

## CURRENT STATUS OF RESISTANCE IN COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) TO SELECTED ACTIVE SUBSTANCES OF INSECTICIDES IN POLAND

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**Abstract:** Chemical plant protection is still an indispensable method in effective potato protection against Colorado potato beetle – CPB – (*Leptinotarsa decemlineata* Say) in Poland. This species is able to develop strong resistance against all active substances used in chemical and biological insecticides. The phenomenon of resistance is variable in time and in space. Therefore the objective of the study was to determine the present susceptibility level of Polish populations of CPB to main groups of insecticides recommended in Poland for CPB control.

**Key words:** Colorado potato beetle, insecticide resistance, pyrethroids, organo-phosphorous, phenylpyrazoles, neonicotinoids, nereistoxin analogues

### INTRODUCTION

Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is still a major pest of potato crops in Poland (Malinowski 2003; Mrówczyński 2000; Pawińska and Mrówczyński 2000; Węgorek and Stachecki 1999). Although the importance of potato production in Poland systematically decreases (over 2 millions hectares in the past), currently it still reaches about 700,000 ha (Statistical Information Centre 2004 – Table 1). The average potato tuber yield in Poland has always been very low (18.8 t per hectare in comparison with the West European countries – average of 40 t/ha) (Statistical Information Centre 2004). There are some important factors affecting potato yield. The main are: inadequate control of pests, diseases, weeds and widespread occurrence and resistance phenomenon of CPB in Poland.

In spite of recommended strategy of integrated pest management (IPM) (EPPO 1999, 2001; Lipa 1984; Pruszyński 2003; Pruszyński and Węgorek 2004), chemical treatment is the main method of potato protection against CPB. The threshold of economic harmfulness of CPB in Poland is considerably exceeded every year. When

the treatment is abandoned the damage may affect as much as 80–90% of the crop (Pruszyński and Węgorzek 1990; Węgorzek 1959). The cost of chemical protection of potato fields against CPB in Poland amounts on average 40€/ha – 30 millions in global scale. In spite of applying insecticides, yield losses resulted from CPB feeding and insecticide resistance are high and amount on average 15–25%. Ongoing over 50 years constant and strong selective pressure of insecticides on Polish CPB populations is a factor, which accelerates the process of increasing resistance of that species. CPB is considered to be one of several Polish pests with the highest likelihood of developing insecticide resistance and this phenomenon in CPB has been a severe problem in Poland for a number of years (Kroczyński et al. 1982, 1989, 1996; Łakocy 1967, 1973, 1977; Pawińska 2000; Pruszyński et al. 1987; Przybysz et al. 1996, 2003; Węgorzek 2003b; Węgorzek and Jörg 2003; Węgorzek et al. 1987). The dynamics and widespread of CPB resistance in Poland (to organochlorines, organophosphates, carbamates, pyrethroids, nereistoxin analogues) and risk of increase of tolerance to neonicotinoids and phenylpyrazoles has created a need for resistance monitoring and elaborating the strategies for the management of CPB resistance to all synthetic insecticides recommended in Poland (Dziennik Ustaw 2004; EU 1991). The studies on

Table 1. *Leptinotarsa decemlineata* control in Poland within the years 1978–2004

Years 1978–2004	Potato field size (thousands of hectares)	% of protection area	Number of treatments
1978	2360	76	1.2
1979	2441	78	1.2
1980	2344	50	1.1
1981	2257	72	1.2
1982	2178	78	1.2
1983	2220	96	1.6
1984	2147	78	1.1
1985	2095	37	1.1
1986	2009	70	1.1
1987	1934	75	1.1
1988	1886	82	1.2
1989	1858	89	1.3
1990	1835	87	1.4
1991	1732	84	1.4
1992	1757	89	1.7
1993	1761	89	1.4
1994	1697	83	1.4
1995	1522	97	1.6
1996	1342	98	1.6
1997	1303	68	1.3
1998	1250	42	1.3
1999	1255	83	1.4
2000	1300	80	1.5
2001	1190	82	1.6
2002	800	85	1.5
2003	766	87	1.4
2004	713	85	1.6
Mean 27 years	Mean 1701.93	Mean 78.52	Mean 1.35

