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RESEARCH

Preliminary Studies on the Susceptibility Level of *Ceutorchynhus assimilis* (Coleoptera: Curculionidae) to Acetamiprid and Chlorpyrifos in Poland and Resistance Mechanisms of the Pest to Acetamiprid

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ABSTRACT. The cabbage seed weevil, *Ceutorchynhus assimilis* (Paykull) (Coleoptera: Curculionidae) is a pest that more and more often causes large financial losses for rapeseed cultivators in Poland and other European countries. One of the reasons of these problems is the resistance of the pest to certain active substances of insecticides. The aim of the study was to assess the susceptibility level of the pest to chlorpyrifos, an organophosphate substance, and acetamiprid, a neonicotinoid, and to determine its enzymatic mechanisms of susceptibility to acetamiprid using synergists, i.e., blockers of particular enzyme groups. The presented research is the first to discuss the mechanisms of the resistance of the cabbage seed weevil to acetamiprid. The achieved results showed medium, high, or very high resistance of the cabbage seed weevil to acetamiprid and its lack of resistance to chlorpyrifos. The research on the mechanisms of the resistance of the pest to acetamiprid revealed the participation of hydrolytic enzymes blocked by S,S,S-tributylphosphorotrithioate and glutathione transferases blocked by diethyl malonate in the metabolism of acetamiprid. The results did not show the participation of oxidative enzymes and esterases blocked by piperonyl butoxide in the detoxification of acetamiprid.

Key Words: cabbage seed weevil, acetamiprid, chlorpyrifos, resistance, resistance mechanism

Both Poland and other European countries strive to create integrated farm plant production programs. One of the more difficult problems is the control of agrophages that developed a certain level of resistance to the active substances present in pesticides (insecticides, herbicides, and fungicides) from different chemical groups. This phenomenon causes large financial losses and affects the natural environment.

Because of their resistance to some insecticides, it is currently very difficult to control two rapeseed pests that are of significant importance to the economy—the pollen beetle, *Meligethes aeneus* (F.) (Coleoptera: Nitidulidae), and cabbage seed weevil, *Ceutorchynhus assimilis* (Paykull) (Coleoptera: Curculionidae) (Heimbach et al. 2006a,b, Nauen 2007, Węgorek et al. 2009c, Zamojska et al. 2010). In the case of these pests, agronomic conditions need to be accounted for, including the increased area of rapeseed cultivation and the insecticide use history. Intensive chemical oilseed rape protection in Poland started in the 50s of the 20th century. To date, over 40 active substances have been used. Organochlorines and carbamates were withdrawn in 1998 and nereistoxine analogs in 2006. Nowadays, there are five insecticide chemical groups used in oilseed rape protection in Poland: neonicotinoids (with acetamiprid as the only active substance, used since 2004 and tiachloprid used in the mixture with deltamethrin), pyrethroids (10 active substances with deltamethrin as the first one, used since 1981), organophosphates (with chlorpyrifos as the only active substance used since 2000), oxadiazines (with indoxacarb as the only active substance, used since 2013), and azometine pyridines (with pymetrozine as the only active substance, used since 2013) (Węgorek 2009, recommendations of the Polish Ministry of Agriculture). Regarding the last 10 yr, pyrethroids (deltamethrin, cypermethrin, alpha-cypermethrin, lambda-cyhalothrin, esfenvalerate, beta-cyfluthrin, zeta-cypermethrin, gamma-cyhalothrin, and tau-fluvalinate) and neonicotinoids (acetamiprid and tiachloprid) have mainly been used for *C. assimilis* control. Out of all insecticide active substances mentioned, tau-fluvalinate and acetamiprid are the most often used ones because they are not toxic to bees in the recommended doses (in Poland the period of controlling *C. assimilis* coincides with an intensive flight of bees). Depending on the density of oilseed rape pest populations, pyrethroids are used two or three times a season against cabbage seed weevil and pollen beetle but only once a season against *C. assimilis*. Acetamiprid is usually used only once a season. Chlorpyrifos does not exert selective pressure on *C. assimilis* because, due to its toxicity to bees, it is used only in the first treatment against the pollen beetle. All these recommendations result from the strategy preventing resistance and relying mainly on using a given active substance only once per season (Węgorek et al. 2013). In Poland, rapeseed is currently cultivated on 800 ha and is treated chemically numerous times each year.

The level of the susceptibility of the pollen beetle to many insecticides, both in Poland and in other countries, has been studied and monitored for a long time (Łąkocy 1969, Węgorek 2005 a,b, Hansen 2008, Richardson 2008, Węgorek and Zamoyska 2008, Węgorek et al. 2009c, Zamojska et al. 2010). However, the data on the susceptibility of the cabbage seed weevil to active substances present in insecticides are limited and relate only to pyrethroids (Garthwaite et al. 1995, Buntin 1999, Heimbach et al. 2006a). Data on chlorpyrifos and acetamiprid are scarce (Zamojska et al. 2010).

