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JOURNAL OF PLANT PROTECTION RESEARCH Vol. 53, No. 1 (2013)

DOI: 10.2478/jppr-2013-0013

SUSCEPTIBILITY LEVEL OF THE POLLEN BEETLE (*MELIGETHES AENEUS* F.) TO SELECTED PYRETHROIDS IN POLAND, AND RESISTANCE MECHANISMS OF THE PEST TO DELTAMETHRIN

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Received: September 20, 2012
Accepted: January 31, 2013

Abstract: Pollen beetle susceptibility level to different insecticides has been investigated in Poland for several years. So far, high resistance to many of the insecticides' active substances has been found. The work presents the current status of the pollen beetle susceptibility level to deltamethrin and beta-cyfluthrin, pointing to the beetle's medium, high or very high resistance to both active substances. Also, pollen beetle resistance mechanisms to deltamethrin were investigated. Research, conducted with the use of oxidases, esterases and glutathione transferases blockers, showed that all three enzymes groups are involved in the pollen beetle resistance mechanisms to deltamethrin. The main role was played by oxidases and to a lower degree: esterases and glutathione transferases, respectively.

Key words: pollen beetle, pyrethroids, resistance, resistance mechanisms, synergists

INTRODUCTION

Insect resistance to insecticides is one of the main problems in plant protection resulting in great yield losses. Presently one of the most important insect pests, known for its resistance to many active substances of insecticides, is the pollen beetle (*Meligethes aeneus* F.). The susceptibility level of the pest to different insecticides, especially to pyrethroids, has been widely investigated (Węgorek 2005; Hansen 2008; Richardson 2008; Węgorek and Zamojska 2008; Węgorek *et al*. 2009; Zamojska *et al*. 2010). The constant decrease in the susceptibility level of the pest to many active substances creates the need to monitor the resistance phenomenon. Deltamethrin and beta-cyfluthrin are very popular insecticides from the pyrethroid group which have been used against the pollen beetle since the beginning of the eighties and since the mid-nineties, respectively.

One of the most important insect resistance mechanisms to insecticides is the biotransformation of active substances involving three main enzyme groups: monooxigenases, esterases, and glutathione transferases (Ahmad 1986; Yu 1988; Malinowski 2003; Terra and Fereira 2005). The most common pollen beetle resistance mechanism to pyrethroids is the one based on monooxygenases, however, it is known that esterases also play a role in this process (Węgorek 2009; Philippou *et al*. 2010; Węgorek *et al*. 2011a).

The first aim of the study was to determine the current status of the pollen beetle susceptibility level to deltamethrin and beta-cyfluthrin. The second aim was to investigate the participation of all three enzyme groups (oxidases, esterases and glutathion transferases) in the process of the pest resistance to deltamethrin. To achieve the second aim, three enzymes blockers were used (Malinowski 2003), among them, S,S,S-tributylphosphorotrithioate (DEF) as an esterases blocker, and diethyl malonate (DEM) as a transferases blocker which were used on the pollen beetle for the first time.

MATERIALS AND METHODS

In the research, Insecticide Resistance Action Committee (IRAC) Susceptibility Test Method No. 7 was used. The method, thoroughly described earlier (Węgorek *et al*. 2011a), was adapted with slight modifications. Instead of filter paper, plant material (oilseed rape leaves and inflorescences) was used and 20 g of plant material was put inside each glass container used for testing.

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 the product): recommended dose (in the years 2008–2010. Presently it is not recommended

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against pollen beetle, but still recommended against cabbage seed weevil, which is most often present on rape fields together with pollen beetle in Poland): 0.25 l/ha, recommended concentration 31.25 ppm,
- deltamethrin (Decis 2.5 EC – 2.5% of active substance): recommended dose: 0.2 l/ha, recommended concentration: 25 ppm.

Pollen beetle adults, together with the untreated plant material, were collected for testing from three populations in the Wielkopolska region in Poland: Września, Wałcz and Krotoszyn, in the years 2008–2010. Laboratory conditions and statistical calculations were consistent with the ones described for the colorado potato beetle in the Journal of Plant Protection Research (Węgorek *et al*. 2011b; Zamojska *et al*. 2011). Base on the percent mortality of the pollen beetle at each dose, lethal concentrations LC_{50} and LC95 were calculated.