CPB susceptibility to main classes of insecticides were performed mainly in the Institute of Plant Protection in Poland, the Plant Breeding and Acclimatisation Institute – Department in Bonin and the Institute of Industrial Organic Chemistry in Warsaw. Nowadays a lot of consideration is taken to neonicotinoids and phenylpyrazoles because the importance of both classes of insecticides for CPB control in Poland systematically increases (Węgorzek et al. 2003). Insecticides from these groups are relatively new for the control of CPB in Poland (fipronil – 1996, acetamiprid – 1996, imidacloprid – 1998, thiamethoxam – 1999, thiacloprid – 2001) (Colliot et al. 1992; Elbert et al. 1996; Elbert and Overbeck 1990; Leicht 1996; Mrówczyński et al. 2000; Pawińska et al. 1996; Sobótka 1998; Yamamoto et al. 1995). Research presented in this study concerns laboratory investigations with the objective to define actual susceptibility level of CPB to selected active substances. They were performed in the Institute of Plant Protection in Poznań and provided important information on CPB resistance to insecticides applied currently in Poland.

## MATERIALS AND METHODS

Following commercial products were used for the field and laboratory investigations:

- Neonicotinoids: Confidor 200 SL, active substance – imidacloprid; Actara 25 WG, active substance – thiamethoxam; Mospilan 20 SP, active substance – acetamiprid;
- Phenylpyrazoles: Regent 200 SC, active substance – fipronil;
- Nereistoxin analogues: Bancol 50 WP, active substance – bensultap;
- Organophosphorous: Ultracid 40 EC, active substance – methidathion;
- Pyrethroids: Decis 2,5 EC, active substance – deltamethrin; Karate 025 EC, active substance – lambda-cyhalothrin, Fastac 100 EC, active substance – alpha-cypermethrin; Cyperkil Super 25 EC, active substance – cypermethrin.

CPB larvae ( $L_2$ ) and beetles were collected from 4 distant fields representing populations occurring in the Wielkopolska region (Western Poland): Bolewice, Rogalinek, Skoki, Winna-Góra.

Laboratory tests were conducted in the years 2002–2004, using IRAC method number 7.

Representative samples of insects from field CPB populations (larvae or beetles), suitable for immediate testing were collected and transported in large isolators. The insects were not subjected to temperature, humidity or starvation stress after collection. To conduct the laboratory tests non-infested, untreated leaves were collected from the same fields as insects were. Accurate dilutions of the tested compounds from commercially available products were prepared. For initial studies 5–7 insecticides doses were used. In some cases doses based on presently recommended field rates were used. Leaves were dipped separately in the tested liquids for 5 seconds, stirred gently and next hung on a string until the leaf surface was dry. At first leaves were immersed in “untreated liquid” (control) and next in the dilutions of tested liquids in a range from the lowest to the highest dose. The treated surface-dry leaves were placed in 15-cm diameter Petri dishes. Discs of dry filter paper were placed inside to help to maintain proper conditions. Equal num-

bers (10 for one dish) of L<sub>2</sub> or L<sub>3</sub> larvae or adult beetles were placed in each Petri dish, ensuring that a total of 90 larvae or beetles were used per treatment. Petri dishes were stored in laboratory conditions with mean temperature of 22–25°C and photoperiod of 16 hours day and 8 hours night. In case of rapidly acting compounds, a final assessment of larval mortalities was made after 48 h. For slowly acting compounds (e.g. bensultap) first assessment was made after 72 h, when the leaves were replaced with fresh, untreated ones. The dishes were kept until final assessment, i.e. either next 48 h or until larvae in the “untreated” (control) container moulted again. Results were expressed as percent mortality for each dose, and corrected with the use of Abbott’s formula as needed (when mortality in the control exceeded 10%) (Abbot 1925). At each assessment, larvae and beetles were categorised as: (a) alive – unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch or high temp. (30–35°C during 10 minutes), or (b) dead or affected, giving an abnormal response to stimulation or showing abnormal growth. Corrected Mortality =  $100 \times (P-C/100-C)$  where P = % mortality in a given treatment, C = % mortality in controls. LC<sub>50</sub> and LC<sub>95</sub> were calculated using Finney probit analysis method (Finney 1952) and expressed in ppm.

