Meligethes aeneus (Coleoptera: Nitidulidae) Resistance to Lambda-Cyhalothrin in the Czech Republic in 2012 and 2013

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Abstract

SEIDENGLANZ M., POSLUŠNÁ J., ROTREKL J., KOLAŘÍK P., HRUDOVÁ E., TÓTH P., HAVEL J., BERNARDOVÁ M. (2015): *Meligethes aeneus* (Coleoptera: Nitidulidae) **resistance to lambda-cyhalothrin in the Czech Republic in 2012 and 2013**. Plant Protect. Sci., 51: 94–107.

Susceptibility of *Meligethes aeneus* populations from the Czech Republic and Slovakia was tested with lambda-cyhalothrin (93 populations in 2012, 82 populations in 2013) using IRAC method No. 011 Version 3. Resistant populations predominated in both years. The mean percentage mortalities for a common European field rate of 7.5 g a.i./ha were 60.95% in 2012 and 61.36% in 2013 (according to Abbott's formula). The LC $_{50}$ values estimated for the tested populations exceeded the European field rate in many cases (22.09% of populations in 2012 and 17.14% in 2013). Only in 10.47% and 7.14% of populations the LC $_{90}$ values were below the European field rate (in 2012 and 2013, respectively). Slovak populations seemed to be somewhat less resistant compared to the Czech ones.

Keywords: pollen beetles; pyrethroid resistance; esteric pyrethroids; adult vial tests; IRAC

At present, pyrethroid-resistant populations of pollen beetles (Meligethes aeneus F., Coleoptrera: Nitidulidae) are probably the most dominant in West and Central Europe and are becoming established in the North and East (SLATER et al. 2011). Pollen beetle resistance to pyrethroids is a reality in Europe and will affect future oilseed rape production. Deterioration of the problem is associated with extension of oilseed rape area, and also with reducing the number of spray solutions is reduced (ZLOF 2008). The first case of reduced susceptibility of pollen beetles to pyrethroids in Europe was reported in 1999 in the Champagne region, in northeastern France (BALLANGER et al. 2007). Based on this evidence resistance monitoring activities were initiated in many other European countries (ZIMMER

& Nauen 2011a,b). Consequently, resistant pollen beetles were also discovered in 2000 in Switzerland (Derron et al. 2004) and in Sweden (Djurberg & GUSTAFSSON 2007), in 2003 in Finland (TIILIKAINEN & HOKKANEN 2008), in 2004 in Poland (WEGOREK 2005; Wegorek et al. 2006, 2009; Philippou et al. 2011), and in 2007 in Luxembourg (EICKERMANN et al. 2008). The first cases of pyrethroid resistance in Denmark were described in 2000 and 2001 and confirmed in 2003 (HANSEN 2003, 2008). Since 2002, when the first resistant pollen beetles were observed in Germany, the number of positive recordings has rapidly increased in this country (Heimbach 2005; Nauen 2005, 2007; Heimbach & Müller 2006; Тніеме at al. 2006, 2008; Неімвасн et al. 2007). In 2006 more than 50% of the winter oilseed rape

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QJ1230077.

acreage in Germany was affected (Тніеме et al. 2008). It is clear that the main oilseed-rape-growing areas of Europe are affected by this problem. The UK (and maybe Austria) is slightly less affected by the phenomenon and it seems that Meligethes populations from Ukraine and some Baltic states (Estonia and Latvia) remain fully susceptible to pyrethroids (RICHARDSON 2008; VEROmann & Toome 2011; Zimmer & Nauen 2011a,b). In 2007 resistant populations of pollen beetles were also recorded for the first time in the Czech Republic (Ko-COUREK et al. 2007; Stará et al. 2010; Seidenglanz et al. 2011, 2012, 2013). However, we cannot exclude the possibility that the resistant populations appeared even earlier in the Czech Republic, because winter oilseed rape has been grown very intensively, the number of insecticidal sprays usually applied to the crop during one season is comparable with the situation in Germany, and the acreages of this crop have been continually increasing in the country over the recent fifteen years (KOCOUREK 2013a). Seemingly in 2011 the situation got markedly worse in the Czech Republic. Resistant and highly resistant populations became very frequent across the whole country at that time. Suddenly they were not limited only to northern regions as they had been in previous years (SEIDENGLANZ *et al.* 2012, 2014).

The aim of this paper is to illustrate the change in resistance levels of *Meligethes aeneus* from the Czech Republic (and Slovakia in 2012, too) to esteric pyrethroids (lambda-cyhalothrin used in tests) in the course of two years (2012–2013) and compare the situation with results recorded in the previous monitoring period (2009–2011) (SEIDENGLANZ *et al.* 2011, 2012, 2013, 2014). IRAC (Insecticide Resistance Action Committee; www.irac-online.org) laboratory test No. 11 Ver. 3 was used.

