https://doi.org/10.17221/53/2022-PPS

Pyrethroid susceptibility and oxidative detoxification mechanism in Colorado potato beetle and western corn rootworm

Daria Dworzańska, Joanna Zamojska, Paweł Węgorek, Paweł K. Bereś, Sławomir Drzewiecki*

Institute of Plant Protection – National Research Institute, Poznań, Poland

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

Citation: Dworzańska D., Zamojska J., Węgorek P., Bereś P.K., Drzewiecki S. (2023): Pyrethroid susceptibility and oxidative detoxification mechanism in Colorado potato beetle and western corn rootworm. Plant Protect. Sci., 59: 174–184.

Abstract: The Colorado potato beetle (CPB) and the western corn rootworm (WCR) are among the world's most expansive insect pests. The aim of the study was to assess, for the first time in Poland, the levels of susceptibility to deltamethrin and tau-fluvalinate in the WCR and to determine the current status of susceptibility to those active substances in the CPB. In addition, the role of oxidative metabolism in the detoxification of pyrethroids was determined using piperonyl butoxide (PBO). The study found pyrethroid resistance in the CPB populations. The WCR populations exhibited "high susceptibility", "susceptibility", "low resistance" and "medium resistance" to the pyrethroids tested. Experiments using PBO showed that oxidative enzymes are involved in the deltamethrin detoxification mechanism in the CPB, although the effectiveness of this mechanism in Poland is decreasing. PBO was found to be highly toxic to the WCR populations. The findings from this study should be taken into account when developing a strategy to prevent the development of resistance in the pest species. The results for the toxicity of PBO to Polish WCR populations demonstrated that the enzymes inhibited by PBO are involved in important metabolic processes, which should be considered when developing innovative control methods for this pest.

Keywords: *Leptinotarsa decemlineata* Say; *Diabrotica virgifera virgifera* LeConte; deltamethrin; tau-fluvalinate; susceptibility levels; oxidative mechanism; piperonyl butoxide

The Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) and the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (WCR) are both members of the Chrysomelidae family of beetles. The beetles are considered to be among the world's most expansive invasive species. They have spread rapidly and continue to spread to new areas, causing serious damage to economically important crops such as potato (CPB) and maize (WCR).

The CPB is now established throughout most of North America, Europe, and many parts of Asia (CABI 2020). In the second half of the 20^{th} century, the spread of the CPB revolutionised the global phytopharmaceutical industry. The active substances of insecticides from different chemical groups were employed to manage the pest. Over the years, the pest has developed all the resistance mechanisms known in insects (i.e. physiological and behavioural resistance mechanisms as well as resistance mechanisms related to nervous tissue insensitivity) against the active substances of insecticides from all chemical groups that are used

https://doi.org/10.17221/53/2022-PPS

to control it (Alyokhyn 2008; Whalon et al. 2008; Pélissié et al. 2022). Actually, the most common resistance mechanism to the active substances of insecticides in the CPB is oxidative metabolism. The mechanism is currently known to be responsible for the detoxification of, among others, pyrethroid compounds (Sharif et al. 2007).

The WCR is now mainly found in North America and Europe (CABI 2020). It appeared in Europe much later than the CPB. In Poland, the WCR was first recorded in 2005 in the Podkarpacie Province (southeast of Poland). The species has since spread rapidly and quite extensively (Bereś et al. 2015). Today, the WCR continues to expand its range, spreading towards the north of Poland. In North America, to which the species is native, the problems with the protection of maize crops against the WCR are gradually becoming more serious, mainly due to the increasing resistance of the pest to different active substances of insecticides from different chemical groups, mainly including carbamates and organophosphorus compounds (Souza 2019). As a result of the frequent use of pyrethroids for the chemical treatment of maize, the WCR has also developed resistance to active substances from the pyrethroid chemical group (Pereira et al. 2017). Furthermore, like the CPB, the WCR has developed resistance to the Cry protein produced by transgenic maize varieties having *Bacillus thuringiensis* genes (Gassmann et al. 2016). In Poland, no cases of resistance in this species have been observed yet. However, farmers increasingly report problems relating to the effective protection of maize crops (Bereś, unpublished data). It may be assumed that the harmful effects of the WCR in the current agro-climatic conditions in Poland will intensify year by year, and the problems with maize crop protection will keep on aggravating, as is the case in the United States, to which the species is native (Souza 2019).

Pyrethroids, which were introduced into widespread use at the beginning of the 1980s, continue to play a dominant role in plant protection in Poland and many other countries around the world. According to the Polish Ministry of Agriculture and Rural Development, a total of 40 active substances of insecticides are currently registered in the Polish market, 11 of which are pyrethroid active substances. Polysubstrate monooxygenases, whose dominant parts are cytochrome P450 proteins, play the most important role in the enzymatic detoxification process in insects (Malinowski 2003).