Resistance coefficient values were calculated as follows:

resistance coefficient (RC) = LC_{95}/recommended field dose (with the assumption that the recommended field dose had resulted in 100% mortality of insects at registration time).

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 pollen beetle resistance to deltamethrin were tested using synergists which block three main enzyme groups (Malinowski 2003). The synergists used were:
- piperonyl butoxide (PBO) – oxidases blocker
- S,S,S-tributylphosphorotrithioate (DEF) – esterases blocker
- diethyl malonate (DEM) – glutathione transferases blocker.

The method was thoroughly described in the Journal of Plant Protection Research (Zamojska *et al*. 2011). The only difference is that PBO concentration as well as DEF and DEM concentrations were always 100 ppm. It was investigated earlier that these concentrations do not cause any insect mortality when they are applied alone, without insecticides.

The synergism coefficient (SC) values were 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 insecticide:
SC < 1 – antagonism
SC = 1 – the lack of synergism and the lack of antagonism
SC > 1 – synergism.

RESULTS

The pollen beetle susceptibility level to deltamethrin and beta-cyfluthrin was presented in table 1. Most often there was high resistance of the pest to these active substances. The range of LC_{50} for deltamethrin was from 20.12 ppm (Września in 2009) to 43.53 ppm (Września in 2008). LC_{50} values exceeded the concentration recommended in Poland (25 ppm) in seven cases (in 2008: Września and Krotoszyn; in 2009: Wałcz and Krotoszyn; in 2010: Września, Wałcz and Krotoszyn). The highest LC_{95} value (809.16 ppm) exceeded the recommended concentration 32 times, and was recorded for the population of Września in 2009. The lowest LC_{95} value (107.89 ppm), exceeded the recommended concentration 4.3 times, and was noted in Wałcz in 2008. Thus, all LC_{95} values exceeded the recommended concentration many times. Resistance coefficient values for deltamethrin, presented in table 1, point to the population of Września in 2008 and 2009 and the population of Krotoszyn in 2010, as having the highest resistance. High resistance was noted for Września in 2010, Wałcz in both 2009 and 2010, and Krotoszyn in 2008 and 2009. Only in one case – Wałcz in 2008, was there medium resistance recorded. There was no low resistance or the lack of resistance stated.

For beta-cyfluthrin, the LC_{50} values were between 16.35 ppm (Wałcz in 2009) and 133.38 ppm (Września in 2010). The values exceeded the recommended concentration (31.25 ppm) in five cases (in 2008: Września and Krotoszyn; in 2009: Września; in 2010: Września, Wałcz and Krotoszyn). The highest LC_{95} value was noted for Września in 2010: 7653.6 ppm. This value was over 200 times above the recommended concentration. The lowest LC_{95} value (191.56) exceeded the recommended concentration 5 times (Krotoszyn in 2008). Resistance coefficient values revealed very high resistance for Września in 2008, 2009, and 2010, and for Wałcz in 2010. Medium resistance was recorded only once – for Wałcz in 2008. In the remaining cases, high resistance to beta-cyfluthrin was stated.

Research on cooperation between oxidative enzyme blocker PBO and deltamethrin (Table 2, Fig. 1) showed a very strong synergistic action between the two substances. Synergism coefficient values calculated for LC_{50} were between 255.36 (Krotoszyn in 2009) and 821.39 (Wałcz in 2009). Synergism coefficient values calculated for LC_{95}, also signaled strong synergism ranging between 11.3 (Krotoszyn in 2009) and 765.52 (Września in 2009).

Research on cooperation between deltamethrin and esterases blocker DEF also showed synergistic action, however, it was much less intensive than in the case of PBO. Synergism coefficient values for LC_{50} were placed between 1.87 (Września in 2009) and 7.25 (Wałcz in 2010). Synergism coefficient values for LC_{95} were very similar: between 3.96 (Wałcz in 2010) and 10.26 (Września in 2009).