MATERIAL AND METHODS

Samples of *Meligethes* adults (*M. aeneus* highly predominated in the catches) from different localities in the Czech Republic (as well as in Slovakia in

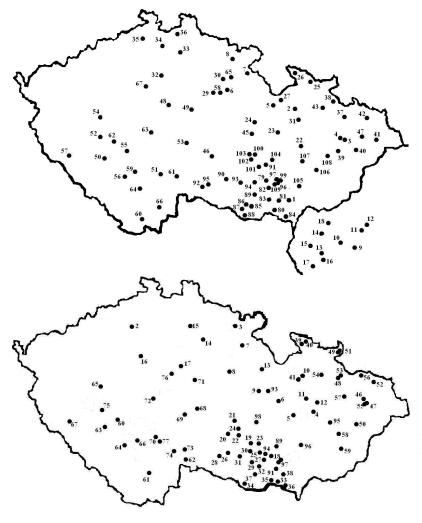


Figure 1. The spots on the map of the Czech Republic (CZ) and Slovakia (SK) mark the places where the *Meligethes* populations tested in 2012 were sampled. A total of 93 populations (83 CZ and 10 SK ones) were compared. The numbers of the spots corresponds with the population numbers used in Table 1 and in Figures 3A, B and C

Figure 2. The spots on the map of the Czech Republic mark the places where the *Meligethes* populations tested in 2013 were sampled. A total of 82 populations were compared. The numbers of the spots corresponds with the population numbers used in Table 2 and on Figures 4A, B and C

 $Table\ 1.\ Susceptibility\ of\ pollen\ beetles\ from\ Czech\ Republic\ and\ Slovakia\ after\ 24\ h\ exposure\ to\ lambda-cyhalothrin\ in\ 2012\ (93\ samples\ tested\ with\ an\ adult\ vial\ test,\ method:\ IRAC\ 011\ version\ 3)$

San	nple		PRI	LC_{50}	95% c.l.	RR	LC_{90}	95% c.l.	RR
No.	locality (district, state)	date	Ь	(g a.i./ha) ^a	7570 C.I.	(LC ₅₀)	(g a.i./ha) ^b	7570 C.I.	(LC ₉₀)
1	Klobouky u Brna (BV, CZ)	23.4.	5	11.96	4.65 - 53.58	234.53	69.72	23.13-4686.95	116.40
2	Rapotín (SU, CZ)	25.4.	5	9.75	6.71-14.55	191.14	41.51	25.23-96.77	69.29
3	Pohoř Odry (NJ, CZ)	2.5.	5	XXX			XXX		
4	Odry (NJ, CZ)	2.5.	5	6.56	3.31-15.09	128.53	36.44	15.66-264.36	60.83
5	Nekoř-Bredůvka (ÚO, CZ)	4.5.	4	4.31	2.27 - 7.81	84.55	37.69	17.56-178.75	62.92
6	Hradec Králové (HK, CZ)	4.5.	4	2.22	1.38 - 3.37	43.61	16.28	9.44-39.94	27.18
7	Náchod (NA, CZ)	4.5.	4	3.03	1.87 - 4.95	59.47	14.60	8.23-39.07	24.37
8	Trutnov I (TU, CZ)	9.5.	4	3.94	2.17 - 7.41	77.24	31.56	14.50-142.14	52.68
9	Nové Sady (NR, SK)	10.5.	4	1.59	1.09-2.22	31.24	10.35	6.81-18.83	17.28
10	Velké Kostolany (PN, SK)	9.5.	3	0.77	0.30 - 1.43	15.16	10.32	4.91-45.22	17.23
11	Prašice (TO, SK)	10.5.	3	1.34	0.70 - 2.29	26.24	6.71	3.69-19.77	11.21
12	Libichava (BN, SK)	10.5.	3	0.54	0.19-0.99	10.67	8.91	4.33-38.72	14.87
13	Budmerice (PK, SK)	9.5.	3	1.23	0.88 - 1.67	24.02	4.56	3.14-7.98	7.62
14	Jablonica (SE, SK)	10.5.	3	0.92	0.63-1.26	17.96	4.16	2.80-7.61	6.94
15	Plavecké Podhradie (MA, SK)	10.5.	4	1.67	1.00-2.61	32.69	7.82	4.63-19.50	13.06
16	Báhoň (PK, SK)	9.5.	4	1.67	1.15 - 2.43	32.75	11.04	6.52-25.91	18.44
17	Slovenský Grob (PK, SK)	9.5.	3	2.62	1.47 - 4.40	51.27	11.83	6.63-33.17	19.75
18	Turá Lúka (MY, SK)	10.5.	3	1.25	0.87 - 1.76	24.45	5.11	3.33-10.16	8.52
22	Senice na Hané (OL, CZ)	11.5.	4	3.72	2.57-5.36	73.02	20.28	12.64-41.86	33.85
23	Moravská Třebová (SY, CZ)	11.5.	4	2.63	1.41-4.58	51.51	51.60	22.89-215.76	86.15
24	Litomyšl (SY, CZ)	11.5.	4	2.21	1.36-3.27	43.41	9.05	5.69-21.16	15.10
25	Mikulovice u Jeseníku (JE, CZ)	18.5.	4	3.06	1.56-5.78	60.06	20.71	9.89-89.29	34.57
26	Javorník (JE, CZ)	18.5.	4	5.35	3.12-9.22	104.86	24.67	13.34-77.52	41.19
27	Lichkov (ÚO, CZ)	18.5.	4	2.41	1.51 - 3.72	47.20	21.83	12.26-53.10	36.44
29	Budín u Nechanic (HK, CZ)	25.5.	4	4.87	3.33-7.09	95.51	17.48	11.21-36.60	29.18
30	Hněvčeves (HK, CZ)	25.5.	4	3.31	1.64-6.57	64.94	25.70	11.43-141.77	42.91
31	Libina (SU, CZ)	29.5.	4	2.84	1.78-4.43	55.61	26.23	14.37-67.93	43.79
32	Chorušice (ME, CZ)	29.5.	4	2.46	1.46-3.99	48.20	29.27	14.81-92.24	48.86
33	Český Dub (LB, CZ)	29.5.	4	3.46	1.58-6.90	67.78	53.49	21.09-380.88	89.30
34	Brniště (CL, CZ)	30.5.	4	3.64	2.08-6.21	71.33	22.67	11.90-72.18	37.84
35	Markvartice (DC, CZ)	30.5.	5	4.08	1.69-11.17	80.00	29.60	10.90-419.45	49.42
36	Hrádek nad Nisou (LB, CZ)	30.5.	4	XXX			XXX		
37	Kylešovice (OP, CZ)	3.5.	5	XXX			XXX		
38	Krnov (OP, CZ)	8.5.	5	13.33	8.94-21.50	261.31	104.42	53.85-318.79	174.33
39	Hranice (PR, CZ)	10.5.		6.48	3.36-10.24	127.02	29.48	16.49-152.46	49.22
40	Hodslavice (NJ, CZ)	10.5.	5	10.26	6.18-21.10	201.14	33.28	17.28-161.91	55.57
41	Nošovice (FM, CZ)	11.5.		1.68	1.24-2.31	32.84	4.36	3.01-8.43	7.28
42	Markvartovice (OV, CZ)	15.5.		XXX			XXX		
43	Nové Heřminovy (BR, CZ)	17.5.		XXX			XXX		
45	Polička (SY, CZ)	6.6.		5.27	3.14-9.29	103.24	41.75	20.18-156.58	69.70
46	Petrovice (HB, CZ)	6.6.		5.17	3.10-9.02	101.39	123.21	49.88-599.31	205.68
47	Mošnov (OV, CZ)	12.6.		4.67	2.60-7.52	91.55	25.89	14.24-91.08	43.23