The strategies for pest resistance prevention that are implemented in Poland lead to changes in the levels of susceptibility of the pests to the active substances of insecticides, as demonstrated by a long-term increase and subsequent decrease in the level of pyrethroid resistance in the CPB (Zamojska et al. 2011). The aim of the experiments presented in this paper was to assess, for the first time in Poland, the level of WCR susceptibility to deltamethrin and tau-fluvalinate and to determine the current status of susceptibility to those active substances in the CPB. In light of the changes in pyrethroid susceptibility in the CPB, an attempt was also made to determine the current role of oxidative metabolism in pyrethroid detoxification in this species and to compare the results with the findings for the WCR, whose pyrethroid resistance has not been scientifically demonstrated in Poland.

MATERIAL AND METHODS

The study was conducted between 2017 and 2019 in laboratory conditions on adult CPBs and WCRs from populations found in different regions of Poland:

CPB: Września (Wielkopolska Province, northwest of Poland), Szczecinek (West Pomerania Province, northwest of Poland), Bożków (Lower Silesia Province, southwest of Poland).

WCR: Nienadówka (Podkarpacie Province, southeast of Poland), Krzeczowice (Podkarpacie Province, southeast of Poland), Sośnicowice (Silesia Province, south of Poland).

The distance between particular CPB populations was about 200 km (Września–Szczecinek) and slightly above 300 km (Września–Bożków). The longest distance was between Bożków and Szczecinek – 435 km. The aim of selecting the populations mentioned above was to investigate whether CPB insecticide susceptibility levels are variable among distant populations. WCR individuals were first detected in the southeast of Poland (Nienadówka, Krzeczowice). The insecticide selection pressure has been continuous ever since. From this area, the beetles pushed on toward Poland's west and north regions. Sośnicowice is a population settled 300 km west of Nienadówka and Krzeczowice.

https://doi.org/10.17221/53/2022-PPS

It accounts for a much shorter period of insecticide selection pressure on WCR in Sośnicowice which can result in significant differences in the insects' susceptibility levels.

The collected insects were transported to a laboratory, kept in a climatic chamber at a temperature of around 10 °C, humidity 60%, photoperiod 12 h/12 h (day/night), and subjected to tests the following day. Two pyrethroid active substances were tested (Table 1): deltamethrin: Decis Mega 50EW (active substance content – 4.8%), tau-fluvalinate: Mavrik Vita 240 EW (active substance content – 22.06%).

Tau-fluvalinate was chosen because of its different chemical structure when compared to other pyrethroids. Moreover, both tested insect species had never undergone selective pressure by tau-fluvalinate. Active substance concentrations were expressed in ppm. Between five and eight concentrations were used for each active substance. In each case, double the recommended dose was adopted as the starting dose due to the preliminary research, which showed that it was possible to achieve 100% mortality of the insect in some cases. It was assumed that 200 L of water would be used per hectare. In the case of tau-fluvalinate, the recommended dose was based on the recommendations for the pollen beetle.

The synergist used in the investigation of an oxidative detoxification mechanism: piperonyl butoxide (PBO) – an inhibitor of oxidative enzymes and some hydrolytic enzymes: PBO technical (active substance content – 90%).

The choice of PBO as the inhibitor of P450 was based on the fact that this synergist is the most frequently used oxidative enzyme inhibitor by other scientists discovering insects' resistance to insecticides (Pereira et al. 2017; Souza 2019). The inhibitor is a well-known mechanism of action (Jones 1998) and has also been used in previous, own research (Węgorek 2004).

Table 1. Recommended doses (RD) of the active substances tested, expressed in ppm

Active substance	RD (ppm)		RD used in the experiments (ppm)
	CPB	WCR*	CPB + WCR
Deltamethrin	36	36*	36
Tau-fluvalinate	–	–	220**

CPB – Colorado potato beetle; WCR – western corn rootworm

*The substance was withdrawn from recommendations for WCR control in Poland; the dose given relates to previous recommendations

**Substance not currently recommended for CPB and WCR control; recommendations for the pollen beetle were applied in the tests

Determination of the levels of insect susceptibility to the pyrethroid active substances tested

The tests to determine the levels of susceptibility to the active substances were carried out using the contact-and-stomach IRAC (www.irac-online.org) test method no. 7, which is recommended both for adults and larvae. The presented studies involved beetles as the most resistant insect developmental stage. The method involves exposing the insects studied to treated plant material from host plants. In the present study, CPBs were exposed to treated potato leaves, whereas WCRs were exposed to treated maize leaves. The leaves were dipped for 5 s in aqueous solutions of the test substances, dried at room temperature, and placed in glass containers. In the experiments with CPBs, the containers used were Petri dishes with a diameter of 20 cm, with 80% of their bottom surface covered with potato leaves. Twenty CPBs were then placed in each Petri dish lined with plant material. In the experiments with WCRs, maize leaves, each measuring 21 cm^2, were placed in 1-litre glass containers. Fifty beetles were then placed in each container. The containers were covered with mill gauze, whereas Petri dishes were covered with dishes of the same diameter, ensuring that there was a gap allowing continuous access of air. Three replications were performed for each of the concentrations tested. For control versions, plant material dipped in water without the addition of an insecticide was used.

The temperature in the laboratory was 18–20 °C and the photoperiod was maintained at 16 : 8 (light : dark).