As the resistance mechanisms are different in various species, with each of the mechanisms having its own characteristics, it is necessary to identify the mechanisms in agricultural and forest pests that are extremely important from the point of view of the economy, when designing plant protection strategies. The basic resistance mechanisms in pests include slower permeation of toxins through the coverings of insects, enzymatic detoxification of insecticides by enzyme systems, including especially mixed-function oxidases, hydrolases, and transferases (Malinowski 2003, Terra and Ferreira 2005), lowered susceptibility of target parts of the pests to insecticides, and behavioral mechanisms (Malinowski 2003, Węgorek 2009). There is no data in literature on the resistance mechanisms of the cabbage seed weevil to the active substances of insecticides.

The aim of the research was to specify the level of susceptibility of the cabbage seed weevil to chlorpyrifos, an organophosphate substance, and acetamiprid, a neonicotinoid, and to determine the participation of enzymatic systems in the resistance mechanisms of the pest to acetamiprid using the blockers of particular enzyme groups. The presented studies are the first to discuss the mechanisms of the cabbage seed weevil to acetamiprid.

Materials and Methods

In the research, IRAC Susceptibility Test Method 7 was used. The method was described earlier for studies on pollen beetle (Węgorek et al. 2011a). The only differences included the tested species, plant material used (leaves and flower clusters of rape), and the number of the beetles placed in one container. In the case of the research presented in this publication, 50 cabbage seed weevils were placed in each container. The insects used in the research and the plant material were collected from fields that were not treated chemically at three locations in the Wielkopolska province: Września, Wałcz, and Krotoszyn in 2008–2010. Tested populations have a very similar insecticide use history.

Insecticides (Commercially Available Products). Insecticide concentrations in ppm were calculated, assuming that 200 liters of water would be used per hectare.

Neonicotinoids (contact-gastric action): acetamiprid (Mospilan 20 SP with 20% of active substance): recommended dose of 0.12 kg/ha and recommended concentration: 120 ppm.

Organophosphate (contact-gastric and gas action): chlorpyrifos (Pyrinex 480 EC with 480 g/liter of active substance). Pyrinex 480 EC is not recommended for controlling cabbage seed weevils. The calculations were based on the recommended dose of 0.6 liter/ha (recommended concentration: 1,440 ppm) and the recommendations for the pollen beetle feeding on the winter rapeseed, often together with the cabbage seed weevil.

Laboratory conditions 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). Also, statistical calculations were the same as the ones described in the mentioned publications—based on the percent mortality of the cabbage seed weevil at each dose, lethal concentrations LC50 and LC95 were calculated.

Resistance coefficient (RC) values were calculated as follows:

RC = LC95/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—lack of resistance; RC = 1.1–2—low resistance; RC = 2.1–5—medium resistance; RC = 5.1–10—high resistance; and RC > 10—very high resistance.

The enzymatic mechanisms of the resistance of the cabbage seed weevil to acetamiprid were determined using the method described for the Colorado beetle in the *Journal of Plant Protection Research* (Węgorek et al. 2011b, Zamojska et al. 2011), with the aforementioned changes. The research used synergists blocking three main groups of enzymes taking part in the metabolisms of toxins: piperonyl butoxide (PBO)—mainly oxidases blocker; S,S,S-tributylphosphorotrithioate (DEF)—esterases blocker; and diethyl malonate (DEM)—glutathione transferases blocker.

The dose of each if the synergists used was 100 ppm. Earlier research showed that the dose is nontoxic for the studied beetles. Inhibitors were applied at the same time as 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 assess synergism between deltamethrin and a given insecticide: SC < 1—antagonism; SC = 1—lack of synergism and the lack of antagonism; and SC > 1—synergism.

Results

The cabbage seed weevil susceptibility level to chlorpyrifos and acetamiprid is presented in Table 1.

The LC50 values for acetamiprid were high, ranging from 86.89 ppm (Wałcz population in 2009) to 267.02 ppm (Września population in 2010). The lowest value was thus three times lower than the highest one. The biggest difference in the values was found in the Wałcz population (the lowest value was 1.59 times lower than the highest one) in 2008 and 2009. Except for the Wałcz population in 2009, the LC50 values slightly exceeded the concentration of acetamiprid recommended in Poland, with the values being from 1.01 to 2.23 times higher than the recommended dose. The LC95 values of acetamiprid in all the populations and in all the years of research exceeded the recommended dose, with the maximum value being 43 times higher than recommended dose (Września population in 2010) and the lowest value being 3.5 times higher than the recommended dose (Wałcz population in 2009). The LC95 values ranged from 422.46 to 5,753.4 ppm. The biggest difference between the values (with the lowest value being 4.44 times lower than the highest one) within one population was found in the research on the Września population in 2009 and 2010. The calculated values of the RC are presented in Table 1. In only one case, the resistance to acetamiprid was medium (Wałcz population in 2009). In the remaining cases, the resistance was high (2008: Wałcz and Krotoszyn and 2010: Wałcz) or very high (2008: Września; 2009: Września and Krotoszyn; and 2010: Września and Krotoszyn).