Table 1 to be continued

San	nple			LC ₅₀		RR	LC ₉₀		RR
	locality (district, state)	date	PRI	(g a.i./ha) ^a	95% c.l.	(LC ₅₀)	(g a.i./ha) ^b	95% c.l.	(LC_{90})
48	Tismice (KO, CZ)	22.4.	5	5.24	2.24-11.86	102.65	44.87	17.52-613.41	74.91
49	Nebovidy (KO, CZ)	27.4.	5	20.94	12.72-43.64	410.67	174.98	72.26-1082.07	292.11
50	Kasejovice (PJ, CZ)	28.4.	4	0.85	0.46 - 1.38	16.73	6.80	3.76-19.18	11.35
51	Želeč (TA, CZ)	29.4.	4	XXX			XXX		
52	Příkosice (RO, CZ)	29.4.	4	1.97	1.06-3.17	38.65	11.77	6.67-33.58	19.65
53	Křivsoudov (BN, CZ)	29.4.	5	9.46	5.22-20.85	185.47	188.74	62.97-1713.21	315.10
54	Kladruby (RO, CZ)	29.4.	4	1.99	1.05 - 3.47	38.96	27.03	12.49-112.83	45.12
55	Zalužany (PB, CZ)	30.4.	4	4.50	2.33-8.81	88.31	18.36	9.28-82.55	30.65
56	Kestřany (PI, CZ)	30.4.	4	3.75	2.77 - 5.07	73.49	9.30	6.61-16.61	15.53
57	Koloveč (DO, CZ)	30.4.	4	2.32	0.67 - 6.15	45.49	294.87	52.58-51890.80	492.27
58	Libčany (HK, CZ)	30.4.	5	11.72	8.48-16.11	229.71	26.76	18.84-54.48	44.68
59	Kluky (PI, CZ)	30.4.	4	4.60	3.32-6.39	90.16	13.39	9.09-26.08	22.35
60	Zubčice (CK, CZ)	25.6.	3	0.80	0.45 - 1.26	15.59	5.90	3.33-15.90	9.84
61	Deštná (JH, CZ)	30.4.	4	3.65	2.36-5.58	71.51	12.97	8.01-30.24	21.65
62	Vranovice u Rožmitálu p. Třemšínem (PB, CZ)	30.4.	3	1.64	1.10-2.42	32.06	7.75	4.76–17.02	12.93
63	Neveklov (BN, CZ)	30.4.	4	2.84	1.47 - 5.63	55.71	18.44	8.48-91.00	30.78
64	Dříteň (CB, CZ)	30.4.	3	1.03	0.60-1.63	20.25	6.60	3.77-17.31	11.02
65	Zaloňov (NA, CZ)	1.5.	5	7.34	5.20-10.71	143.92	27.92	17.53-60.90	46.62
66	Borovany (CB, CZ)	1.5.	3	2.85	2.09 - 3.89	55.84	7.40	5.18-13.66	12.35
67	Dřínov (ME, CZ)	11.6.	4	3.86	2.42 - 6.05	75.63	13.00	7.92-32.50	21.71
79	Troubsko (BO, CZ)	23.4.	5	11.78	4.50-77.03	230.92	713.53	97.36-556473.03	1191.20
80	Dolní Dunajovice (BV, CZ)	23.4.	4	1.50	0.70-2.74	29.49	27.56	11.97-136.76	46.01
81	Velké Němčice (BV, CZ)	23.4.	4	7.56	2.50-50.73	148.22	373.90	53.98-655360.23	624.20
82	Syrovice (BO, CZ)	23.4.	5	14.26	7.78-34.73	279.57	269.08	84.36-3015.96	449.22
83	Smolín (BO, CZ)	23.4.	5	7.38	2.61-39.55	144.67	190.16	36.75-74677.56	317.45
84	Lednice (BV, CZ)	23.4.	4	3.08	1.82 - 5.22	60.35	36.40	17.41-129.53	60.77
85	Vitonice (ZN, CZ)	27.4.	5	10.21	4.66 - 35.42	200.27	655.66	118.87-50483.65	1094.59
86	Tvořihráz (ZN, CZ)	27.4.	5	68.31	21.23-1894.85	1339.41	6249.71	468.46-37784705.80	10433.57
87	Suchohrdly (ZN, CZ)	27.4.	4	7.91	4.02 - 19.53	155.00	270.97	72.80-4867.60	452.37
88	Hatě (ZN, CZ)	27.4.	4	4.02	2.33 - 7.10	78.75	56.48	24.96-243.47	94.28
89	Rokytnice n. Rokytnou (TR, CZ)	27.4.	4	4.14	2.08 - 8.40	81.25	103.86	35.74-950.14	173.40
90	Krahulov (TR, CZ)	30.4.	4	1.07	0.46 - 1.98	21.00	23.74	10.17-123.21	39.63
91	Újezd u Černé Hory (BK, CZ)	30.4.	4	5.32	2.47 - 13.43	104.25	289.33	67.54-9129.11	483.03
92	Mysletice (JI, CZ)	30.4.	4	3.32	1.57-6.76	65.10	58.46	21.88-461.31	97.60
93	Studenec (TR, CZ)	30.4.	5	7.17	3.43-19.42	140.67	359.01	81.52-11936.66	599.34
94	Vysoké Popovice (BO, CZ)	30.4.	4	5.73	2.49 - 15.94	112.25	144.65	38.29-4534.26	241.49
95	Telč (JI, CZ)	30.4.	4	1.91	0.84 - 3.74	37.43	25.15	10.48-161.76	41.98
96	Popovice u Rajhradu I (BO, CZ)	22.4.	4	10.07	6.48-16.58	197.53	70.47	36.47-225.96	117.65
97	Brno Chrlice (BM, CZ)	1.5.	4	3.86	1.83 - 8.54	75.75	164.77	46.41-2682.71	275.08
98	Radvanice, Zaječí Důl I (PR, CZ)	8.5.	5	20.72	9.17-98.22	406.24	1081.20	178.63-115421.99	1805.01
99	Brno Tuřany (BM, CZ)	16.5.	4	1.85	1.08 - 3.02	36.27	18.89	9.72-58.94	31.54
100	Bystřice n. Perštejnem- Lesoňovice (ZR, CZ)	20.5.	5	XXX			XXX		