A similar situation was recorded in the research on the cooperation between deltamethrin and glutathione transferases' blocker DEM. Synergism coefficient values calculated for LC_{50} ranged between 1.46 (Września in 2009) and 3.89 (Wałcz in 2009). The values calculated for LC_{95} did not differ significantly and were placed between 1.99 (Krotoszyn in 2009) and 7.95 (Wałcz in 2009). Such results distinctly point to the synergistic action of both substances.

Table 1. Susceptibility level of pollen beetle adults to deltamethrin and beta-cyfluthrin. Results expressed in LC_{50}, LC_{95}, and Resistance Coefficient (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	44.05 (28.16–130.22)	20.91 (11.56–41.38)	32.68 (21.22–65.20)	326.95	123.44	191.56	10.46 very high	3.95 medium	6.12 high
	deltamethrin	45.53 (32.98–79.53)	20.64 (15.52–29.10)	26.94 (18.99–45.05)	284.75	107.81	228.19	11.39 very high	4.31 medium	9.12 high
2009	beta-cyfluthrin	35.51 (27.15–47.97)	16.35 (13.19–19.60)	30.99 (21.16–45.58)	443.23	167.73	182.37	14.18 very high	5.36 high	5.83 high
	deltamethrin	20.12 (13.54–35.54)	35.32 (19.71–169.68)	28.09 (21.05–42.01)	809.16	231.92	141.30	32.36 very high	9.27 high	5.65 high
2010	beta-cyfluthrin	133.38 (76.65–379.98)	30.84 (18.42–77.06)	50.39 (42.76–62.13)	7653.6	912.5	255.22	244.91 very high	29.2 very high	8.16 high
	deltamethrin	25.27 (17.19–45.05)	29.68 (17.41–98.55)	28.58 (23.57–36.01)	215.68	134.55	297.24	8.62 high	5.37 high	11.88 very high

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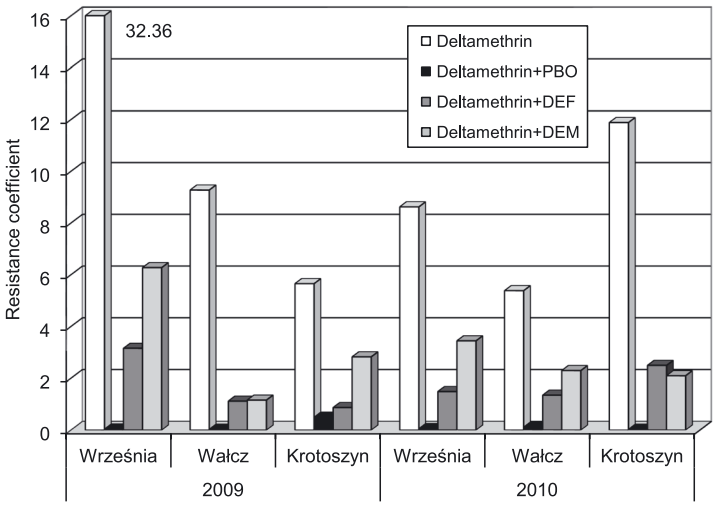
Table 2. The influence of PBO, DEF, and DEM on deltamethrin action in the pollen beetle adults in the years 2009–2010. Results expressed in LC_{50}, LC_{95} and coefficients: RC and SC