Detection of the oxidative detoxification mechanism for pyrethroids

In the study, experiments were conducted to investigate the presence of a mechanism based on the activity of enzymes inhibited by piperonyl butoxide, i.e., oxidative enzymes and some hydrolytic enzymes. The experiments were carried out using deltamethrin.

https://doi.org/10.17221/53/2022-PPS

In the first stage, the levels of insect susceptibility to PBO were determined using the method described above.

In the case of the CPB, in the second stage of the experiments, the synergist was used at a concentration of 100 ppm, which was found in preliminary tests to be non-toxic to this species. In each case, the same dose of the synergist (100 ppm) was added to the test concentrations. Parallel tests with insecticides without the addition of synergists were carried out in each case.

In the case of the WCR, the second stage of the experiments was not initiated due to the toxicity of the synergist to this species.

Evaluation of experiments and analysis of results

As the mortality of insects in all the control versions ranged between 0% and 2%, there was no need to apply additional calculation methods. The mortality of the insects was determined after 24 h, and the results were expressed in the percentage of dead beetles. Based on the mortality results, concentrations lethal to 50% and 95% of the insects (LC50 and LC95) were calculated. Calculations were performed using POLO PLUS software (version 1.0) based on Finney's logarithmic-probit method. For these calculations, mortality data covering 0% and 100% are not necessary (Finney 1952).

The levels of insect susceptibility to insecticides were classified using the revised IRAC classification (www.irac-online.org) based on insect mortality at 100% and 25% of the recommended dose. Table 2 summarises the classification criteria adopted.

Table 2. Criteria for classifying the levels of insecticide susceptibility in insects based on the revised IRAC classification (IRAC 2020, revised)

Insecticide dose (%)	Pest mortality (%)	Classification
100	100	high susceptibility
25	10	
100	100	susceptibility
25	< 100	
100	90–99	low resistance
100	60–89	medium resistance
100	40–59	high resistance
100	< 40	very high resistance

RESULTS

Insect susceptibility to the active substances of insecticides: Deltamethrin

Colorado potato beetle (Figure 1; Table 3). The mortality rate of CPBs exposed to the recommended dose of deltamethrin (36 ppm) did not reach 100%, ranging between 50% (Szczecinek, 2019) and 90% (Września, 2017). There were only two cases (Bożków and Września, 2017) when the use of double the recommended dose resulted in 100% mortality of the insects studied. In other cases, insect mortality at this dose level ranged between 60% (Szczecinek, 2019) and 90% (Września, 2018). The use of 25% of the recommended dose resulted in a mortality rate ranging from 15% (Września, 2019) to 50% (Września, 2017; Bożków, 2018). The IRAC classification did not indicate the susceptibility of the pest in any of the cases. The levels of insect resistance determined on that basis ranged

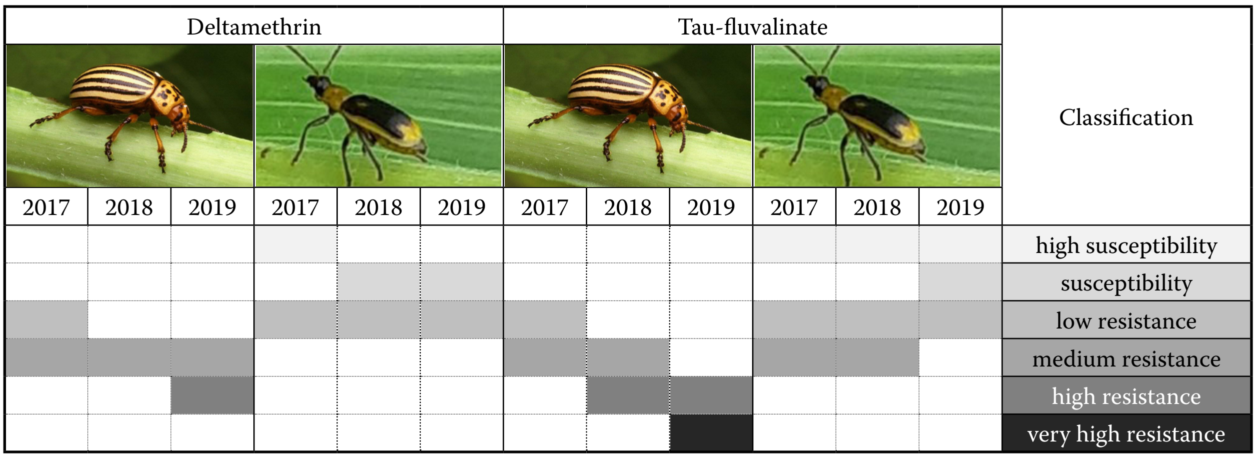

Figure 1. Comparison of Colorado potato beetle and western corn rootworm resistance levels based on the revised IRAC classification (www.irac-online.org)

https://doi.org/10.17221/53/2022-PPS

Table 3. Susceptibility levels to deltamethrin in the Colorado potato beetle in 2017–2019 and the impact of piperonyl butoxide (PBO) on the effect of deltamethrin in 2018, and 2019