Table 1 to be continued

Sample		PRI	LC ₅₀	95% c.l.	RR	LC ₉₀	95% c.l.	RR
No. locality (district, state)	date	Ы	(g a.i./ha)a	95% C.I.	(LC_{50})	(g a.i./ĥa) ^b	95% C.I.	(LC_{90})
101 Tišnov (BO, CZ)	23.5.	5	15.55	6.56-80.60	304.96	577.59	100.99-86886.38	964.25
102 Horní Rozsíčka (ZR, CZ)	30.5.	5	13.70	6.32-50.74	268.67	733.42	135.76-49634.24	1224.41
103 Rozsochy (ZR, CZ)	3.6.	4	2.13	1.10 - 3.83	41.71	40.39	17.15-197.72	67.43
104 Krhov (TR, CZ)	6.6.	4	2.52	1.40 - 4.46	49.47	40.36	17.61-183.07	67.37
105 Bučovice (VY, CZ)	10.6.	4	6.17	3.64-10.94	120.90	30.73	15.97-107.54	51.31
106 Kojetín (PR, CZ)	25.6.	4	8.40	3.70-27.60	164.69	177.79	44.93-3123.56	296.81
107 Vranovice-Kelčice (PV, CZ)	2.7.	4	5.30	3.40 - 8.42	103.96	38.29	20.84-103.46	63.92
108 Radvanice, Zaječí Důl II (PR, CZ)	8.7.	5	8.67	5.33-15.56	169.90	84.45	38.63-336.83	140.99
109 Popovice u Rajhradu II (BO, CZ)	15.7.	4	0.86	0.10-2.37	16.88	29.79	8.36-1904.03	49.73
Means		4.17	5.83		114.46	173.21		289.17

PRI – Pyrethroid Resistance Index (1–5) stated according to method IRAC 011 v. 3; c.l. – confidence limits; at he lowest LC $_{50}$ recorded during the five years lasting monitoring (2009–2013) served as a base for RR (LC $_{50}$) calculations; minimal LC $_{50}$ (2009–2013) = 0.051 g a.i./ha (95% c. limits = 0.00–0.21 g a.i/ha); population No. 36_{2010} ; he lowest LC $_{90}$ recorded during the five years lasting monitoring (2009–2013) served as a base for RR (LC $_{90}$) calculations; minimal LC $_{90}$ (2009–2013) = 0.599 g a.i./ha (95% c. limits = 0.41–1.56 g a.i./ha); populations No. 90, 92, 93, 94, and 108 collected in 2009; RR – resistance ratio was not possible to estimate correctly the LC $_{50}$ and LC $_{90}$ values for the populations No. 3, 36, 37, 42, 43, 51, and 100

2012) were collected mainly from winter oilseed rape fields (in some cases spring rape, white mustard and poppy fields were used, too) in the course of April, May, June, and July 2012 and 2013 (Figures 1 and 2; Tables 1 and 2). Each of the *Meligethes* samples was tested with lambda-cyhalothrin; a total of 93 populations in 2012 and 82 populations in 2013 were tested and compared. Lambda-cyhalothrin (analytical standard; batch number: HUD6A 3514) was obtained from Syngenta Czech Ltd. (Prague, Czech Republic).

