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SUSCEPTIBILITY LEVEL OF THE COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) TO CHLORPYRIFOS AND ACETAMIPRID IN POLAND AND RESISTANCE MECHANISMS OF THE PEST TO CHLORPYRIFOS

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Abstract: Nowadays, neonicotinoids play an essential role in the control of the Colorado potato beetle (CPB) in Poland. Taking into consideration that CPB shows some resistance to pyrethroids and the main role of oxidative metabolism in this resistance, research was conducted to estimate CPB susceptibility level to chlorpyrifos and acetamiprid. The results pointed to a lack of CPB resistance to acetamiprid and a weak susceptibility level to chlorpyrifos by the CPB. For this reason, the second part of the experiment was aimed at detecting the resistance mechanisms of the CPB to chlorpyrifos. Results showed that none of the tested enzyme groups (oxidases, esterases and glutathione transferases) are the reason for CPB resistance to chlorpyrifos. The experiments revealed an increase in the beetles survival after adding oxidative enzyme blocker to chlorpyrifos.

Key words: Colorado potato beetle, acetamiprid, chlorpyrifos, resistance, resistance mechanisms, synergetics

INTRODUCTION

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) developed a level of resistance to many insecticides in Poland (Kroczyński *et al*. 1989; Węgorek 1994, 2005, 2007; Malinowski 2003). This species has developed different resistance mechanisms, including: enhanced metabolism involving monooxygenases, esterases, glutathione transferases, target site insensitivity, increase of excretion, reduced penetration and behavioral resistance. The monooxygenase system is the most common mechanism in the CPB (Slicox *et al*. 1985; Argentine *et al*. 1995; Hoy and Head 1995; Malinowski 2003; Stankovic *et al*. 2004; Mota-Sanchez *et al*. 2006; Sharif *et al*. 2007). The insect's metabolism can lead to the production of a metabolite that is less or more toxic to the insect than the parent compound. For example oxi-metabolite of chlorpyrifos is much more toxic than chlorpyrifos. Also, thiamethoxam, bensultap and indoxacarb are known to be pre-insecticides (Richter *et al*. 1989; Różański 1992; Malinowski 2003; Nauen *et al*. 2003)

The introduction of neonicotinoids in 1998, reduced the problem of CPB resistance in Poland. Since the registration of acetamiprid, there has been a constant monitoring of the CPB susceptibility level to this active substance and other substances from the mentioned neonicotinoid group. This monitoring is necessary, because there are several cases of identified CPB resistance to imidacloprid and acetamiprid in the world (Olson *et al*. 2000; Zhao *et al*. 2000; Mota-Sanchez *et al*. 2006; Alyokhin *et al*. 2007; Baker *et al*. 2007).

The aim of the study was to estimate the current status of the CPB susceptibility level to acetamiprid and chlorpyrifos, and to detect the resistance mechanisms of CPB to chlorpyrifos with the use of three different enzyme blockers.

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 with the assumption that 200 l of water would be used per hectare.

- acetamiprid (Mospilan 20 SP – 20% of active substance): recommended dose: 0,08 kg/ha, recommended concentration 80 ppm,
- chlorpyrifos (Pyrinex 480 EC – 480 g of active substance/1 l of product): recommended dose: 0,6 l/ha (this concerns a different product containing chlorpyrifos), recommended concentration: 1,440 ppm.

In the years: 2008–2010, Colorado potato beetle adults, and not-infested, untreated potato leaves were collected for testing from three populations in the Wielkopolska region: Września, Wałcz and Krotoszyn. A final assessment

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for lethal effects of the active substance in the insecticides were determined after 24 hours of application, and expressed as percent mortality of Colorado potato beetles at each dose. Tests were performed in laboratory conditions at 20–22°C at a photoperiod of 16:8 (L:D).

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.

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 – 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 CPB adults resistance to chlorpyrifos were tested using synergists which block three main enzymes groups. The synergists 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 the 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. LC_{50}, LC_{95} and RC were calculated on the basis of the results of these experiments. Additionally, the synergism coefficient (SC) was calculated as follows:

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

The following criteria were accepted to assess synergism between chlorpyrifos and a given synergist:
SC < 1 – antagonism,
SC = 1 – the lack of synergism and the lack of antagonism.
SC > 1 – synergism.

RESULTS

Results of research on the CPB adults susceptibility level to acetamiprid revealed the high toxicity of this active substance to CPB (Table 1). LC_{50} values ranged from 1.29 ppm (Krotoszyn in 2009) to 10.79 ppm (Wałcz in 2008). Because the recommended concentration of acetamiprid in Poland is 80 ppm, this dose was not exceeded in any of the experiments. Acetamiprid's good action was also visible after calculating LC_{95} values. In all the experiments these values were below the recommended concentration and ranged from 13.09 ppm (Września in 2009) to 52.96 ppm (Wałcz in 2008). In the case of the CPB, all the experiments showed a lack of resistance to acetamiprid, because resistance coefficient values were always below.

