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CHANGES IN THE COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) SUSCEPTIBILITY LEVEL TO PYRETHROIDS AND THE PEST RESISTANCE MECHANISMS TO DELTAMETHRIN

Joanna Zamojska, Paweł Węgorek, Marek Mrówczyński*

Institute of Plant Protection – National Research Institute
Władysława Węgorka 20, 60-318 Poznań, Poland

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Abstract: Monitoring of Colorado potato beetle (CPB) resistance to different insecticides has been conducted in Poland for years. This work presents the current status of the CPB susceptibility level to deltamethrin and beta-cyfluthrin, noting the important increase of pyrethroid toxicity towards the CPB, when compared with earlier data. The work also presents research on CPB resistance mechanisms to pyrethroids. The resistance research was conducted using different enzymes blockers: piperonyl butoxide, S,S,S-tributylphosphorotrithioate and diethyl malonate.

Key words: Colorado potato beetle, pyrethroids, resistance, resistance mechanisms, synergetics

INTRODUCTION

Pyrethroids have been the most numerously represented insecticide group used for Colorado potato beetle (*Leptinotarsa decemlineata* Say) control, since 1981 in Poland. The first introduced pyrethroid active substance was deltamethrin, which has been in use for 30 years (Zamojska 2011). After recording too high a level of Colorado potato beetle (CPB) resistance to selected pyrethroid insecticides (Węgorek 2005), new strategies of potato protection against the CPB were introduced (Węgorek *et al*. 1998). Pyrethroid use was limited to only one treatment per season and using insecticides from other chemical groups of neonicotinoids and phenylpyrazoles, to which the pest had not developed resistance in Poland, was recommended. The very high effectiveness of insecticides from these chemical groups as well as their very high popularity among farmers caused the selective pressure of pyrethroids be much weaker. This is the reason for testing the current status of CPB susceptibility level to pyrethroids (deltamethrin and beta-cyfluthrin) as well as the deltamethrin detoxification mechanisms of this insect.

Insect resistance to insecticides is based mainly on detoxification metabolism engaging three enzyme groups: oxidases, esterases and glutathione transferases (Schoknecht and Otto 1989; Malinowski 2003; Alyokhin *et al*. 2008). Oxidative metabolism participation in CPB pyrethroid resistance was noted in Poland (Węgorek 2004). In this study, participation of oxidases as well as the remaining enzyme groups, was tested.

The aim of the study was to detect the direction of susceptibility level changes which will surely help strategy modification and modernization.

MATERIALS AND METHODS

IRAC Susceptibility Test Method No. 7 was used for testing (Węgorek 2005).

Insecticides (commercially available products)

Insecticide concentrations in ppm were calculated, assuming that 200 l of water would be used per hectare.
- beta-cyfluthrin (Bulldock 025 EC – 25 g of active substance/1 l of product): recommended dose: 0.3 l/ha, recommended concentration 37.5 ppm,
- deltamethrin (Decis 2.5 EC – 2.5% of active substance): recommended dose: 0.3 l/ha, recommended concentration: 37.5 ppm.

In the years: 2008–2010, Colorado potato beetle adults and non-infested, untreated potato leaves were collected for testing from three populations in the Wielkopolska region: Września, Wałcz and Krotoszyn. Tests were performed in laboratory conditions at 20–22°C and at a photoperiod of 16:8 (L:D).

A final assessment – lethal effects of the active substance of the insecticides was determined after 24 hours of application and expressed as percent mortality of the CPB at each dose. Lethal concentrations LC_{50} and LC_{95} were calculated using a computer program based on Finney's probit analysis method (Finney 1952) and expressed in the ppm concentration of an active substance.

*Corresponding address:
j.zamojska@iorpib.poznan.pl

To asses the resistance of a given population, the resistance coefficient was calculated as follows:

resistance coefficient (RC) = LC_{95}/recommended field dose

Recommended field concentrations, giving about 100% mortality, were established during field experiments for the registration of a given insecticide. The following criteria for resistance assessment were assumed:

RC ≤ 1 – the lack of resistance,
RC = 1.1–2 – low resistance,
RC = 2.1–5 – medium resistance,
RC = 5.1–10 – high resistance,
RC > 10 – very high resistance.