Dose (ppm)	2017			2018						2019					
	deltamethrin			deltamethrin			deltamethrin + PBO			deltamethrin			deltamethrin + PBO		
	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek
Insect mortality (%)															
72.00	100	100	70	85	90	70	100	98	99	85	65	60	100	98	100
36.00	70	90	70	70	75	70	100	95	98	60	55	50	98	98	98
18.00	60	50	50	60	65	45	98	95	95	45	50	35	95	95	98
9.00	40	50	30	50	40	30	95	90	95	30	15	20	90	95	95
4.50	40	20	10	25	40	25	80	90	90	0	10	10	85	90	90
2.25	0	10	10	10	5	25	80	90	90	0	0	5	85	88	90
Dose (ppm)															
LC50	11.80 (4.33–30.17)	10.81 (6.48–17.78)	21.61 (14.45–35.84)	11.93 (7.68–16.76)	9.55 (4.18–15.41)	20.15 (11.64–37.33)	0.83 (0.07–1.74)	0.01 (0–0.12)	0.03 (0–0.25)	22.95 (12.53–47.83)	30.77 (16.32–105.8)	40.07 (31.73–54.31)	0.25 (0.03–0.67)	0.04 (0–0.25)	0.10 (0.002–0.42)
LC95	89.24	64.07	311.50	220.70	179.16	538.71	9.90	24.70	11.00	139.26	414.69	772.26	15.31	13.09	8.02

from "low" (Września, 2017) through "medium" (in most other cases) to "high" (Września and Szczecinek, 2019). In the case of the Szczecinek population in 2019, the calculated LC50 value (40.07 ppm) was higher than the recommended dose. The lowest LC50 value (9.55 ppm) was recorded for the Września population in 2018. In all the cases, the LC95 values were higher than the recommended dose, ranging from 64.07 ppm (Września, 2017) to 772.26 ppm (Szczecinek, 2019).

Western corn rootworm (Table 4). The use of the recommended dose of deltamethrin (36 ppm) resulted in mortality ranging between 92% (Nienadówka, 2018) and 100% (Sośnicowice, 2017– 2019) in the western corn rootworms studied. The use of double the recommended dose caused mortality of between 98% (Nienadówka, 2019) and, in most cases, 100%. The mortality rate of the insects at 25% of the recommended dose was also high, ranging from 85% (Krzeczowice and Nienadówka, 2018, Sośnicowice, 2019) to 100% (Sośnicowice, 2017). The IRAC classification, based on insect mortality at 100% and 25% of the recommended dose, showed "high susceptibility" (Sośnicowice, 2017), "susceptibility" (Sośnicowice, 2018 and 2019), and, in other cases, "low resistance" of the insects analysed. Based on the data obtained, it was impossible to calculate LC50 and LC95 values for the Sośnicowice population in all the years of study. In other cases, none of the LC50 values recorded was higher than the recommended dose. All the values were below 1. The LC95 values ranged from 10.9 ppm (Nienadówka, 2017) to 43.28 ppm (Nienadówka, 2018). The LC95 value for the Nienadówka population in 2018 was the only LC95 value that was higher than the recommended dose.

Insect susceptibility to the active substances of insecticides: Tau-fluvalinate

Colorado potato beetle (Table 5). The mortality of beetles exposed to the recommended dose of tau-fluvalinate (220.60 ppm) ranged between 20% (Bożków, 2019) and 90% (Września, 2017). The use of double the recommended dose resulted in mortality of between 35% (Bożków, 2019) and 90% (Września, 2018). With 25% of the recommended dose (55.15 ppm), the lowest mortality (10%) was observed for the Września population in 2017 and for the Bożków population in 2019. In contrast, the highest mortality (50%) was recorded for the Szczecinek population in 2018.

https://doi.org/10.17221/53/2022-PPS

Table 4. Susceptibility levels to deltamethrin in the western corn rootworm

Dose (ppm)	2017			2018			2019		
	Krzeczowice	Nienadówka	Sośnicowice	Krzeczowice	Nienadówka	Sośnicowice	Krzeczowice	Nienadówka	Sośnicowice
Insect mortality (%)									
72.00	100	99	100	99	100	100	100	98	100
36.00	99	99	100	95	92	100	95	98	100
18.00	95	95	100	90	90	100	95	95	100
9.00	90	95	100	85	85	98	90	90	85
4.50	88	90	98	80	80	98	90	90	85
2.25	85	88	98	–	80	98	85	90	85
1.13	80	70	–	–	–	–	–	–	–
0.56	80	55	–	–	–	–	–	–	–
Dose (ppm)									
LC50	0.14 (0.025–0.347)	0.25 (0.078–0.485)	–	0.79 (0.123–1.807)	0.26 (0.000–1.233)	–	0.09 (0.000–0.577)	0.02 (0.000–0.201)	–
LC95	13.34	10.90	–	32.09	43.28	–	18.18	16.83	–