Value	Substance	2009			2010		
		Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn
LC_{50} [ppm] (confidence intervals, p = 0.95)	deltamethrin	20.12 (13.54–35.54)	35.32 (19.71–169.68)	28.09 (21.05–42.01)	25.27 (17.19–45.05)	29.68 (17.41–98.95)	28.58 (23.58–36.31)
	deltamethrin + PBO	0.078 (0.053–0.105)	0.043 (0.028–0.060)	0.11 (0.070–0.159)	0.072 (0.057–0.088)	0.083 (0.064–0.104)	0.038 (0.024–0.053)
	deltamethrin + DEF	10.75 (8.00–15.73)	5.91 (3.12–10.58)	5.97 (3.47–10.30)	4.83 (3.17–6.99)	4.09 (1.54–7.72)	9.37 (6.62–14.47)
	deltamethrin + DEM	13.72 (7.69–53.31)	9.06 (8.16–10.09)	11.37 (7.34–22.04)	7.48 (6.27–9.01)	9.76 (4.96–32.05)	9.53 (7.29–13.10)
SC for LC_{50}	deltamethrin + PBO	257.94	821.39	255.36	350.97	357.59	752.10
	deltamethrin + DEF	1.87	5.97	4.70	5.23	7.25	3.05
	deltamethrin + DEM	1.46	3.89	2.47	3.37	3.04	2.99
LC_{95} [ppm]	deltamethrin	809.16	231.92	141.30	215.68	134.45	297.24
	deltamethrin + PBO	1.057	0.890	12.503	1.46	2.95	0.82
	deltamethrin + DEF	78.85	28.18	21.83	37.48	33.90	62.25
	deltamethrin + DEM	156.85	29.16	70.66	86.04	57.37	52.38
SC for LC_{95}	deltamethrin + PBO	765.52	260.58	11.30	147.72	45.57	362.48
	deltamethrin + DEF	10.26	8.23	6.47	5.75	3.96	4.77
	deltamethrin + DEM	5.16	7.95	1.99	2.50	2.34	5.67
RC and resistance classification	deltamethrin	32.36 b. wysoka	9.27 wysoka	5.65 wysoka	8.62 wysoka	5.38 wysoka	11.88 b. wysoka
	deltamethrin + PBO	0.042 brak	0.035 brak	0.50 brak	0.058 brak	0.118 brak	0.032 brak
	deltamethrin + DEF	3.15 średnia	1.12 niska	0.87 brak	1.49 niska	1.35 niska	2.49 średnia
	deltamethrin + DEM	6.27 wysoka	1.16 niska	2.82 średnia	3.44 średnia	2.29 średnia	2.09 średnia

PBO – piperonyl butoxide; DEF – S,S,S-tributylphosphorotrithioate; DEM – diethyl malonate



PBO – piperonyl butoxide; DEF – S,S,S-tributylphosphorotrithioate; DEM – diethyl malonate

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

The presented results showed the highest synergistic cooperation between deltamethrin and PBO. It can be concluded, that the pollen beetle resistance mechanism to deltamethrin mainly involves oxidative enzymes, but the participation of esterases and glutathione transferases is also evident.

DISCUSSION

The results of our research showed a continuing high pollen beetle resistance to the tested pyrethroids: deltamethrin and beta-cyfluthrin. This is confirmed by the results of other authors (Detourne *et al*. 2002; Ballanger and Delorme 2005; Heimbach *et al*. 2006a, 2006b; Kazachkova 2007; Slater and Nauen 2007; Hansen 2008; Richardson 2008; Tillikainen and Hokkanen 2008; Węgorek 2009). There has been a widespread problem with pollen beetle resistance to pyrethroids in Europe during the last ten years (Detourne *et al*. 2002; Hansen 2003; Węgorek 2005; Heimbach *et al*. 2006a; Richardson 2008; Tillikainen and Hokkanen 2008;). Field research in Poland noted that at registration time, the effectiveness of pyrethroids against the pollen beetle was very high (Witkowski *et al*. 1988, 1989a 1989b; Pruszyński and Mrówczyński 1990; Mrówczyński *et al*. 1997; Seta *et al*. 1997). A significant decrease in the pollen beetle susceptibility level to different pyrethroid active substances was found by Węgorek (Węgorek 2009). The research presented by Węgorek showed that LC_{50} values for deltamethrin ranged from 47 ppm to 87 ppm, LC_{95} from 164 ppm to 360 ppm, and that the resistance coefficient values ranged from 6 to 14. In our research, LC_{50} values for deltamethrin were between 20 ppm and 46 ppm, LC_{95} values were between 108 ppm and 809 ppm, and resistance coefficient values were between 4 and 32. Similar slight differences were also visible in the results of research on beta-cyfluthrin. It can be concluded, that pollen beetle resistance level to deltamethrin and beta-cyfluthrin has maintained the same, high level for the last few years. When taking into consideration such a high resistance level, an ineffectiveness of the treatments in the fields can be expected.