## RESULTS AND DISCUSSION

The results of laboratory tests are presented in Tables 2–11.

CPB is the example of insect species that can develop strong resistance mechanisms to all active substances used to control it all over the world (Clark and Argentine 1997; Forgash 1985; French et al. 1992; Heim et al. 1990; Solomennikowa et al. 1990; Tisler and Zehnder 1990; Whalon et al. 1997). Also in our study, populations from all four areas demonstrated high level of resistance to pyrethroid group (Tables 8, 9, 10, and 11), and some level of resistance to nereistoxine analogues (Table 6) and organophosphorous insecticides (Table 7). Survival at recommended concentrations in case of pyrethroids, nereistoxine analogues and organophosphorous indicated occurrence of resistance to these active substances in examined populations. In laboratory studies (2002–2004) pyrethroid insecticides were less effective in controlling either CPB larvae or beetles but beetles showed better resistance (Tables 8, 9, 10, and 11). The results of insecticide susceptibility monitoring indicated that CPB larvae do not show resistance to phenylpyrazoles (fipronil) and neonicotinoids (acetamiprid, thiamethoxam, imidacloprid) group (Tables 2, 3, 4, and 5). In case of CPB beetles survival of 1–2% was recorded in all tested populations, even when applying recommended field doses. The results indicated that CPB populations tolerant to pyrethroids, nereistoxine analogue and organophosphorous were no cross-resistant to neonicotinoids (Tables 2, 3, and 4) and phenylpyrazoles insecticide (Table 5). The resistance mechanism of surviving beetles is not known, although some symptoms point at insensibility of nervous tissue in the place of biological activity of active substance. However, it requires further researches.

The results suggest that at present situation of CPB resistance limitations in use of pyrethroid and nereistoxin analogues group is indispensable in Poland. Also, rational application of all recommended insecticides and their rotation including dif-

ferent modes of their molecular action is necessary (Pruszyński et al. 2003; Węgorzek 2004; Węgorzek et al. 1998).

#### Neonicotinoids:

Table 2. Imidacloprid action against *L. decemlineata*; the range of recommended doses in Poland: 42–167 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	0.8	1.2	1.8	2.9	3.3	3.5	3.7	4.8	5.3	14.4	15.4	13.4
Rogalinek	0.4	1.1	1.5	2.9	3.0	3.5	2.9	3.2	3.4	11.9	13.3	10.3
Skoki	0.6	2.8	1.1	2.0	2.8	3.3	3.9	3.9	3.5	13.4	13.5	10.4
Winna Góra	0.6	2.9	1.8	2.8	2.9	3.2	3.5	3.8	3.9	15.7	16.2	20.8

Table 3. Acetamiprid action against *L. decemlineata*; the range of recommended doses in Poland: 20–133 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	0.2	0.3	0.4	2.6	2.9	2.3	1.8	2.2	2.5	10.8	15.7	20.7
Rogalinek	0.1	0.3	0.4	2.1	2.6	2.7	1.8	2.1	2.5	17.9	20.8	26.6
Skoki	0.5	0.5	0.7	1.8	2.8	2.0	1.8	1.9	2.0	11.2	13.2	15.0
Winna Góra	0.1	0.3	0.6	1.9	2.9	3.3	2.2	2.5	3.0	6.7	8.8	10.8