Table 5. Susceptibility levels to tau-fluvalinate in the Colorado potato beetle

Dose (ppm)	2017			2018			2019		
	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek	Bożków	Września	Szczecinek
Insect mortality (%)									
441.20	–	–	–	80	90	80	35	70	65
220.60	80	90	70	50	65	70	20	55	40
110.30	50	50	60	50	50	60	15	30	30
55.15	30	10	40	40	40	50	10	25	25
27.58	5	0	10	20	20	40	5	10	5
13.79	0	0	0	10	10	10	5	10	5
Dose (ppm)									
LC50	103.60 (82.21–136.18)	110.85 (101.35–121.49)	96.96 (58.88–204.12)	125.31 (78.98–229.50)	95.38 (72.15–128.45)	71.57 (40.07–122.62)	1 500.70 (779.55–4 793.10)	197.18 (139.36–319.20)	262.54 (173.39–512.37)
LC95	428.63	263.26	560.40	2 704.30	1 101.89	1 715.10	105 750.00	3 590.90	4 357.60

The IRAC classification indicated "low resistance" (for the Września population in 2017), "medium resistance", "high resistance" and "very high resistance" (for the Bożków population in 2019). The LC50 values were usually lower than the recommended dose. The lowest LC50 value (71.57 ppm) was recorded for the Szczecinek population in 2018. In two cases (Bożków and Szczecinek, 2019), the LC50 values were higher than the recommended dose. The highest LC50 value (1 500.70 ppm) was recorded for the Bożków population in 2019. In all the cases, the LC95 values were higher than the recommended dose, ranging between 263.26 ppm (Września, 2017) and 105 750 ppm (Bożków, 2019).

Western corn rootworm (Table 6). The mortality of the WCRs exposed to the recommended dose of tau-fluvalinate (220.60 ppm) ranged from 80% (Krzeczowice, 2018) to 100% (Sośnicowice, 2017–2019 and Krzeczowice, 2019). With double the recommended dose, 100% mortality was also observed in insects from the Nienadówka population in 2018 and 2019. The mortality rate of insects exposed to 25% of the recommended dose was slightly different, ranging from 60% (Krzeczowice, 2018) to 100% (Sośnicowice, 2017–2019).

https://doi.org/10.17221/53/2022-PPS

Table 6. Susceptibility levels to tau-fluvalinate in the western corn rootworm

Dose (ppm)	2017			2018			2019		
	Krzeczowice	Nienadówka	Sośnicowice	Krzeczowice	Nienadówka	Sośnicowice	Krzeczowice	Nienadówka	Sośnicowice
Insect mortality (%)									
441.20	90	95	100	80	100	100	100	100	100
220.60	90	85	100	80	98	100	100	90	100
110.30	85	80	100	70	98	100	95	90	100
55.15	80	75	100	60	92	100	90	80	100
27.58	70	70	99	60	88	100	80	80	95
13.79	55	70	98	60	–	99	80	70	85
Dose (ppm)									
LC50	3.70	7.26	–	13.15	3.44	–	4.10	3.84	5.80
	(0.20–11.01)	(1.23–16.01)	–	(1.90–28.08)	(0.25–8.95)	–	(0.23–86.87)	(0.04–11.99)	(1.94–8.76)
LC95	1 023.40	905.42	–	8 474.80	70.82	–	86.87	296.28	24.16

The IRAC classification indicated "high susceptibility" (Sośnicowice, 2017–2019), "susceptibility", "low resistance" and, in the case of the Nienadówka population in 2017 and the Krzeczowice population in 2018, "medium resistance". The data obtained did not allow for the calculation of the LC50 and LC95 values for the Sośnicowice population in 2017 and 2018. In all the cases, the LC50 values were significantly lower than the recommended dose, ranging between 3.44 ppm (Nienadówka, 2018) and 13.15 ppm (Krzeczowice, 2018). The lowest LC95 value (24.16 ppm) was recorded for the Sośnicowice population in 2019. The highest LC95 value (8 474.8 ppm) was recorded for the Krzeczowice population in 2018.

Detection of a pyrethroid detoxification mechanism based on the activity of enzymes inhibited by piperonyl butoxide: Colorado potato beetle

In the tests carried out to determine the level of CPB susceptibility to PBO at a concentration of 100 ppm, no mortality was observed in any of the populations studied.

Impact of PBO on the level of CPB susceptibility to deltamethrin (Table 3; Figure 2). Following the addition of PBO to the recommended dose of deltamethrin, the mortality of the beetles at 100% of the recommended dose increased to 95–100% in 2018 and to 98% in 2019. The addition of PBO to 25% of the recommended dose of deltamethrin resulted in mortality of 90–95% both in 2018 and 2019. The IRAC classification indicated "susceptibility" (Bożków, 2018) and "low resistance" of the beetles (in all other cases).

Following the addition of PBO, the LC50 values for all populations in the two years of the experiments were significantly lower than the recommended dose, ranging from 0.01 ppm (Września, 2018) to 0.83 ppm (Bożków, 2018). The LC95 values for all populations in the two years of the experiments were significantly lower than the recommended dose, ranging from 8.02 ppm (Szczecinek, 2019) to 24.70 ppm (Września, 2018).