The LC50 levels of chlorpyrifos ranged from 1.8 Września population in 2008) to 7.86 ppm (Wałcz population in 2010). The biggest difference within one population was found in the case of the Września population in 2008 and 2010, with the lowest value being twice lower than the highest one. The highest value of LC50 was 183 times lower than the

Table 1. Susceptibility level of cabbage seed weevil adults to chlorpyrifos and acetamiprid

Year	Active substance	LC50 (ppm) (confidence intervals, $P = 0.95$)			LC95 (ppm)			RC and resistance classification		
		Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn	Września	Wałcz	Krotoszyn
2008	Chlorpyrifos	1.80 (1.27–2.30)	4.38 (2.71–6.50)	2.48 (1.93–3.07)	8.35	28.14	10.31	0.006 none	0.019 none	0.007 none
	Acetamiprid	207.25 (142.75–348.73)	136.30 (100.27–193.84)	165.59 (120.91–242.82)	2,396.4	976.27	1,034.22	19.97 very high	8.14 high	8.61 high
2009	Chlorpyrifos	4.49 (3.94–5.06)	5.34 (3.21–7.69)	2.92 (1.85–3.93)	16.59	34.91	20.04	0.011 none	0.024 none	0.014 none
	Acetamiprid	188.49 (140.41–272.47)	86.89 (68.98–109.67)	159.72 (121.86–219.07)	1,295.9	422.46	1,230.12	10.79 very high	3.52 medium	10.25 very high
2010	Chlorpyrifos	3.73 (3.43–4.05)	7.86 (5.40–11.45)	3.01 (2.61–3.43)	7.78	36.23	19.92	0.005 none	0.025 none	0.014 none
	Acetamiprid	267.02 (179.39–478.27)	121.25 (84.23–184.57)	193.80 (137.78–302.93)	5,753.4	890.71	1,223.5	47.94 very high	7.42 high	10.19 very high

Results expressed in LC50, LC95, and RC.

Table 2. The influence of PBO, DEF, and DEM on acetamiprid action in the cabbage seed weevil in the years 2009–2010

Parameter	Substance	2009		2010	
		Września	Krotoszyn	Września	Krotoszyn
LC50 (confidence interval, $P = 0.95$)	Acetamiprid	188.49 (140.4–272.5)	159.72 (121.8–219.07)	267.02 (179.4–478.3)	193.80 (137.7–302.9)
	Acetamiprid + PBO	198.58 (149.1–284.7)	131.27 (106.58–164.7)	203.05 (135.7–361.3)	204.73 (130.13–407.6)
	Acetamiprid + DEF	54.45 (31.36–144.18)	129.66 (84.45–331.59)	51.42 (28.09–165.15)	63.35 (38.39–165.43)
	Acetamiprid + DEM	80.08 (58.0–131.96)	57.21 (37.39–109.95)	51.85 (34.01–95.46)	44.52 (31.77–68.21)
SC for LC50	Acetamiprid + PBO	0.95	1.21	1.31	0.94
	Acetamiprid + DEF	3.46	1.23	5.19	3.06
	Acetamiprid + DEM	2.35	2.79	5.15	4.35
LC95	Acetamiprid	1,295.9	1,230.12	5,753.4	1,223.5
	Acetamiprid + PBO	1,398.7	985.97	3,714.1	1,825.1
	Acetamiprid + DEF	366.43	963.17	581.55	593.99
	Acetamiprid + DEM	585.80	318.04	438.01	324.37
SC for LC95	Acetamiprid + PBO	0.92	1.24	1.55	0.67
	Acetamiprid + DEF	3.53	1.27	9.89	2.06
	Acetamiprid + DEM	2.21	3.86	13.13	3.77
RC and resistance classification	Acetamiprid	10.79 very high	10.25 very high	47.94 very high	10.19 very high
	Acetamiprid + PBO	11.65 very high	8.21 high	30.95 very high	15.21 very high
	Acetamiprid + DEF	3.05 medium	8.02 high	4.81 medium	4.94 medium
	Acetamiprid + DEM	4.88 medium	2.65 medium	3.65 medium	2.70 medium

Results expressed in LC50, LC95, and coefficients: RC and SC.

recommended dose. The LC95 values in the research on the influence of chlorpyrifos on the cabbage seed weevil ranged from 7.78 (Września population in 2010) to 36.23 ppm (Wałcz population in 2010). The biggest difference within one population was found within the Września population (with the highest value being 2.1 times higher than the lowest one) in 2009 and 2010. None of the LC95 values exceeded the dose of 1,440 ppm, with the highest LC95 value being 87 times lower than the recommended dose. The RC values for chlorpyrifos in all the cases indicated that the pest is not resistant to this substance.