Table 1. Susceptibility level of Colorado potato beetle adults to acetamiprid and chlorpyrifos. 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	acetamiprid	5.94 (2.87–8.42)	10.79 (7.14–14.80)	4.85 (1.45–7.98)	23.92	52.96	31.65	0.30 lack of resistance	0.66 lack of resistance	0.40 lack of resistance
	chlorpyrifos	116.65 (96.79–141.31)	90.00 (74.22–108.39)	106.45 (70.63–155.80)	302.80	404.19	459.66	0.21 lack of resistance	0.28 lack of resistance	0.32 lack of resistance
2009	acetamiprid	2.96 (2.19–3.65)	3.95 (2.44–5.52)	1.29 (0.48–2.17)	13.09	38.34	17.53	0.16 lack of resistance	0.48 lack of resistance	0.22 lack of resistance
	chlorpyrifos	229.77 (169.12–315.78)	69.92 (59.97–80.63)	375.78 (259.18–556.91)	1,446.66	483.51	1,828.00	1.004 low	0.33 lack of resistance	1.27 low
2010	acetamiprid	6.47 (3.56–9.21)	8.92 (5.28–12.74)	10.35 (7.03–13.91)	39.01	44.35	52.31	0.48 lack of resistance	0.55 lack of resistance	0.65 lack of resistance
	chlorpyrifos	319.24 (169.57–595.96)	234.42 (196.00–279.21)	250.48 (117.16–492.65)	1,461.6	548.63	979.27	1.01 low	0.38 lack of resistance	0.68 lack of resistance

RC – resistance coefficient

Much higher LC_{50} values were obtained for chlorpyrifos which means worse toxicity for the CPB. LC_{50} values ranged from 69.92 ppm (Wałcz in 2009) to 375.78 ppm (Krotoszyn in 2009). LC_{95} values for chlorpyrifos were much higher than in the case of acetamiprid. The LC_{95} values were between 302.8 ppm (Września in 2008) and 1828 (Krotoszyn in 2009). These values slightly exceed the recommended concentration (1,440 ppm). Resistance coefficient values in the three experiments pointed to low resistance of the pest to chlorpyrifos. Results shown in table 2 also suggest some resistance of the CPB to chlorpyrifos. Percent of insect mortality using 100% of the recommended concentration ranged from 85% (Września in 2010) to 100% (Września in 2008, Wałcz in 2008, Wałcz 2009 and Wałcz 2010).

Table 2. Susceptibility level of Colorado potato beetle adults to chlorpyrifos. Results expressed in percent mortality of insects

Concentration [ppm]	Września			Wałcz			Krotoszyn		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
1,440.0 (100% of recommended concentration)	100	95	85	100	100	100	98	85	90
720	100	85	80	100	98	95	98	75	90
360	100	70	80	95	90	85	92	60	85
180	75	35	30	70	80	35	80	30	45
90	25	15	0	50	60	0	40	0	0
45	10	15	0	28	30	0	20	0	0
22.5	0	0	0	5	20	0	0	0	0
11.25	0	0	0	0	0	0	0	0	0

Table 3. The influence of PBO, DEF and DEM on chlorpyrifos action on Colorado potato beetle beetles 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)	chlorpyrifos	229.7 (169.1–315.7)	69.9 (59.9–80.6)	375.7 (259.2–556.9)	319.2 (169.5–595.9)	234.4 (196.0–279.2)	250.4 (117.1–492.6)
	chlorpyrifos+PBO	781.39 (582.9–1,165.8)	916.79 (684.8–1,402.9)	707.0 (508.1–1,103.8)	784.7 (533.4–1,421.3)	1,000.1 (890.8–1,144.6)	859.8 (768.7–974.2)
	chlorpyrifos+DEF	270.4 (196.5–378.3)	74.27 (47.07–109.01)	228.92 (137.2–369.1)	219.21 (141.7–335.1)	178.8 (139.6–227.2)	148.1 (116.3–187.4)
	chlorpyrifos+DEM	230.38 (162.7–327.5)	123.7 (89.48–168.6)	214.2 (127.2–355.4)	223.12 (137.5–355.8)	181.2 (108.9–290.9)	219.2 (158.9–299.5)
SC for LC_{50}	chlorpyrifos+PBO	0.29	0.07	0.53	0.41	0.23	0.29
	chlorpyrifos+DEF	0.85	0.94	1.64	1.45	1.31	1.69
	chlorpyrifos+DEM	0.99	0.56	1.75	1.43	1.29	1.14
LC_{95} [ppm]	chlorpyrifos	1,446.6	483.5	1,828.0	1,461.6	548.6	979.2
	chlorpyrifos+PBO	4,758.2	4,150.1	3,733.8	4,699.2	3,205.0	2,827.5
	chlorpyrifos+DEF	1,209.55	313.86	750.71	1,090.3	394.89	708.59
	chlorpyrifos+DEM	1,361.15	582.4	1,091.6	1,077.0	760.2	798.2
SC for LC_{95}	chlorpyrifos+PBO	0.30	0.11	0.49	0.31	0.17	0.34
	chlorpyrifos+DEF	1.19	1.54	2.43	1.34	1.38	1.38
	chlorpyrifos+DEM	1.06	0.83	1.67	1.35	0.72	1.22
RC and resistance classification	chlorpyrifos	1.004 low	0.33 lack of resistance	1.27 low	1.01 lack of resistance	0.38 lack of resistance	0.68 lack of resistance
	chlorpyrifos+PBO	3.3 medium	2.88 medium	2.59 medium	3.26 medium	2.22 medium	1.96 low
	chlorpyrifos+DEF	0.84 lack of resistance	0.22 lack of resistance	0.52 lack of resistance	0.75 lack of resistance	0.27 lack of resistance	0.49 lack of resistance
	chlorpyrifos+DEM	0.94 lack of resistance	0.40 lack of resistance	0.75 lack of resistance	0.74 lack of resistance	0.52 lack of resistance	0.55 lack of resistance