The results show that there is a synergism between deltamethrin and piperonyl butoxide. The findings allow the conclusion that the main mechanism responsible for deltamethrin resistance in the CPB is the one based on enzymes inhibited by piperonyl butoxide, i.e. oxidative enzymes and some hydrolytic enzymes.

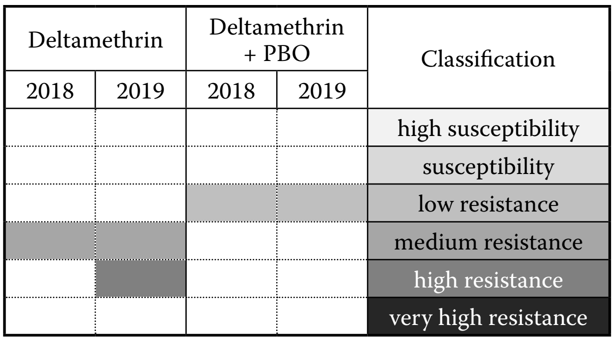

Figure 2. Comparison of the range of Colorado potato beetle resistance levels to deltamethrin and deltamethrin in combination with piperonyl butoxide (PBO) based on the revised IRAC classification (www.irac-online.org)

https://doi.org/10.17221/53/2022-PPS

Detection of a pyrethroid detoxification mechanism based on the activity of enzymes inhibited by piperonyl butoxide: Western corn rootworm

The PBO toxicity in this species was found to be on a level of a strong insecticide, and so PBO would not be considered a synergist in such a situation. Regarding the above findings, the decision not to use PBO for determining the oxidative metabolism in the WCR was made. PBO was found to be highly toxic to WCRs. Using 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm resulted in 100% mortality in all the populations studied in the two years of the experiments (Table 7).

DISCUSSION

The experiments carried out in 2017, 2018, and 2019 showed "low resistance" (only in 2017), "medium resistance" and "high resistance" to deltamethrin in the Colorado potato beetles studied. Similar levels of CPB susceptibility were observed for tau-fluvalinate. In 2019, one of the CPB populations studied showed "very high resistance" to this pyrethroid. The WCR populations studied showed "high susceptibility", "susceptibility" and "low resistance" to both the pyrethroid substances tested. In the case of tau-fluvalinate, the WCR beetles also exhibited "medium resistance".

Pyrethroids are the most prevalent chemical group of insecticides used worldwide (Molnár and Rakosy-Tican 2021). In Poland, active substances from this group have been commonly used for plant protection purposes for 40 years. This has resulted in the development of resistant genotypes in many pest insect species, including the CPB. Initially, the pest showed susceptibility to many active substances from this group of chemical compounds, which has been documented by Polish researchers, including Pietkiewicz and Pawińska (1985) (deltamethrin, alphamethrin, cyhalothrin, beta-cyfluthrin) and Mrówczyński et al. (1994) (deltamethrin, alphamethrin, cyhalothrin, beta-cyfluthrin). The first decrease in deltamethrin susceptibility in the CPB was observed in 1994 by Węgorek, who confirmed the resistance of the pest to deltamethrin, lambda-cyhalothrin, alpha-cypermethrin and cypermethrin in his subsequent studies (Węgorek 2005). The study by Węgorek (2005) showed that, in the case of all the pyrethroids tested, 95% mortality (LC95) of CPBs can be achieved at a dose of over 10 000 ppm. The situation improved several years later, probably as a result of a decreased application of pyrethroid active substances in favour of active substances from new chemical groups (including fipronil from the phenylpyrazole family, and acetamiprid from the neonicotinoid family) (Zamojska et al. 2011). A decrease in CPB resistance to pyrethroids was confirmed in a study by Zamojska et al. (2011), who investigated the susceptibility of the insect to deltamethrin and beta-cyfluthrin between 2008 and 2010. In that study, the LC95 values for deltamethrin and beta-cyfluthrin ranged between 44.25 and 183.61 ppm and between 70.83 and 229.38 ppm, respectively. In turn, the LC50 values for deltamethrin and beta-cyfluthrin were 2.68–12.54 ppm and 3.21–14.09 ppm, respectively. Today, the susceptibility of the CPB to pyrethroid active substances is once again decreasing. This is confirmed by the findings from the present study, in which the LC95 values for deltamethrin and tau-fluvalinate were 64.07–772.26 ppm, and 263.26–105 750 ppm, respectively. The LC50 values for the two substances were 9.55–40.07 ppm and 71.57–1 500.70 ppm, respectively.