Mechanisms of the CPB resistance to deltamethrin were tested using synergists which block three main enzyme groups. They are:
- piperonyl butoxide (PBO) – oxidases blocker,
- S,S,S-tributylphosphorotrithioate (DEF) – esterases blocker,
- diethyl malonate (DEM) – glutathione transferases blocker.

PBO concentration was always 100 ppm. DEF and DEM concentrations were always 200 ppm. These concentrations were determined during initial studies as not causing an increase in insect mortality in relation to the control. PBO was used in the form of water dilutions while DEF and DEM were dissolved first in a slight amount of acetone and then the solution was completed with demineralised water. Each time, the proper amount of tested insecticide was added to the same concentration of tested synergist. On the basis of the results of these experiments, LC_{50}, LC_{95} and RC were calculated. Additionally, the synergism coefficient (SC) was calculated as follows:

SC = LC of active substance alone/LC of active substance with a synergist

The following criteria were accepted to asses synergism between deltamethrin and a given synergist:

SC < 1 – antagonism,
SC = 1 – the lack of synergism and the lack of antagonism,
SC > 1 – synergism.

RESULTS

In the conducted studies, LC_{50} values for deltamethrin (Table 1) were between 2.68 ppm (Wałcz in 2009) and 12.54 ppm (Krotoszyn in 2009). There were no cases in which the recommended concentration – 37.5 ppm – was exceeded. The highest LC_{95} value for deltamethrin was recorded for the population of Krotoszyn in 2010 – 183.61 ppm. The recommended concentration – 37.5 ppm – was exceeded 4.9 times. The lowest LC_{95} value – 44.25 ppm – was recorded for the population of Września in 2010, which exceeded the recommended concentration 1.18 times. Resistance coefficient values, calculated for deltamethrin, showed low resistance for the population of Września in all the study years, and medium level of resistance for the populations of Wałcz and Krotoszyn, also in all the study years.

LC_{50} values for beta-cyfluthrin (Table 1) were between 3.21 ppm (Września in 2010) and 14.09 ppm (Wałcz in 2010). The recommended concentration – 37.5 ppm – was not exceeded in any experiment. LC_{95} values calculated for beta-cyfluthrin were between 70.83 ppm (Wałcz in 2008) and 229.38 (Krotoszyn in 2010). In all experiments, the recommended concentration was exceeded. Resistance coefficient values pointed to high resistance in one case (Krotoszyn in 2010), low resistance also in one case (Wałcz in 2008), and medium resistance in all the remaining cases.

Table 1. Susceptibility level of the CPB to deltamethrin and beta-cyfluthrin. Results expressed in LC_{50}, LC_{95} and RC

Year	Active substance	LC_{50} [ppm] (confidence intervals, p = 0,95)			LC_{95} [ppm]			RC and resistance classification		
		Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn
2008	beta-cyfluthrin	11.99 (8.04–16.43)	7.19 (3.69–10.58)	6.84 (2.03–13.40)	125.57	70.83	213.82	3.34 medium	1.88 low	5.7 medium
	deltamethrin	8.57 (5.95–11.88)	7.68 (5.26–10.52)	11.20 (7.29–16.65)	61.95	124.71	153.01	1.65 low	3.32 medium	4.08 medium
2009	beta-cyfluthrin	4.55 (2.79–6.74)	6.04 (4.20–8.06)	7.65 (3.96–12.36)	91.34	82.82	101.55	2.43 medium	2.20 medium	2.79 medium
	deltamethrin	7.54 (5.34–10.14)	2.68 (1.65–3.76)	12.54 (8.27–18.97)	68.83	109.21	147.25	1.83 low	2.91 medium	3.92 medium
2010	beta-cyfluthrin	3.21 (1.52–5.02)	14.09 (6.41–33.91)	8.04 (3.73–13.75)	89.19	177.68	229.38	2.37 medium	4.73 medium	6.11 high
	deltamethrin	5.70 (3.73–7.90)	8.64 (6.36–11.36)	8.63 (6.90–10.56)	44.25	102.83	183.61	1.18 low	2.74 medium	4.89 medium