Table 4. Thiamethoxam action against *L. decemlineata*; the range of recommended doses in Poland: 33–133 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	0.4	0.5	0.3	1.1	1.8	1.3	3.8	4.4	4.7	5.2	5.8	5.5
Rogalinek	0.4	0.8	0.4	1.1	1.8	1.5	2.2	2.7	3.3	4.9	5.7	5.3
Skoki	0.6	0.7	0.5	2.1	2.5	2.8	3.2	3.6	3.3	6.8	6.9	7.3
Winna Góra	0.2	0.6	0.7	1.8	1.8	1.9	2.4	2.4	3.5	4.4	4.8	4.3

#### Phenylpyrazoles:

Table 5. Fipronil action against *L. decemlineata*; the range of recommended doses in Poland: 33–133 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	0.12	0.3	0.4	0.70	0.9	0.7	0.70	0.8	0.5	2.20	2.5	2.7
Rogalinek	0.09	0.1	0.2	0.30	0.8	0.8	0.45	0.5	0.5	1.50	1.8	1.6
Skoki	0.10	0.1	0.1	0.40	0.6	0.8	0.25	0.3	0.6	1.20	1.2	1.0
Winna Góra	0.06	0.09	0.1	0.70	0.7	0.9	0.30	0.4	0.6	1.25	1.3	1.6

## Nereistoxin analogues:

Table 6. Bensultap action against *L. decemlineata*; the range of recommended doses in Poland: 250–1333 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	10.8	15.6	13.9	15.7	20.0	22.9	30.8	24.4	30.6	800.9	500.8	700.0
Rogalinek	11.7	15.4	10.8	28.5	22.8	30.0	129.0	154.9	200.0	>4000	>4000	>4000
Skoki	8.4	15.7	17.3	25.9	30.8	35.3	40.8	43.6	55.8	>4000	>4000	>4000
Winna Góra	7.5	17.0	22.9	30.8	25.1	34.4	67.9	50.6	56.8	266.7	368.9	500.0

## Organophosphorous:

Table 7. Methidathion action against *L. decemlineata*; the range of recommended doses against CPB in Germany: 500–2000 ppm

Population/ Year	Larvae					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004
Bolewice	110.0	115.8	125.5	915.8	1250.0	1005.5
Rogalinek	101.0	53.78	35.32	1353.0	271.94	256.62
Skoki	56.0	35.65	34.81	812.0	405.17	506.59
Winna Góra	58.0	36.55	36.57	700.6	202.95	196.89

## Pyrethroids:

Table 8. Deltamethrin action against *L. decemlineata*; the range of recommended doses in Poland: 8–50 ppm

Population/ Year	Larvae						Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]			LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Bolewice	3.3	4.9	3.2	8.8	16.6	20.2	50.1	55.7	60.2	>10000	>10000	>10000
Rogalinek	2.8	5.1	2.2	12.7	15.8	18.0	40.5	31.2	45.6	>10000	>10000	>10000
Skoki	4.3	4.0	3.6	17.9	14.8	16.8	39.5	45.8	65.9	>10000	>10000	>10000
Winna Góra	5.1	6.8	8.9	20.2	22.9	20.5	19.8	18.9	36.8	>10000	>10000	>10000

Table 9. Lambda-cyhalothrin action against *L. decemlineata*; the range of recommended doses in Poland: 83–500 ppm

Population/ Year	Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004
Bolewice	40.3	43.4	40.6	>10000	>10000	>10000
Rogalinek	20.9	22.9	15.8	>10000	>10000	>10000
Skoki	26.9	30.0	26.7	>10000	>10000	>10000
Winna Góra	54.4	65.8	55.8	>10000	>10000	>10000

Table 10. Alpha-cypermethrin action against *L. decemlineata*; the range of recommended doses in Poland: 13,25–66 ppm

Population/ Year	Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004
Bolevice	26.7	22.7	25.8	>10000	>10000	>10000
Rogalinek	50.7	39.8	45.9	>10000	>10000	>10000
Skoki	45.9	34.4	30.8	>10000	>10000	>10000
Winna Góra	44.6	39.5	50.5	>10000	>10000	>10000

Table 11. Cypermethrin action against *L. decemlineata*; the range of recommended doses in Poland: 42–200 ppm