The 'adult vial test' recommended by the IRAC was used for testing (IRAC method No. 011 v. 3; www.iraconline.org). The inner surfaces of the glass vials (all with the same inside area: 37.97cm²; P-Lab, Prague, Czech Republic) were coated with different concentrations of lambda-cyhalothrin. One ml of solution for each concentration was used per vial. Vials with the solutions were rotated on a roller mixer at room temperature until the acetone completely evaporated. At the start of the coating process, the inner surface of the vials was completely covered with the solution. Five concentrations were prepared per test: 0 g a.i./ha = untreated control; 0.3 g a.i./ha (= 0.003 µg a.i./cm²); $1.5 \text{ g a.i./ha} (= 0.015 \,\mu\text{g a.i./cm}^2); 7.5 \,\text{g a.i./ha} (= 0.075 \,\mu\text{g})$ a.i./cm²), and 37.5 g a.i./ha (= $0.375 \mu g a.i./cm^2$). The rate 7.5 g a.i./ha (= $0.075 \mu g a.i./cm^2$) is the European (and also Czech) lambda-cyhalothrin registered dose (= 100% field rate). For every sample three replicates were used for each tested concentration. Ten (8–12) adult pollen beetles were placed in each vial. The vials with beetles were stored in constant environment facilities at $18 \pm 2^{\circ}\text{C}$ and 16:8 h light: dark. After 24 h the beetles were tipped out of the vials and scored on filter discs. Insects incapable of coordinated movement (IRAC, www.irac-online.org) were scored as dead for computing mortality levels and LC_{50-90} values.

To test significant differences in mean percentage mortalities among the compared *Meligethes* samples induced by the three concentrations tested (0.015, 0.075, and 0.375 μ g a.i./cm²), the analysis of variance (ANOVA) and appropriate post-test (Tukey's test) were performed. On the basis of mortality recorded in control vials (0 g (μ g) a.i./cm²) all mortality figures were corrected according to Abbott's formula (Abbott 1925). The samples in which the level of mortality in untreated controls exceeded 10% were excluded from assessments. In most of the compared samples (86 and 75 in 2012 and 2013 respectively) the level of mortality was zero. The statistical analysis was performed with Statistica v. 10 software (StatSoft, Inc. 1984–2013).

On the basis of the recorded mean percentage mortalities induced by the concentrations 0.015 and 0.075 μg a.i./cm² Pyrethroid Resistance Indices (PRI) for individual samples were calculated (more detailed description in Table 3).

The LC $_{\rm 50-90}$ values were estimated by Probit analysis using Polo Plus software Version 2 (LeOra Software,

Table 2. Susceptibility of pollen beetles from Czech Republic after 24 h exposure to lambda-cyhalothrin in 2013 (82 samples tested with an adult vial test, method: IRAC 011 version 3)