PBO – piperonal butoxide; DEF – S,S,S-tributylphosphorotrithioate; DEM – diethyl malonate; RC –resistance coefficient; SC – synergism coefficient

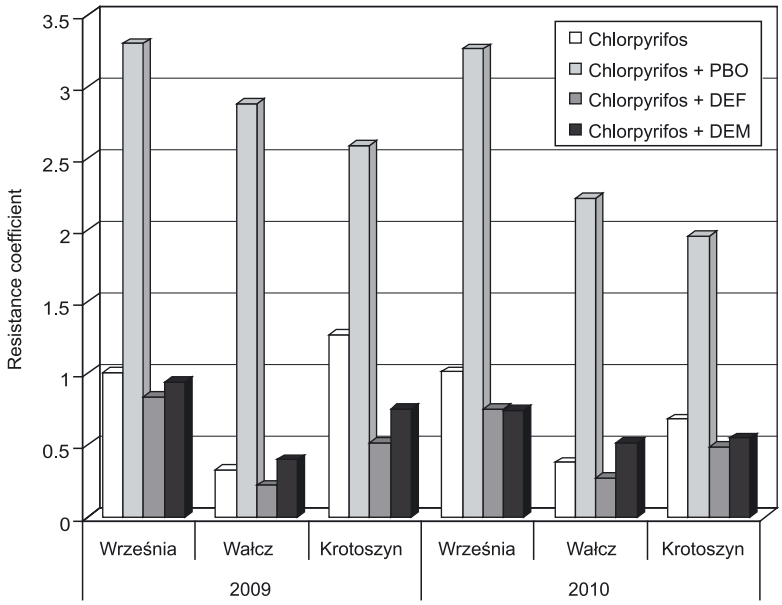

Fig. 1. The influence of synergists on colorado potato beetle adults resistance to chlorpyrifos in the years 2009–2010

Research on the CPB mechanisms for chlorpyrifos detoxification with the use of oxidative enzyme blocker – PBO (Table 3, Fig. 1), showed a decrease of chlorpyrifos toxicity. This decrease is the evidence for antagonism between PBO and chlorpyrifos. Synergism coefficients calculated for LC_{50} were between 0.07 (Wałcz in 2009) and 0.53 (Krotoszyn in 2009), while the same coefficients calculated for LC_{95} ranged from 0.11 (Wałcz in 2009) to 0.49 (Krotoszyn in 2009).

Research on cooperation between chlorpyrifos and DEF, expressed in synergism coefficient values calculated for LC_{50}, revealed a weak antagonism between these two active substances in two cases (Września and Wałcz in 2009), SC values for LC_{50}: 0.85 and 0.94. In the remaining cases, slight synergism was recorded and SC values calculated for LC_{50} were between 1.31 and 1.69. The synergism coefficient calculated for LC_{95} ranged from 1.19 to 2.43, signifying a weak synergism between these substances.

A similar situation was recorded in the research on the cooperation between chlorpyrifos and DEM. Synergism coefficient values calculated for LC_{50} in two cases, signaled slight antagonism (0.56 and 0.99) and in the remaining cases signaled slight synergism (1.14–1.75). Synergism coefficients calculated for LC_{95} showed slight antagonism in two cases (0.82 and 0.72), and slight synergism in the remaining cases (1.06–1.67).