Population/ Year	Beetles					
	LC <sub>50</sub> [ppm]			LC <sub>95</sub> [ppm]		
	2002	2003	2004	2002	2003	2004
Bolevice	11.8	10.3	13.0	67.5	781.1	1015.00
Rogalinek	8.7	6.5	10.69	49.3	468.94	215.77
Skoki	10.5	10.13	10.71	43.2	54.48	55.19
Winna Góra	9.8	15.79	9.05	43.2	160.02	156.64

## STATEMENTS AND CONCLUSIONS

In case of studied pyrethroids, the survival of CPB larvae and beetles in laboratory tests achieved very high level at doses higher than these commercially recommended. The decrease of CPB susceptibility to examined active substances indicates the decline of chemical treatments efficiency under field conditions and points at selection of populations that metabolise high doses of insecticides faster and more effectively. Thus, continuous and widespread use of pyrethroids in Poland can lead to control failure.

CPB strains were more tolerant to nereistoxin analogue (bensultap), and organophosphorous (methidathion) insecticides but were still excellently controlled with imidacloprid, thiamethoxam, acetamiprid, and fipronil. Laboratory investigations gave no indication on resistance or cross-resistance elicited by tested populations of CPB to neonicotinoids and fipronil.

Considering the possibility of occurring high CPB resistance, it is necessary and advisable to control application of insecticides rationally, and to take into account their association with different chemical groups and mechanisms of resistance.

Only systematic monitoring of susceptibility of CPB strains might ensure more effective protection of potato crops in Poland. Therefore one should take into consideration the necessity of CPB susceptibility monitoring by regional plant protection services according to method developed by the Institute of Plant Protection in Poznań in the year 2002 (Pruszyński and Węgorek 2004).

Using neonicotinoid and phenylpyrazole classes of insecticides in Poland we need to remember that we have CPB strains resistant to pyrethroids and more tol-

erant to nereistoxin analogue and organophosphorous. We should also remember that this pest insect is multiple and cross-resistant to five major groups of insecticides in the United States, and also to *Bacillus thuringiensis*, abamectine, imidacloprid and fipronil.

Constant monitoring of CPB susceptibility level to insecticides used in Poland is obligatory now (Dziennik Ustaw 2004). Also studies on mechanisms of CPB resistance to active substances are very useful because they will allow to enterprise a better strategy for delaying CPB resistance (Clarc and Argentine 1997; Węgorek 2003a).

Poland is a member of the European Union since May 2004. In August 2004 Polish Ministry of Agriculture changed some directives concerning researches on effectiveness of plant protection products (Dziennik Ustaw 2004). Presently the registration authorities must take into consideration the resistance risk analysis based on EPPO standards and recommendations (EPPO 2001). So during the registrative investigations, resistance phenomenon of agrophages must be seriously taken into consideration. Also chemical companies should take responsibility for constant control of efficacy of their products and update them if needed in accordance with new results from susceptibility monitoring. The future of chemical protection of potato plants against CPB in Poland will be dependent on many factors, among which the resistance of this species will be one of the most important.

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#### POLISH SUMMARY

#### AKTUALNY STAN ODPORNOŚCI STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY) NA WYBRANE SUBSTANCJE AKTYWNE ŚRODKÓW CHEMICZNYCH ZALECANYCH W POLSCE DO JEJ ZWALCZANIA

Stonka ziemniaczana (*Leptinotarsa decemlineata* Say) jest wciąż głównym szkodnikiem ziemniaka w Polsce. Jej zwalczanie oparte jest na metodzie chemicznej przy użyciu substancji aktywnych należących do różnych grup chemicznych. Stonka ziemniaczana jest zdolna do wykształcenia odporności na każdą z nich i dlatego należy prowadzić stałą kontrolę stopnia wrażliwości tego szkodnika na poszczególne substancje aktywne. Praca przedstawia wyniki monitoringu wrażliwości omawianego szkodnika na wybrane insektycydy z grup środków fosforoorganicznych, pyretroidów, pochodnych nereistoksyny, neonikotynoidów oraz fenylpirazoli.