The results of experiments devoted to the resistance mechanisms of the cabbage seed weevil to acetamiprid are presented in Table 2 and in Fig. 1. The experiments were carried out on two populations: Września and Krotoszyn in 2009 and 2010.

The experiments designed to determine the interaction of acetamiprid and PBO did not show any significant synergistic action of these substances. The SC for LC50 revealed both a slight antagonism in each of the studied populations (Września population in 2009 and Krotoszyn population in 2010), with the values being, respectively, 0.95 and 0.94, and a slight synergism (Krotoszyn population in 2009 and Września population in 2010)—the values were, respectively, 1.21 and 1.31. The values of the SC for LC95 were similarly ambiguous, showing antagonism (Września population in 2009 and Krotoszyn population in 2010) or a slight synergism (Krotoszyn population in 2009 and Września in 2010). The difference between the values was so small that in three cases, it did not result in a change of the classification of resistance, and in one case, the classification of resistance changed from very high to high (Krotoszyn population in 2009). These results prove the limited role of the oxidative metabolism and esterases blocked by PBO in the acetamiprid detoxification processes in the cabbage seed weevil in the studied populations.

The results of experiments devoted to the interaction of DEF and acetamiprid showed a synergism with this substance. In all the experiments, the SC for LC50 was >1, with its values ranging from 1.23 (Krotoszyn population in 2009) to 5.19 (Września population in 2010). The SC for LC95 ranged from 1.27 (Krotoszyn population in 2009) to 9.89 (Września population in 2010). In all the cases, it resulted in a change of the resistance classification from very high to high (Krotoszyn population in 2009) or from very high to medium (in the remaining cases). The results of the research showed a higher mortality rate of the cabbage seed weevil after the addition of DEF to acetamiprid, which indicates that esterases take part in the detoxification of acetamiprid.

The results of experiments devoted to the interaction of acetamiprid, and DEM showed synergism with this active substance. In all the cases, the SC for LC50 was >1, with its values ranging from 2.35 (Września population in 2009) to 5.15 (Września population in 2010). The SC for LC95 ranged from 2.21 (Września population in 2009) to 13.13 (Września population in 2010). In all the experiments, it resulted in a change of the resistance classification of the studied populations from very high to high. The results indicate the participation of glutathione transferases in the processes of acetamiprid detoxification in the studied populations of the cabbage seed weevil.

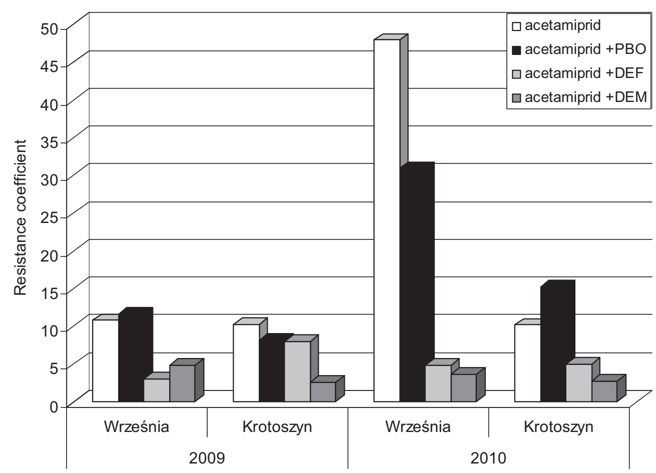

Fig. 1. The influence of synergists on cabbage seed weevil adult resistance to acetamiprid in the years 2009–2010.

To summarize, the experiments concerning the resistance mechanisms of the cabbage seed weevil to acetamiprid prove that the esterases blocked by DEF and glutathione transferases inhibited by DEM take part in the detoxification metabolism of acetamiprid. In turn, the results do not prove the participation of the oxidative enzymes blocked by PBO in the detoxification of acetamiprid.

Discussion

The research on the resistance of the Colorado beetle and pollen beetle in Poland and around the world has a long history, and many aspects of this phenomenon in both these species have been explained. There are not, however, many works discussing the resistance of the cabbage seed weevil to insecticides.

