had low half-life values. Therefore, it is recommended to use cyantraniliprole and chlorantraniliprole based insecticides for control of *G. molesta* in peach orchards.

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