RC – resistance coefficient

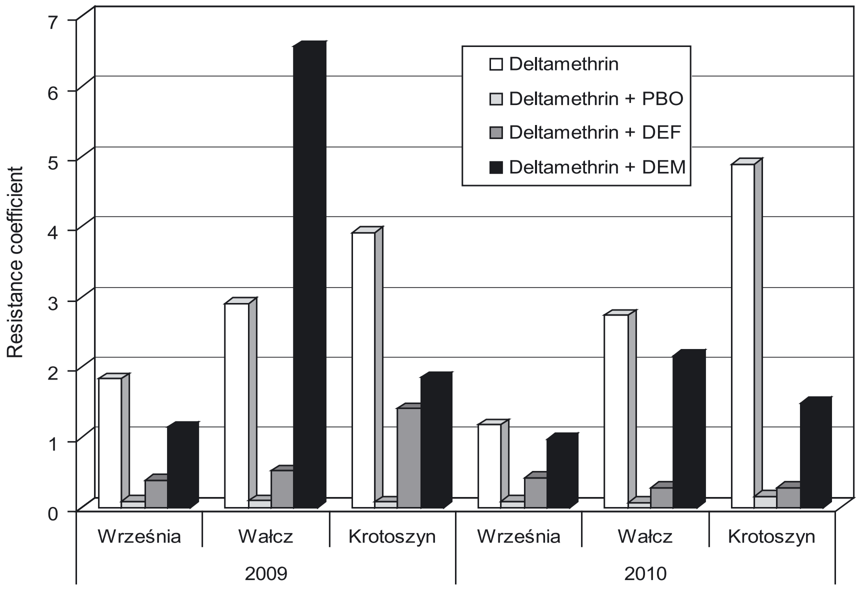

Fig. 1. The influence of synergists on Colorado potato beetle resistance to deltamethrin in the years 2009–2010

Table 2. The influence of PBO, DEF and DEM on deltamethrin action on the Colorado potato beetle in the years 2009–2010. Results expressed in LC_{50}, LC_{95} and coefficients: RC and SC

Value	Substance	2009			2010		
		Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn
LC_{50} [ppm] (confidence intervals, p = 0.95)	deltamethrin	7.54 (5.34–10.14)	2.68 (1.65–3.76)	12.54 (8.27–18.97)	5.70 (3.73–7.90)	8.64 (6.36–11.36)	8.63 (6.90–10.56)
	deltamethrin + PBO	0.28 (0.19–0.36)	0.32 (0.20–0.44)	0.26 (0.11–0.41)	0.35 (0.17–0.54)	0.30 (0.18–0.42)	0.70 (0.38–1.01)
	deltamethrin + DEF	3.15 (1.98–4.64)	3.36 (2.08–4.91)	0.82 (0.21–1.56)	2.7 (2.25–3.23)	2.27 (1.95–2.59)	1.66 (0.77–2.52)
	deltamethrin + DEM	4.61 (3.73–5.5)	12.72 (7.48–26.78)	3.45 (2.11–5.00)	4.72 (4.05–5.46)	3.37 (1.52–5.63)	6.69 (5.63–7.84)
SC for LC_{50}	deltamethrin + PBO	26.92	8.37	48.23	16.28	28.8	12.32
	deltamethrin + DEF	2.39	0.79	15.29	2.11	3.80	5.19
	deltamethrin + DEM	1.63	0.21	3.63	1.20	2.56	1.29
LC_{95} [ppm]	deltamethrin	68.83	109.21	147.25	44.25	102.83	183.61
	deltamethrin + PBO	3.37	3.80	3.06	3.42	2.49	6.05
	deltamethrin + DEF	14.99	19.81	53.21	15.88	10.70	10.57
	deltamethrin + DEM	42.76	246.42	69.53	36.47	81.20	56.02
SC for LC_{95}	deltamethrin + PBO	20.42	28.74	48.12	12.93	41.29	30.34
	deltamethrin + DEF	4.59	5.51	2.76	2.78	9.61	17.37
	deltamethrin + DEM	1.61	0.44	2.11	1.21	1.26	3.27
RC and resistance classification	deltamethrin	1.83 low	2.91 medium	3.92 medium	1.18 low	2.74 medium	4.89 medium
	deltamethrin + PBO	0.089 lack of resistance	0.10 lack of resistance	0.081 lack of resistance	0.091 lack of resistance	0.066 lack of resistance	0.16 lack of resistance
	deltamethrin + DEF	0.39 lack of resistance	0.52 lack of resistance	1.41 low	0.42 lack of resistance	0.28 lack of resistance	0.28 lack of resistance
	deltamethrin + DEM	1.14 low	6.57 high	1.85 low	0.97 lack of resistance	2.16 medium	1.49 low