The results of the research on the level of the susceptibility of the cabbage seed weevil to acetamiprid showed that the susceptibility of the pest is surprisingly low. The dose of acetamiprid recommended in Poland (120 ppm) may even not be sufficient to achieve a mortality rate of 50%. In the presented research, only one experiment with a concentration lower than the recommended one (120 ppm)—87 ppm showed a mortality rate of 50%. To achieve the mortality rate of 95% in the case of the cabbage seed weevil, it was necessary to use acetamiprid doses that were on average 2–12 times higher than the ones for the pollen beetle (Węgorek 2009, J.Z., unpublished data), and in comparison to the Colorado beetle, the concentrations were 20-100 times higher (Węgorek et al. 2011b). It proves that the cabbage seed weevil metabolizes acetamiprid more efficiently than the two other mentioned species and that it is necessary to take it into account when planning rapeseed protection programs. Cabbage seed weevils appear and feed on the rapeseed from the flowering period, i.e., when the highest number of honey bees, tolerating very high doses of acetamiprid, appears at the plantations (J.Z. and P.W., unpublished data). The resistance of the cabbage seed weevil to this insecticide potentially eliminates this active substance from the cabbage seed weevil control program when the harmfulness level values are exceeded significantly. This phenomenon is a negative one and needs to be taken into account when designing rapeseed protection strategies using insecticides. There is no research publications related to the resistance of the discussed pest to acetamiprid, except for the author's own publication, which included limited information on this subject (Zamojska et al. 2010). The resistance of other species to the active substances from the group of neonicotinoids has been presented in many publications in the last 10 yr, including Choi et al. (2001), Daborn et al. (2001), Nauen et al. (2002), Wang et al. (2002), Byrne et al. (2003), Foster et al. (2003), Zewen et al. (2003), Nauen and Denholm (2005), and Gorman et al. (2008).

Resistance level of *C. assimilis* to acetamiprid might be caused by two factors. The first one is an evolutionary-created capability of metabolizing acetamiprid and the other one is a strong selective pressure of acetamiprid in the last 10 yr in Poland. These conclusions derive from the research on other insect species and theories of the cumulation of resistance genes (Malinowski 2003). However, the conclusion requires further research.

The values of the RC showed that the cabbage seed weevil is not resistant to chlorpyrifos. The research performed by Węgorek (2009) proves that the toxic effect of this active substance on the pollen beetle, related to the oxidizing desulfuration of chlorpyrifos, which in turn leads to increased toxicity, persists on fields for about 8 d after the treatment. The residues of chlorpyrifos on rapeseed decrease to <20 ppm on the third day after the treatment. The reaction of the cabbage seed weevil to chlorpyrifos is similar to that of the pollen beetle (Węgorek 2009). The LC50 level in the cabbage seed weevil in this research was similar, ranging from 1.8 to 7.9 ppm, whereas the LC95 level was significantly higher, ranging from 7.8 to 36.2 ppm, compared with the pollen beetle.

Both in Poland and in Europe, research has been conducted on the mechanisms of the resistance of different insect species, most frequently the pollen beetle and Colorado beetle, to pyrethroids and other insecticides (Węgorek 2002, 2004, 2009; Nauen 2007; Skillman 2007; Philippou et al. 2010; Węgorek et al. 2011a,b; Zamojska et al. 2013). Although many publications stress the significant role of the oxidative enzymes blocked by PBO in the detoxification of insecticides (Węgorek 2009, Philippou et al. 2010, Węgorek et al. 2011a, Zamojska et al. 2013), the participation of esterases cannot be excluded, as PBO also blocks this group of enzymes (although to a smaller extent; Gunning et al. 1998, Philippou et al. 2010). The issue of the role of esterases and glutathione transferases in the detoxification of insecticides in many insect species has also been discussed in world literature (Clark et al. 1986, Devonshire and Field 1991, Whyard et al. 1994, Parker et al. 1996, Gunning et al. 1998, Ranson and Hemingway 2005).

The research on the mechanisms of the detoxification of acetamiprid in the cabbage seed weevil did not prove that oxidative enzymes in this species play a significant role in the metabolism of the studied active substance. In turn, it was observed that esterases and glutathione transferases play a certain role in this process. However, the considerable difference in the toxicity of acetamiprid in the cabbage seed weevil compared with the Colorado beetle and pollen beetle (Węgorek 2009, Węgorek et al. 2011b) may stem from the differences in the molecular structure and affinity of the acetylocholine receptor to this toxin. Acetamiprid and other neonicotinoids have been used for the protection of rapeseed and potato since 1999, and the selective pressure to the studied species has not been long or strong. Thus, the cabbage seed weevil was probably better evolutionarily adapted and less susceptible to acetamiprid than the two other species of the beetles. In Poland, the Colorado beetle is not resistant to this insecticide, and the resistance of the pollen beetle is usually low. Both the species display natural differences in the tolerance to this insecticide. In turn, in the United States, the Colorado beetle has developed resistance to other neonicotinoids—relatively high resistance to imidacloprid (Mota-Sanchez et al. 2000, Dively 2006, Alyokhin et al. 2007) and low resistance to thiamethoxam (Grafius 2006). The resistance resulted from the oxidative mechanism blocked by PBO (Mota-Sanchez et al. 2006). The metabolic resistance to acetamiprid was observed in the diamondback moth [*Plutella xylostella* (L.)]. Both PBO and DEF increased the toxicity of this active substance (Ninsin and Tanaka 2005). The fact that the cabbage seed weevil survives the recommended doses of acetamiprid is a negative phenomenon, potentially making it difficult to use this insecticide in the integrated rapeseed protection programs in Poland.