	nple		PRI	LC ₅₀	95% c.l.	RR	LC ₉₀	95% c.l.	RR
	locality (district)	date		(g a.i./ha) ^a		(LC ₅₀)	(g a.i./ha) ^b		(LC ₉₀)
2	Žitonice (LT)	13.5.	5	11.64	7.36–16.66	228.25	48.02	31.04–106.25	80.17
3	Trutnov (TU)	14.5.	4	4.13	2.74-5.33	80.98	8.55	6.63–12.87	14.28
4	Horka (OL)	15.5.	4	2.34	0.35–7.56	45.84	39.52	10.87–6169.78	65.97
5	Konice (PV)	14.5.	4	4.14	2.62-6.48	81.14	16.50	9.87-39.46	27.54
6	Moravská Třebová (SY)	15.5.	4	2.84	1.12-5.29	55.71	39.73	18.79–165.98	66.33
7	Velká Jesenice (NA)	15.5.	4	6.43	4.11–9.21	126.08	31.45	20.46-62.63	52.51
8	Ráby (PA)	15.5.	4	5.21	1.26-8.49	102.06	19.63	11.88–96.43	32.77
9	Litomyšl (SY)	15.5.	4	2.84	1.71 - 4.54	55.76	30.86	16.09-91.60	51.52
10	Rapotín (SU)	17.5.	5	13.24	7.82–26.72	259.61	116.61	49.11-640.53	194.67
11	Uničov (OL)	23.5.	4	6.78	4.90–9.16	132.84	22.66	15.69–40.55	37.83
12	Šternberk (OL)	23.5.	4	4.40	2.86–6.55	86.24	20.38	12.68-43.68	34.02
13	Rybná n. Zdobnicí (RK)	24.5.	4	4.18	3.06-5.71	81.92	11.21	7.80-20.84	18.72
14	Jičín (JC)	24.5.	5	5.80	2.29–16.03	113.63	31.50	12.37-423.04	52.59
15	Turnov (SM)	24.5.	4	2.02	1.14 - 3.20	39.67	14.62	8.50-34.70	24.41
16	Kralupy n. Vltavou (ME)	24.5.	4	5.53	2.44-11.46	108.35	26.69	12.60–147.12	44.55
17	Sadská (NB)	24.5.	4	XXX			XXX		
18	Troubsko (BO)	24.5.	4	0.98	0.11-2.91	19.25	16.77	4.92-2027.36	28.00
19	Přibyslavice (TR)	17.5.	4	4.25	1.86-10.46	83.41	59.38	20.00-792.82	99.14
20	Řehořov (JI)	17.5.	4	2.04	1.22 - 3.32	39.98	20.11	10.34-62.11	33.57
21	Nové Veselí (ZR)	17.5.	4	1.72	0.86-3.19	33.75	15.11	7.04-68.45	25.22
22	Lavičky (ZR)	17.5.	4	0.96	0.47 - 1.65	18.86	13.02	6.42-46.85	21.73
23	Nové Sady (ZR)	17.5.	5	11.26	5.69-28.19	220.75	79.31	30.77-813.95	132.40
24	Zahradiště (ZR)	17.5.	4	1.65	0.41 - 4.23	32.27	44.75	13.06-1452.14	74.71
25	Zakřany (BO)	6.5.	4	1.65	0.85 - 3.08	32.41	11.30	5.42-49.66	18.87
26	Čechočovice (TR)	6.5.	4	4.45	2.12-10.04	87.24	40.50	15.88-325.56	67.61
27	Ivančice (BO)	6.5.	4	2.32	1.59-3.43	45.57	11.29	6.91-24.36	18.84
28	Hory (TR)	6.5.	4	0.79	0.31-1.40	15.53	11.92	5.86-43.16	19.91
29	Dukovany (TR)	6.5.	4	7.33	3.23-21.44	143.67	125.57	35.75-3454.20	209.63
30	Náměšť n. Oslavou (TR)	6.5.	4	2.02	0.44 - 6.57	39.65	50.41	12.18-9750.40	84.16
31	Slavičky (TR)	6.5.	4	1.94	1.18-3.16	37.96	8.80	4.97-24.95	14.68
32	Moravský Krumlov (ZN)	30.4.	4	2.17	1.35 - 3.48	42.63	8.51	4.99-23.10	14.21
33	Perna (BV)	30.4.	4	2.40	1.57 - 3.67	46.98	9.03	5.49-21.66	15.07
34	Znojmo (ZN)	30.4.	4	2.13	2.46 - 3.13	41.82	9.41	5.86-20.08	15.70
35	Novosedly (BV)	30.4.	3	0.52	0.07 - 1.24	10.27	5.55	2.15-116.83	9.26
36	Úvaly (BV)	30.4.	4	2.38	0.61 - 7.04	46.69	86.87	20.27-10207.59	145.03
37	Skalice (ZN)	30.4.	4	4.33	3.06-6.16	84.90	15.07	9.83-30.85	25.16
38	Starovice (BV)	30.4.	4	0.73	0.13-1.77	14.22	23.45	7.53-558.63	39.15
39	Bernartice (JE)	12.7.	4	5.68	2.59-13.21	111.41	21.80	10.20-177.29	36.39
40	Uhelná (JE)	12.7.	3	2.85	1.56-5.52	55.88	8.17	4.49-38.13	13.64
41	Vikýřovice (SU)	12.7.	4	XXX			XXX		
42	Trutnov II (TU)	16.7.	4	5.02	3.73-6.44	98.33	12.10	9.09-20.32	20.19
46	Bravantice (NJ)	1.7.	5	13.78	3.39-28.56	270.14	86.25	37.80-1075.70	143.99
47	Mošnov II (NJ)	1.7.	5	7.44	5.71-9.77	145.94	21.30	15.17-36.93	35.56
48	Sosnová I (OP)	24.6.	4	0.99	0.42 - 1.82	19.45	8.20	3.98-34.61	13.69
49	Bohušov u Osoblahy I (BR)	24.6.	5	9.32	4.26-31.38	182.67	52.09	19.16-1432.10	86.95