PBO – piperonyl butoxide; DEF – S,S,S-tributylphosphorotrithioate; DEM – diethylmalonate; RC – resistance coefficient; SC – synergism coefficient

Results presented in table 2 and on figure 1 show that, in the case of the CPB, the main role is played by oxidases, out of all the enzymes taking part in the deltamethrin metabolism. Also, esterases take part in detoxification of this active substance. Results do not show the participation of glutathione transferases in deltamethrin detoxification in the CPB.

The synergism coefficient for deltamethrin and PBO, calculated for LC_{50} values, ranged between 8.37 (Wałcz in 2009) and 48.23 (Krotoszyn in 2009). For deltamethrin with DEF, this coefficient ranged from 0.79 (Wałcz in 2009) to 15.29 (Krotoszyn in 2009). For deltamethrin with DEM, synergism coefficient values, calculated for LC_{50}, were between 0.21 (Wałcz in 2009) and 3.63 (Krotoszyn in 2009).

Synergism coefficient values for deltamethrin with PBO, calculated for LC_{95} values, ranged from 12.93 (Września in 2010) to 48.12 (Krotoszyn in 2009). For deltamethrin with DEF, these values were between 2.76 (Krotoszyn in 2009) and 17.37 (Krotoszyn in 2010). The same values for deltamethrin with DEM, ranged from 0.44 (Wałcz in 2009) to 3.27 (Krotoszyn in 2010).

Synergism between deltamethrin and PBO changed the resistance classification for deltamethrin from low or medium resistance to the lack of resistance, in all cases. In the case of deltamethrin with DEF, (except for one case, in which resistance classification changed from medium to low) (Krotoszyn in 2009), in all the remaining cases, resistance level was changed to the lack of resistance. As for deltamethrin with DEM, weak antagonism was recorded in one case (Wałcz in 2009), and no change was recorded in two cases (Września in 2009 and Wałcz in 2010). In three cases, slight synergism was recorded.

DISCUSSION

In the presented studies, the CPB showed some level of resistance to pyrethroids, which is consistent with results obtained by other authors over the past 20 years (Węgorek 1994, 2004, 2005; Peric *et al*. 1997; Hilton *et al*. 1998; Miyo *et al*. 1999; Alyokhin *et al*. 2008; Whalon *et al*. 2008). The CPB susceptibility level at the beginning of pyrethroid use in Poland, was much higher than in the presented study (Malinowski and Kroczyński 1980). High effectivenes of deltamethrin and beta-cyfluthrin in CPB control was recorded also by Mrówczyński (Mrówczyński *et al*. 1994) and Pietkiewicz and Pawińska (1985). Differences in toxicity between these two active substances were not high in those studies.