Pollen beetle resistance mechanisms to pyrethroids and other insecticide active substances have already been investigated, both in Poland and in other European countries (Nauen 2007; Skillman 2007; Węgorek 2009; Phillippou *et al*. 2010; Węgorek *et al*. 2011a). The main mechanism of resistance to pyrethroids was found to be the one based on oxidative enzymes. In our research, blocking oxidative enzymes with PBO resulted in a much better effectiveness of deltamethrin. Similar results on cooperation between PBO and pyrethroid active substances (esfenvalerate, lambda-cyhalothrin, zeta-cypermethrin, beta-cyfluthrin, cypermethrin and tau-fluvalinate) against the pollen beetle were obtained earlier (Węgorek 2009; Węgorek *et al*. 2011a). Such results do not exclude participation of esterases, because PBO is also known to be an esterases blocker (Gunning *et al*. 1998). However, other scientific papers emphasis that PBO is mainly an oxidative enzymes blocker and only slightly inhibits esterases (Schoknecht and Otto 1989; Malinowski 2003). In light of this, our results showing high synergism between deltamethrin and PBO prove that not only oxidases, but also some esterases take part in the pyrethroid detoxificatoion.

In our research, an increase in deltamethrin toxicity was also found after blocking esterases with DEF. This effect was not as high as in the case of PBO but it can be concluded that the resistance mechanism based on esterases, is also involved in deltamethrin detoxification. So far, in Poland, the participation of esterases in the pollen beetle resistance mechanisms has been tested only with the use of carbaryl. The results did not clearly indicate that esterases blocked with carbaryl so as to participate in the pyrethroid detoxification (Węgorek 2009; Węgorek *et al*. 2011a). Such results do not exclude the participation of other esterases. The results of the research presented in this study prove the participation of esterases blocked with DEF, in the deltamethrin detoxification of the pollen beetle for the first time. DEF may also block acetylcho-

linesterase, and so it may act as an insecticide. However the concentrations of the blockers used in this study had been estimated before starting the experiments, as the ones that did not cause any insect mortality when used without insecticides. The role of esterases in insecticide detoxification mechanisms in different insect pests was also shown by other authors (Devonshire and Field 1991; Whyard *et al*. 1994; Parker *et al*. 1996; Gunning *et al*. 1998).

The results of the presented studies also confirmed the role of glutathione transferases – enzymes taking part in the second phase of the biotransformation of toxins in the pollen beetle resistance mechanisms to deltamethrin. It is important to note, though, that the synergism coefficient was much lower than in the case of PBO and DEF, proving the slight participation of glutathione transferases in deltamethrin detoxification. Little is known about the role of glutathione transferases (GST) in pyrethroids detoxification. Generally, highly specialized insects which feed on a small group of plants, most often have only one form of GST isoensymes, while poliphagues usually have 4–9 forms of GST isoenzymes (Ottea and Plapp 1984; Clark *et al*. 1986; Ranson and Hemingway 2005). Probably, the narrow feeding specialization of the pollen beetle is the reason for the slight role of glutathione transferases in deltamethrin detoxification.

Resistance to different insecticide active substances, created by different insect species, is usually based on several mechanisms (Pospischil *et al*. 1999; McAbee *et al*. 2004). The presented research proves that the pollen beetle has a very strong metabolism potential that may create even more problems in rapeseed protection in the future. Resistance mechanisms, based of the already proven activity of the enzymes, can be used in plant protection by choosing active substances, whose metabolites (arising under the influence of enzymes responsible for resistance) are much stronger toxins than the starting material. Nowadays in Poland, this has been done in the case of chlorpiryfos, whose metabolite is a much stronger insecticide. That is why constant monitoring of the susceptibility level, detecting resistance mechanisms, and searching for synergists that could be used in overcoming resistance, should be stable directions of scientific research.

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