The fact that oxidative enzymes are not an important factor responsible for acetamiprid detoxification in the tested insect species can be the result of the lack of isoenzymes capable of metabolizing the acetamiprid molecule. Oxidative enzymes are very common in detoxification processes in different insect species, but their effectiveness is dependent on different enzymes versions. For example, the western honey bee [*Aphis mellifera* (L.)], similar to the cabbage seed weevil, is resistant to high doses of acetamiprid and is susceptible to most pyrethroids and chlorpyrifos (Zamojska et al. 2010, J.Z. and P.W., unpublished data). It has been reported that PBO will sensitize honeybees to the effects of some pyrethroid insecticides (Johnson et al. 2006), but not to tau-fluvalinate (Moores et al. 2012). The level of synergism with PBO is dependent on molecular structure of the active substance in pyrethroids (Gunning et al. 2007). Our experiments suggest similar metabolism processes in the cabbage seed weevil. Also, different esterases' isoenzymes can be of various effectiveness to different active substances of insecticides (Capiner 2008, Montella et al. 2012). This may be the reason why esterases responsible for acetamiprid detoxification are not involved in chlorpyrifos resistance development. This result proves that insect resistance is a greatly complex matter.

The problem of the resistance of any pest species is a complicated one and should be analyzed as a separate issue and research subject. The conclusions of the research on one species cannot be used in the case of other species. One characteristic of many of the studied species is the participation of at least a couple detoxification metabolic pathways with different levels of expression (Pospischil et al. 1999, McAbee et al. 2004). The results of the research are of particular importance for the protection of rapeseed, in the case of which the cabbage seed weevil and pollen beetle have to be controlled at the same time using active substances, to which the two pests react differently. The developed integrated production and plant protection programs are and will be based on monitoring and preventing the resistance of the pests.

References Cited

Alyokhin, A., G. Dively, M. Patterson, C. Castaldo, D. Rogers, M. Mahoney, and J. Wollam. 2007. Resistance and cross-resistance to imidacloprid and thiamethoxam in the Colorado potato beetle. Pest Manag. Sci. 63: 2–41.

Buntin, G. D. 1999. Cabbage seedpod weevil control and damage loss assessment in winter canola using insecticides. *In* 10th International Rapeseed Congress, Canberra, Australia. (http://www.regional.org.au/au/gcirc/3/115.htm).

Byrne, F. J., S. Castle, N. Prabhaker, and N. Toscano. 2003. Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. Pest Manag. Sci. 59: 347–352.

Capiner, J. R. 2008. Encyclopedia of entomology. Springer, Dordrecht, The Netherlands.

Choi, B. R., S. W. Lee, and J. K. Yoo. 2001. Resistance mechanisms of green peach aphid *Myzus persicae* (Homoptera: Aphididae) to imidacloprid. Kor. J. Appl. Entomol. 40: 265–271.

Clark, A., N. Shamaan, M. Sinclair, and W. Dauterman. 1986. Insecticide metabolism by multiple glutathione S-transferases in two strain of the house fly, *Musca domestica* L. Pestic. Biochem. Physiol. 25: 169–175.

Daborn, P., S. Boundy, J. Yen, B. Pittendrigh, and R. Ffrench-Constant 2001. DDT resistance in *Drosophila* correlates with Cyp6g1 over-expression and confers cross-resistance to the neoni cotinoid imidacloprid. Mol. Genet. Genom. 266: 556–563.

Devonshire, A. L., and L. M. Field. 1991. Gene amplifications and insecticide resistance. Ann. Rev. Entomol. 36: 1–24.

Dively, P. G. 2006. Management of resistance to imidacloprid in Colorado potato beetles in the Northeastern U.S., Great Lakes fruit, vegetable and farm market EXPO. Michigan State of University Collage of Human Medicine, Grand Rapids, MI.

Foster, S., I. Denholm, and R. Thompson. 2003. Variation in response to neonicotinoid insecticides in peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae). Pest Manag. Sci. 59: 166–173.

Garthwaite, D. G., M. R. Thomas, and M. Hunt. 1995. Pesticide usage survey: arable farm crops 1994. Report 127, Ministry of Agriculture, Fisheries and Food, London, United Kingdom.

Gorman, K., Z. Liu, I. Denholm, K. Bruggen, and R. Nauen. 2008. Neonicotinoid resistance in rice brown planthopper *Nilaparvata lugens*. Pest Manag. Sci. 64(11): 1122–1125.

Grafius, J. E. 2006. The Michigan experience with managing Colorado potato beetle, Great Lakes fruit vegetable and farm market EXPO. Michigan State of University Collage of Human Medicine, Grand Rapids, MI.