Table 1 to be continued

Sar	nple			LC ₅₀	050/ 1	RR	LC ₉₀	050/ 1	RR
No	locality (district)	date	PRI	(g a.i./ha) ^a	95% c.l.	$(LC_{50})^{c}$	(g a.i./ha) ^b	95% c.l.	$(LC_{90})^{c}$
50	Valašské Meziříčí (VS)	29.5.	4	3.25	1.81-5.72	63.75	24.94	12.21-98.47	41.64
51	Bohušov u Osoblahy II (BR)	27.5.	5	XXX			XXX		
52	Šilheřovice (OP)	26.5.	5	6.75	4.50-9.44	132.43	19.15	12.99-41.53	31.97
53	Sosnová II (OP)	23.5.	3	2.21	1.57-3.03	43.29	6.52	4.52 - 11.87	10.89
54	Rudná p. Pradědem (BR)	22.5.	4	4.26	3.23-5.45	83.55	8.61	6.60-13.02	14.37
55	Mošnov I (NJ)	20.5.	5	8.98	3.25-47.17	176.02	70.24	20.22-9658.15	117.26
56	Rohov (OP)	15.5.	4	XXX			XXX		
57	Vítkov (OP)	14.5.	5	9.02	4.03-28.12	176.78	49.49	18.64-946.62	82.62
58	Hlinsko p. Hostýnem (KM)	10.5.	4	2.30	1.02-4.26	45.08	11.14	5.71-49.84	18.60
59	Zlín (ZL)	10.5.	4	5.60	1.77 - 14.92	109.86	23.50	10.07-481.50	39.23
60	Bubovice (PB)	6.5.	4	4.12	2.47-6.95	80.78	13.86	7.96-45.71	23.13
61	České Budějovice (CB)	8.5.	4	4.92	3.31-7.51	96.55	26.05	15.23-62.41	43.49
62	Hospříz (JH)	20.5.	4	7.85	5.35-11.50	153.90	33.91	21.11-74.04	56.61
63	Kasejovice (PJ)	9.5.	4	2.72	1.72-4.31	53.31	21.07	11.42-57.63	35.18
64	Kestřany (PI)	28.4.	4	XXX			XXX		
65	Kladruby (RO)	6.5.	4	3.06	1.73-5.408	59.94	46.28	20.19-209.78	77.26
66	Kluky (PI)	28.4.	5	XXX			XXX		
67	Koloveč (DO)	15.5.	4	6.01	2.65-17.09	117.82	237.08	55.24-9439.78	395.78
68	Kožlí (HB)	15.5.	3	0.65	0.32 - 1.08	12.71	6.38	3.40-20.12	10.64
69	Křivsoudov (BN)	13.5.	5	12.35	8.09-20.12	242.08	74.26	39.40-235.11	123.96
70	Malšice (TA)	12.5.	5	XXX			XXX		
71	Nebovidy (KO)	6.5.	5	XXX			XXX		
72	Neveklov (BN)	28.4.	4	4.90	2.81-8.66	96.00	24.65	12.90-83.24	41.15
73	Nová Včelnice (JH)	13.5.	4	XXX			XXX		
74	Pluhův Žďár (JH)	20.5.	5	XXX			XXX		
75	Příkosice (RO)	6.5.	3	1.54	1.09-2.20	30.27	5.38	3.51-10.95	8.98
76	Tismice (KO)	30.4.	5	64.47	15.32-24763.86	1264.20	30760.21	776.78-0.00	51352.61
77	Želeč (TA)	12.5.	4	XXX			XXX		
89	Brno-Mokrá Hora (BO)	23.4.	4	3.01	1.23-5.85	58.96	21.42	9.85-143.62	35.76
90	Žabčice (BO)	29.4.	5	8.06	4.66 - 13.72	158.08	56.81	28.43-232.19	94.83
91	Rajhrad (BO)	1.5.	5	5.01	2.32-9.95	98.14	38.53	16.87-298.46	64.32
93	Česká Třebová-Semanín (UO)	12.5.	5	8.96	4.29 - 22.57	175.71	205.64	58.63-4970.81	343.31
94	Rosice u Brna (BO)	19.5.	5	7.02	3.39-15.92	137.57	139.94	44.69-2184.03	233.62
95	Radvanice (PR)	19.5.	4	6.82	2.79-18.87	133.78	141.79	39.05-6213.83	236.71
96	Vyškov (VY)	2.6.	4	5.03	1.98-14.38	98.67	344.50	67.65-32836.63	575.12
97	Popovice (BO)	9.6.	3	1.51	1.01-2.25	29.67	7.30	4.45-16.40	12.18
98	Bystřice p. Pernštejnem (ZR)	15.7.	5	XXX			XXX		
Me	ans		4.2	5.50		107.84	482.47		805.45

PRI – Pyrethroid Resistance Index (1–5) stated according to method IRAC 011 v. 3; c.l. – confidence limits; athe lowest LC recorded during the five years lasting monitoring (2009–2013) served as a base for RR (LC so) calculations; minimal LC (2009–2013) = 0.051 g a.i./ha (95% confidence limits = 0.00–0.21 g a.i./ha); population No. 36_{2010} ; bthe lowest LC recorded during the five years lasting monitoring (2009–2013) served as a base for RR (LC so) calculations; minimal LC (2009–2013) = 0.599 g a.i./ha (95% confidence limits = 0.41–1.56 g a.i./ha); populations No. 90, 92, 93, 94, and 108 collected in 2009; RR – resistance ratio was not possible to estimate correctly the LC so and LC so values for the populations No. 17, 41, 51, 56, 64, 66, 70, 71, 73, 74, 77, and 98