Despite numerous global records of insecticide resistance in the WCR (Pereira et al. 2017; Souza 2019), no cases of resistance in this pest have been reported in Poland. The results of the present study showed that based on the IRAC classification, the level of pyrethroid resistance in the WCR populations studied ranged between "high susceptibility" and "medium resist-

Table 7. Mortality (%) of western corn rootworms exposed to piperonyl butoxide

Dose (ppm)	2018			2019		
	Krzeczowice	Nienadówka	Sośnicowice	Krzeczowice	Nienadówka	Sośnicowice
100.0	100	100	100	100	100	100
50.0	100	100	100	100	100	100
25.0	100	100	100	100	100	100
12.5	100	100	100	100	100	100

https://doi.org/10.17221/53/2022-PPS

ance". In all the experiments carried out between 2017 and 2019, slight differences were observed in susceptibility levels between WCR populations from the southeast of Poland (Nienadówka, Krzeczowice) and populations from the southwest of Poland (Sośnicowice), which showed higher susceptibility to the same active substances of insecticides. This is most probably because the pest was first recorded in the southeast of Poland (Bereś et al. 2015), which resulted in a longer period of selection pressure from insecticides in this region.

Knowledge of insecticide resistance mechanisms in insects is a key element in controlling their resistance and choosing adequate tools to counteract this phenomenon and its harmful economic and environmental effects. Each insect species has different genetic, biological, and environmental characteristics promoting the development of a specific resistance mechanism. Therefore, a given insect species may have one or several resistance mechanisms simultaneously involved in the biotransformation, detoxification, and neutralisation of one or several types of toxins (Malinowski 2003).

According to Wilkinson (1983), oxidative metabolism is one of animals' and plants' most common metabolic processes. There are many examples worldwide, also in Poland, of insects in which oxidative enzymes play a key role in the detoxification of insecticides, including the large pine weevil (*Hylobius abietis* L.) (Dobrowolski 1998), the pollen beetle (*Meligethes aeneus* F.) (Slater and Nauen 2007), the CPB (*Leptinotarsa decemlineata* L.) (Węgorek 2004; Zamojska et al. 2011) and the WCR (Pereira et al. 2017; Souza et al. 2021). There is a very large number of studies confirming the key role of oxidative metabolism in the detoxification of insecticides from different chemical groups with different modes of action (including carbofuran, carbaryl, permethrin, deltamethrin, abamectin and imidacloprid) in the CPB (Węgorek 2004; Mota-Sanchez et al. 2006; Zamojska et al. 2011). Malinowski (2003) and Porter and Coon (1991) believe that the ability to metabolise substances from different chemical groups stems from the presence of numerous isoenzymatic forms of cytochrome P450.

In the present study, an attempt was made to diagnose detoxification mechanisms responsible for deltamethrin resistance in the CPB with the use of PBO, an inhibitor of oxidative enzymes. PBO forms a strong complex with cytochrome P450 (Jones 1998). The complex prevents polysubstrate monooxygenases from catalysing a reaction producing less toxic compounds than the parent substances. As a result, an insecticide reaches its target in higher concentrations (Malinowski 2003). The use of PBO resulted in increased mortality of CPBs exposed to deltamethrin in all the populations studied, which was consistent with the results of earlier studies (Zamojska et al. 2011) and showed that oxidative enzymes are involved in the detoxification mechanism in this pest. However, a comparison of the findings from the present study with the results of a study by Węgorek (2004) shows that the effectiveness of this mechanism in the CPB in Poland is decreasing.

In the present study, PBO was found to be highly toxic to the WCR populations studied. The use of even as low a concentration of PBO as 12.5 ppm resulted in 100% mortality of the beetles. This is contrary to the findings from a study by Pereira et al. (2017), in which PBO was used to investigate pyrethroid resistance mechanisms in the WCR. In the case of the Polish WCR populations studied, the enzymes inhibited by PBO are likely to play an essential role in other processes crucial for normal body functions. Thus, even a slight inhibition of the enzymes results in the mortality of the insects.

When developing strategies for preventing resistance development in any insect species, it is essential to focus not only on its current susceptibility level to the active substances of insecticides but also on potential resistance mechanisms and metabolism of insecticides in the insect's organism. In this context, the results for the toxicity of PBO to Polish WCR populations also confirm the involvement of PBO-inhibited enzymes in important metabolic processes in the pest. They may guide further studies on developing innovative pest control methods.

The great similarity in the susceptibility levels observed in the WCR populations indicates that the Polish populations of the insect have low genetic diversity. This may be because the populations developed from a small gene pool of the first insects that migrated to Poland. In order to confirm this thesis, a larger number of WCR populations in Poland and Europe should be tested.

https://doi.org/10.17221/53/2022-PPS

REFERENCES

Alyokhin A., Baker M., Mota-Sanchez D., Dively G., Grafius E. (2008): Colorado potato beetle resistance to insecticides. American Journal of Potato Research, 85: 395–413.

Bereś K.P., Drzewiecki S., Fiedler Ż. (2015): Zachodnia kukurydziana stonka korzeniowa (*Diabrotica virgifera virgifera* LeConte) – występowanie, biologia, szkodliwość oraz strategia zwalczania [The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) – distribution, biology, harmfulness and control strategy]. Poznań, Instytut Ochrony Roślin – PIB. Polish.

CABI (2020): Crop Protection Compendium. Available at: https://www.cabi.org/ (accessed Apr 25, 2020).