Gunning, R., G. Moores, and A. Devonshire. 1998. Inhibition of resistance related esterases by piperonyl butoxide in *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Aphis gossypii* (Hemiptera: Aphididae), pp. 215-226. *In* D. Jones (ed.), Piperonyl butoxide: the insecticide synergist. Academic Press, San Diego, CA.

Gunning, R. V., G. D. Moores, P. Jewess, A. L. Boyes, A. L. Devonshire, and B.P.S. Khambay. 2007. Use of pyrethroid analogues to identify key structural features for enhanced esterase resistance in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pest Manag. Sci. 63: 569–575.

Hansen, L. M. 2008. Occurrence of insecticide resistant pollen beetles (*Meligethes aeneus* F.) in Danish oilseed rape (*Brassica napus* L.) crops. OEPP/EPPO Bull. 38: 5–98.

Heimbach, U., A. Müller, and T. Thieme. 2006a. First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany. Nachrichtenbl. Pflanzenschutz 58: 1–5.

Heimbach, U., A. Müller, and T. Thieme. 2006b. First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany: an extended abstract. IOBC/wprs Bull. 29: 131–134.

Johnson, R. M., Z. Wen, M. A. Schuler, and M. R. Berenbaum. 2006. Mediation of pyrethroid insecticide toxicity to honey bees (Hymenoptera: Apidae) by cytochrome P450 monooxygenases. J. Econ. Entomol. 99: 1046–1050.

Łąkocy, A. 1969. Observations regarding the resistance of the pollen beetle (Meligethes aeneus F.) and the Colorado beetle (Leptinotarsa decemlineata Say) to DDT in Poland. Res. Pub. Inst. Plant Prot. 9: 157–170.

Malinowski, H. 2003. Resistance of insects to insecticides. Mechanisms of its development and possibilities of counteracting the resistance. Wieś Jutra Publishing House, Warsaw, Poland.

McAbee, R. D., K. D. Kang, M. A. Stanich, J. A. Christiansen, C. E. Wheelock, A. D. Inman, B. D. Hammock, and A. J. Cornel. 2004. Pyrethroid tolerance in *Culex pipiens pipiens* var *molestus* from Marin County, California. Pest Manag. Sci. 60: 359–368.

Montella, R. I., R. Schama, and D. Valle. 2012. The classification of esterases: an important gene family involved in insecticide resistance-A Review. Memorias do Instituto Oswaldo Cruz 107: 437–449.

Moores, G. D., P. Węgorek, J. Zamojska, L. Field, and D. Philippou. 2012. The effect of a piperonyl butoxide/tau-fluvalinate mixture on pollen beetle (*Meligethes aeneus*) and honey bees (*Apis mellifera*). Pest Manag. Sci. 68: 795–800.

Mota-Sanchez, D., M. E. Whalon, E. J. Grafius, and R. M. Hollingworth. 2000. Resistance of Colorado potato beetle to imidacloprid. Res. Pest Manag. 11: 31–33.

Mota-Sanchez, D., R. M. Hollingworth, E. J. Grafius, and D. D. Moyer. 2006. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Pest Manag. Sci. 62: 30–37.

Nauen, R. 2007. Insecticide mode of action, incidence and mechanisms of resistance. *In* Presentation Abstract, EPPO Workshop on Insecticide Resistance of *Meligethes* ssp. (Pollen Beetle) on Oilseed Rape, 3–5 September 2007, Berlin, Germany. (http://www.archives.eppo.org/MEETINGS/(2007)).

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. 58: 200–215.

Nauen, R., N. Stumpf, and A. Elbert 2002. Toxocological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 58: 868–874.

Ninsin, K. D., and T. Tanaka. 2005. Synergism and stability of acetamiprid resistance in a laboratory colony of *Plutella xylostella*. Pest Manag. Sci. 61: 723–727.

Parker, A. G., P. M. Campbell, M. E. Spackman, M. J. Russel, and J. G. Oakeshott. 1996. Comparison of an esterase associated with organophosphate resistance in *Lucilia cuprina* with an orthologue not associated with resistance in *Drosophila melanogaster*. Pestic. Biochem. Physiol. 55: 85–95.

Philippou, D., L. Field, P. Węgorek, J. Zamojska, M. Andrews, R. Slater, and G. Moores. 2010. Characterising metabolic resistance in pyrethroid—insensitive pollen beetle (*Meligethes aeneus* F.) from Poland and Switzerland. Pest Manag. Sci. 67: 239-243.

Pospischil, R., K. Szomm, M. Londershausen, I. Schroder, A. Turberg, and R. Fuchs. 1999. Multiple resistance in the Larger House Fly *Musca domestica* in Germany. Pestic. Sci. 48: 333–341.

Ranson, E., and J. Hemingway. 2005. Glutathione transferases, pp. 403-410. *In* L. Gilbert, K. Iatrou, and S. Gill (eds.), Comprehensive molecular insect science. Elsevier B5, Oxford, United Kingdom.