Such results disclose distinct, antagonistic action between chlorpyrifos and oxidases blocker (PBO). Results on cooperation between chlorpyrifos and DEF or DEM are not univocal. It cannot be concluded that esterases or glutathione transferases play a role in chlorpyrifos detoxification in the CPB.

DISCUSSION

Our research showed the Colorado potato beetle adults reveals a high level of susceptibility to acetamiprid. The pest tolerates the active substance chlorpyrifos much more. In Poland, research on the CPB susceptibility level to acetamiprid was conducted in the years 2002–2004 (Węgorek 2005). The research in 2002–2004 showed that LC_{50} ranged from 1.8 to 3 ppm and LC_{95} from 6.7 to 26.6 ppm. In our presented study, LC_{50} was between 1.29 and 10.79 ppm and LC_{95} between 13.09 and 52.96. So, there was a slight decrease in the CPB susceptibility level to this active substance. However, the pest is still efficiently controlled with acetamiprid at the recommended dose. Since there is a tendency for the susceptibility level to decrease, constant monitoring of the CPB susceptibility level is needed. It is important to bear in mind that in other countries, the CPB has built up a resistance to acetamiprid and other neonicotinoids, (Olson *et al*. 2000; Zhao *et al*. 2000; Mota-Sanchez *et al*. 2006; Alyokhin *et al*. 2007; Baker *et al*. 2007). Considering the species evolution, its high susceptibility to neonicotinoids is surprising, because neonicotinoids are derivates of an alcaloid produced by plants from *Solanace* family.

Results indicating lower CPB susceptibility level to chlorpyrifos are surprising in the light of the resistance of the species to pyrethroids, based on an oxidative metabolism (Węgorek 2004). For other species, like the pollen beetle which is also resistant to pyrethroids, negative cross-resistance was recorded between chlorpyrifos and pyrethroids (Węgorek 2009), because oxi-metabolite of chlorpyrifos is a very strong acetylcholinesterase inhibitor (Różański 1992). Thus, a similar reaction was expected in the case of the CPB. Instead of confirming this phenomenon, the results showed a decreased susceptibility level of the CPB to chlorpyrifos. Moreover, the presented studies did not show the participation of oxidases, esterases and glutathione transferases in the CPB resistance to chlotpyrifos. Thus, it can be surmised that there is a mechanism of resistance based on modified acetylcholinesterase in this species. Modified enzyme may have a worse affinity to chlorpyrifos and chlorpyrifos oxi-metabolite. Resistance of the CPB to chlorpyrifos, based on acetylcholinesterase's insensitivity, was recorded by Ioannidis *et al*. (1992). Such a mechanism, in the case of carbamates, was also recorded by Stankovic *et al*. (2004).

Blocking oxidative enzymes with the use of PBO caused an increase in the CPB survival in the presented studies, which can be explained by the decrease in chlorpyrifos oxi-metabolite production. In this case, PBO did not act in a synergetic way but as an antagonist.

Developing of a resistance mechanism, based on oxidative enzymes, is beneficial in the case of a lot of natural and synthetic active substances. Such a mechanism can have negative consequences for the CPB when considering that toxins act similarly to chlorpyrifos, and metabolites have a higher affinity to the acetylcholinesterase of this species.

The research confirms the participation of at least several ways for detoxification metabolism in the CPB beetles, that have a great influence both on susceptibility level to insecticides and development of resistance mechanisms.

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POLISH SUMMARY

POZIOM WRAŻLIWOŚCI STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY) NA CHLOROPIRYFOS I ACETAMIPRYD W POLSCE ORAZ MECHANIZMY ODPORNOŚCI SZKODNIKA NA CHLOROPIRYFOS

Stonka ziemniaczana (*Leptinotarsa decemlineata* Say) wykształciła odporność na 52 różne substancje aktywne, należące do wszystkich grup insektycydów. Biorąc pod

uwagę wykształcenie u stonki ziemniaczanej w Polsce odporność na pyretroidy oraz główną rolę metabolizmu oksydacyjnego w tej odporności, prowadzone są badania mające na celu oszacowanie poziomu wrażliwości stonki ziemniaczanej na chloropiryfos i acetamipryd. Wyniki badań wykazały brak odporności stonki ziemniaczanej na acetamipryd i słaby poziom wrażliwości szkodnika na chloropiryfos. Z tego powodu, druga część badań miała na celu określenie mechanizmów odporności stonki na chloropiryfos. Wyniki wykazały, że żadna z badanych grup enzymów (oksydazy, esterazy i transferazy glutationu) nie przyczynia się do jej odporności na chloropiryfos. Doświadczenia udowodniły wzrost przeżywalności chrząszczy stonki po dodaniu do chloropiryfosu blokera enzymów oksydacyjnych. Antagonizm ten tłumaczony jest zmniejszeniem poziomu metabolitu tlenowego w organizmie szkodnika, który jest silniejszą toksyną od substancji macierzystej.