Dobrowolski M. (1998): Mechanisms of resistance to insecticides in large pine weevil (*Hylobiusabietis* L). In: European Society for New Methods in Agricultural Research (ESNA) XXVIIIth Annual Meeting and International Union of Radioecology (IUR) Working Group Soil-to-Plant Transfer Annual Meeting, August 26–29, 1998, Brno, Czech Republic: 194.

Finney D.J. (1952): Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve. Cambridge, The University Press.

Gassmann A.J., Shrestha R.B., Jakka S.R.K., Dunbar M.W., Clifton E.H., Paolino A.R., Ingber D.A., French B.W., Masloski K.E., Dounda J.W., Clair C.R.S. (2016): Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): Root injury in the field and larval survival in plant-based bioassays. Journal of Economic Entomology, 109: 1872–1880.

Jones D. (1998): Piperonyl Butoxide. The Insecticide Synergist. London, Academic Press.

Malinowski H. (2003): Odporność owadów na insektycydy. Mechanizmy powstawania i możliwości przeciwdziałania [Insect resistance to insecticides. Developmental mechanisms and prevention possibilities]. Warszawa, Wydawnictwo Wieś Jutra. Polish.

Molnár I., Rakosy-Tican E. (2021): Difficulties in potato pest control: The case of pyrethroids on colorado potato beetle. Agronomy, 11: 1920. doi: 10.3390/agronomy11101920

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

Mrówczyński M., Ciesielski F., Widerski K., Wachowiak H., Urban M., Bubniewicz P., Głazek M., Pietryga J., Banaszak H., Pawińska M. (1994): Badania nad zastosowaniem beta-cyflutryny (Bulldock 025 EC) w zwalczaniu szkodników upraw rolniczych [Research on the use of beta-cyfluthrin (Bulldock 025 EC) in the control of crop pests]. In: Materiały XXXIV Sesji Naukowej Instytutu Ochrony Roślin Cz. II [Materials of the 34th Scientific Session of the Institute of Plant Protection, Part. II]. February 9–11, 1994, Poznań, Poland: 41–45.

Pélissié B., Chen Y.H., Cohen Z.P., Crossley M.S., Hawthorne D.J., Izzo V., Schoville S.D. (2022): Genome resequencing reveals rapid, repeated evolution in the Colorado potato beetle. Molecular Biology and Evolution, 39: msac016. doi: 10.1093/molbev/msac016

Pereira A.E., Souza D., Zukoff S.N., Meinke L.J., Siegfried B.D. (2017): Cross-resistance and synergism bioassays suggest multiple mechanisms of pyrethroid resistance in western corn rootworm populations. PLoS One, 12: e0179311. doi: 10.1371/journal.pone.0179311

Pietkiewicz J., Pawińska M. (1985): Skuteczność działania stonkobójczego niektórych pyretroidów w zależności od temperatury powietrza [The effectiveness of certain pyrethroids in controlling the Colorado potato beetle depending on the air temperature]. In: Materiały XXV Sesji Naukowej Instytutu Ochrony Roślin [Materials of the 25th Scientific Session of the Institute of Plant Protection]. February 13–15, 1985, Poznań, Poland: 451–457. Polish.

Porter T.D., Coon M.J. (1991): Cytochrom P–450. Multiplicity of forms, substrates, and catalytic and regulatory mechanisms. Journal of Biological Chemistry, 266: 13469–13472.

Sharif M., Hejazi M.J., Mohammadi A., Rashidi M.R. (2007): Resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, to endosulfan in East Azarbaijan and Ardabil provinces of Iran. Journal of Insect Science, 7: 31. doi: 10.1673/031.007.3101

Slater R., Nauen R. (2007): The development and nature of pyrtehroid resistance in the pollen beetle (*Meligethes aeneus*) in Europe. In: Presentation Abstract, EPPO Workshop on insecticide resistance of *Meligethes* ssp. (pollen beetle) on oilseed rape. September 3–5, 2007, Berlin, Germany.

Souza D. (2019): Characterization of pyrethroid resistance in the western corn rootworm *Diabrotica virgifera virgifera* LeConte [PhD. thesis]. Lincoln, University of Nebraska.

Souza D., Siegfried B.D., Meinke L.J., Miller N.J. (2021): Molecular characterization of western corn rootworm pyrethroid resistance. Pest Management Science, 77: 860–868.

Węgorek P. (2004): Enzymy oksydacyjne jako główny czynnik odporności stonki ziemniaczanej (*Leptinotarsa decemlineata* Say) na pyretroidy w zachodniej Polsce [Oxidative enzymes as a key factor in the resistance of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) to pyrethroids in western Poland]. Postępy w

https://doi.org/10.17221/53/2022-PPS

Ochronie Roślin, 44: 1208–1211. Polish with English abstract.

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

Whalon M.E., Mota-Sanchez D., Hollingworth R.M. (2008): Global Pesticide Resistance in Arthopods. Trowbridge, Cromwell Press.

Wilkinson C.F. (1983): Role of mixed-function oxidases in insecticide resistance. In: Georghiou G.P., Saito T. (eds). Pest Resistance to Pesticides. New York, Plenum Press: 175–206.

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

Received: May 2, 2022
Accepted: March 16, 2023
Published online: May 11, 2023