Richardson, D. M. 2008. Summary of findings from a participant country pollen beetle questionnaire. OEPP/EPPO Bull. 38: 68–72.

Skillman, W. S. 2007. Use of the synergist piperonyl butoxide in combination with a pyrethroid insecticide lambda-cyhalothrin against metabolically resistant pollen beetle, *Meligethes aeneus*. *In* Presentation Abstract, EPPO Workshop on Insecticide Resistance of Meligethes ssp. (Pollen Beetle) on Oilseed rape, 3-5 September 2007, Berlin, Germany. (http://www.archives.eppo.org/MEETINGS/(2007)).

Terra, W., and C. Ferreira. 2005. Biochemistry of digestion, vol. 4, pp. 171–224. *In* L. Gilbert, K. Iatrou, and S. Gill (eds.), Comprehensive molecular insect science. Elsevier, Oxford, United Kingdom.

Wang, K. Y., T. X. Liu, C. H. Yu, X. Y. Jiang, and M. Q. Yi. 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to fenvalerate and imidacloprid and activities of detoxification enzymes on cotton and cucumber. J. Econ. Entomol. 95: 407–413.

Węgorek, P. 2002. Resistance of the Colorado beetle in Poland to some insecticides and possibilities of preventing this phenomenon. Ph.D. dissertation, Institute of Plant Protection, Poznań, Poland.

Węgorek, P. 2004. Oxidative enzymes as the main factor in the resistance of the Colorado beetle (*Leptinotarsa decemlineata* Say) to pyrethroids in Western Poland. Prog. Plant Prot. (Postępy Ochrony Roślin) 44: 1208–1211.

Węgorek, P. 2005a. Current status of resistance in Colorado potato beetle (*Leptinotarsa decemlineata* Say) to selected active substances of insecticides in Poland. J. Plant Prot. Res. 45: 309–319.

Węgorek, P. 2005b. Preliminary data on resistance appearance of Pollen beetle PB (*Meligethes aeneus* F.) to selected pyrethroids, organophosphorus and chloronicotinyls insecticides, in 2004 year in Poland. Resistant Pest Manag. Newsl. 14: 19–21.

Węgorek, P. 2009. Research on the resistance of the pollen beetle (Meligethes aeneus F.) to insecticides. Research publications, Institute of Plant Protection, National Research Institute, Poznan, Poland.

Węgorek, P., and J. Zamoyska. 2008. Current status of resistance in pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. OEPP/EPPO Bull. 38: 91–94.

Węgorek, P., M. Mrówczyński, and J. Zamojska. 2009c. Resistance of pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. J. Plant Prot. Res. 49: 119–127.

Węgorek, P., J. Zamojska, and M. Mrówczyński. 2011a. High resistance to pyrethroid insecticides in Polish pollen beetle (*Meligethes aeneus* F.)—the role of oxidative metabolism. Phytoparasitica 39: 43–49.

Węgorek, P., J. Zamojska, and M. Mrówczyński. 2011b. Susceptibility level of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) to chlorpyrifos and acetamiprid in Poland and resistance mechanisms of the pest to chlorpyrifos. J. Plant Prot. Res. 51: 279–284.

Węgorek, P., M. Korbas, J. Zamojska, R. Kierzek, J. Piszczek, and K. Pieczul. 2013. Agrophage resistance to pesticides, pp. 153. *In* M. Mrówczyński (ed.), Integrated protection of crops. Basics of integrated protection. National Agricultural and Forest Publishing House, Poznan, Poland.

Whyard, S., A. Downe, and V. Walker. 1994. Isolation of an esterase conferring insecticide resistance in the mosquito *Culvex tarsalis*. Insect Biochem. Mol. Biol. 24: 819–827.

Zamojska, J., P. Węgorek, and M. Mrówczyński. 2010. Current level of the resistance of the selected insect species in Poland to insecticides. Prog. Plant Prot. (Postępy Ochrony Roślin) 50: 1205–1212.

Zamojska, J., P. Węgorek, and M. Mrówczyński. 2011. Changes in the Colorado potato beetle (*Leptinotarsa decemlineata* Say) susceptibility level to pyrethroids and the pest resistance mechanisms to deltamethrin. J. Plant Prot. Res. 51: 294–299.

Zamojska, J., P. Węgorek, and M. Mrówczyński. 2013. Susceptibility level of the pollen beetle (*Meligethes aeneus* F.) to selected pyrethroids in Poland and resistance mechanisms of the pest to deltamethrin. J. Plant Prot. Res. 53: 89–95.

Zewen, L., H. Zhaojun, W. Yinchang, Z. Lingchun, Z. Hongwei, and L. Chengjun. 2003. Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms. Pest Manag. Sci. 59: 1355–1359.

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