The first records on the decrease of CPB susceptibility level to pyrethroids in Poland, concerned deltamethrin (Węgorek 1994). When comparing results obtained by Węgorek in the years 2002–2004 when CPB resistance to pyrethroids was very high (Węgorek 2005), with the present results of our study, an increase in the CPB susceptibility level is visible. In research from 2002–2004, deltamethrin, lambda-cyhalothrin, alpha-cypermethrin and cypermethrin LC_{95} concentration were quite a bit above 10,000 ppm, in all experiments. In this presented study LC_{95} for deltamethrin was from 44.25 ppm to 183.61 ppm, and for beta-cyfluthrin from 70.83 ppm to 229.38 ppm. Also, LC_{50} levels in the presented study were much lower than in the studies from the years 2002–2004. It can be concluded that in recent years CPB resistance to pyrethroids decreased. This decrease can be explained by the wide use of fipronil, from the phenylopyrazoles, (presently withdrawn) and the wide use of active substances from the neonicotinoids group (imidacloprid, acetamiprid, tiachloprid, chlotianidin and thiametoxam) in recent years in Poland. The return of a higher susceptibility to pyrethroids in Polish CPB populations, can also be caused by the fact that insects which are resistant to insecticides are often less adapted to the environment (Alyokhin and Ferro 1999; Baker *et al*. 2007; Benkovskaya 2006).

In different parts of the world, the CPB developed resistance to more than 50 active substances from different chemical groups. The presented data show that decreasing the pyrethroids' selective pressure had a very good effect. This process can be used in strategies preventing the resistance phenomenon. For several years, the CPB susceptibility level to pyrethroids increased to a level which now ensures effectiveness of pyrethroid chemical treatments.

From the presented results, it can be concluded that the main detoxification mechanism for pyrethroids in the CPB, is oxidative metabolism. After adding PBO to deltamethrin in all the experiments, a greater increase in insects mortality was recorded. Similar results of cooperation between PBO and pyrethroids, were obtained by Węgorek (2004). Other authors obtained synergism between PBO and insecticides from different chemical groups (Rose and Brindley 1985; Mota-Sanchez 2003, Mota-Sanchez *et al*. 2006; Sharif *et al*. 2007). It can be concluded, that CPB is a species using oxidative enzymes for detoxification of insecticides of different mechanisms of action. The results on synergism between deltamethrin and PBO do not exclude the role played by esterases, because PBO is, in some species, regarded also to be an esterase blocker (Gunning *et al*. 1998).

Also, the esterase blocker DEF, caused increased mortality of the CPB in the presented studies. Such experiments using DEF, were conducted for the first time in Poland. It can be assumed that a detoxification mechanism based on this enzymes group, is also present in the CPB. Other authors have indicated the role of esterases in detoxification of pyrethroids in the CPB (Argentine *et al*. 1995; Lee and Clark 1996). Malinowski (1988) stated that increased metabolism, based on esterases, most often acts in addition to other resistance mechanisms.

The participation of glutathione transferases in deltamethrin metabolism was much lower than in case of oxidases and esterases. The presented results are similar to results of other authors (Argentine *et al*. 1994; Shariff *et al*. 2007).

From our study, it can be concluded, that there are several ways of pyrethroid detoxification in CPB. The cause of significant differences in the CPB susceptibility levels, often within a population, is the high genetic polymorphism of this species (Argentine *et al*. 1989).

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POLISH SUMMARY

ZMIANY WE WRAŻLIWOŚCI STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY) NA PYRETROIDY I MECHANIZMY ODPORNOŚCI SZKODNIKA NA DELTAMETRYNĘ

Monitoring poziomu odporności stonki ziemniaczanej na różne substancje aktywne insektycydów jest w Polsce prowadzony od wielu lat. Praca przedstawia obecny poziom odporności stonki ziemniaczanej na deltametrynę i beta-cyflutrynę, wskazując na znaczny wzrost toksyczności pyretroidów względem stonki ziemniaczanej. Wyniki badań porównywano z otrzymywanymi w latach wcześniejszych.

Omówiono również wyniki badań nad mechanizmami odporności stonki ziemniaczanej na pyretroidy, prowadzonych przy użyciu blokerów różnych grup enzymów: butoksylan piperonylu, S,S,S-tributylotrifosforan, dietylomalenian.