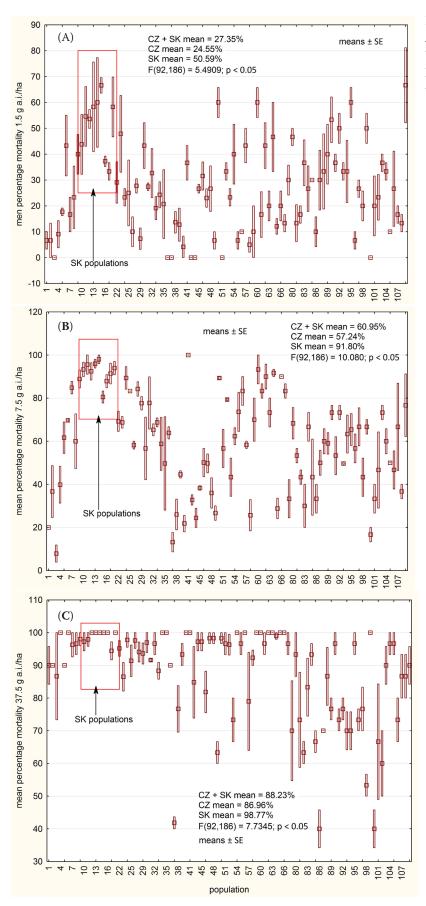


Figure 3. Mean percentage mortalities of *Meligethes* (collected in 2012) induced by 1.5 g (A), 7.5 g (B) and 37.5 g (C) of lambda-cyhalothrin per ha (SK populations = Slovak populations)

Table 3. Classification of *Meligethes* populations by pyrethroid resistance indices (PRI) assigned according to method IRAC 011 v. 3. Not only 2012 and 2013 *Meligethes* collections are compared in the table. Classifications of Czech *Meligethes* collections from 2009 (111 populations tested), 2010 (125 populations tested), and 2011 (102 populations tested) are also included in the table (CZ = Czech Republic; SK = Slovakia)

Insecticide	C	Portion of populations with certain PRI (%)							
insecticide	Season	1	2	3	4	5			
	2009	11.71	18.02	25.23	33.33	11.71			
	2010	4.00	22.40	21.60	43.20	8.80			
	2011	0.00	3.92	13.73	61.77	20.59			
Lambda-cyhalothrin	2012 (CZ+SK)	0.00	1.08	11.83	55.91	31.18			
	2012 (CZ)	0.00	1.21	4.82	59.04	34.94			
	2012 (SK)	0.00	0.00	70.00	30.00	0.00			
	2013	0.00	0.00	7.32	65.85	26.83			

PRI 1 – highly susceptible population (mean percentage mortalities induced with the both rates of 7.5 and 1.5 g a.i./ha are 100% according to Abbott's formula); PRI 2 – susceptible population (mean percentage mortality induced with 7.5 g a.i./ha is 100%, mean percentage mortality induced with the rate of 1.5 g a.i./ha is lower); PRI 3 – moderately resistant population (mean percentage mortality induced with 7.5 g a.i./ha ranges between 90–99.99%); PRI 4 – resistant population (mean percentage mortality induced with 7.5 g a.i./ha ranges between 50–89.99%); PRI 5 – highly resistant population (mean percentage mortality induced with 7.5 g a.i./ha is below 50% according to Abbott's formula)

Berkeley, USA). Zero mortalities were recorded in the populations No. 3, 36, 37, 42, 43, 51, and 100 (assemblage collected in 2012) and in the populations No. 17, 41, 51, 56, 64, 66, 70, 71, 73, 74, 77, and 98 (assemblage from 2013) after their exposure to the two lowest concentrations tested (0.003 and 0.015 μg a.i./cm²). So, that was not possible to estimate correctly the LC $_{50}$ and LC $_{90}$ values for the populations. The populations were excluded from all LC $_{50}$ and LC $_{90}$ calculations and comparisons.

Resistance ratio (RR) calculations are related to the lowest LC_{50} and LC_{90} values which have been recorded in *Meligethes* collections from the beginning of monitoring in 2009 (it still continues). The lowest LC_{50} was recorded in the Czech collection from 2010 (0.051 g a.i./ha) and the lowest LD_{90} was recorded in the Czech collection from 2009 (0.599 g a.i./ha).

RESULTS

In 2012, no highly susceptible *Meligethes* populations were recorded (PRI 1) in the collection and the proportion of susceptible populations (PRI 2) was negligible (1.08%, which is one population out of 93 populations tested). Resistant populations (PRI 4) predominated. The collection contained a great proportion of highly resistant populations (PRI 5), too (Tables 1 and 3). The mean percentage mortality

induced by a concentration equivalent to the common European field rate (7.5 g a.i./ha) recorded for the whole assemblage was 60.95% (expressed according to Abbott's formula). The effects of this concentration on individual populations varied significantly ($F_{92,186}$ = 10.080, P = 0.0000). The variability was relatively high, mortality ranged from 7.87% to 100% in the assemblage. The Slovak (SK) populations showed markedly higher susceptibility (and also lower variability) in comparison with the Czech (CZ) ones (Figure 3B). Concentration equivalent to a fifth of the common field rate (1.5 g a.i./ha) showed high variability in its effects on individual populations, too ($F_{92,186}$ = 5.4909, P = 0.000). The mean percentage mortalities ranged from 0.00% to 66.67% within the collection (CZ + SK mean: 27.35%). Again, in many cases the effects of the concentration on the Slovak populations were significantly higher than on the Czech ones (Figure 3A). Concentration equivalent to the rate of 37.5 g a.i./ha showed somewhat more stable effects in comparison with the lower concentrations. However, there were several populations with very low susceptibility even to the highest tested concentration (Figure 3C). The LC₅₀ values often exceeded the European field rate (22.09% of populations in the collection). 98.84% of populations in the assemblage exceeded the RR LC_{50} value of 15, 87.21% the value of 30, 68.61% the value of 50, and 37.21% of populations exceeded the RR LC_{50} value of 100. In the Slovak part

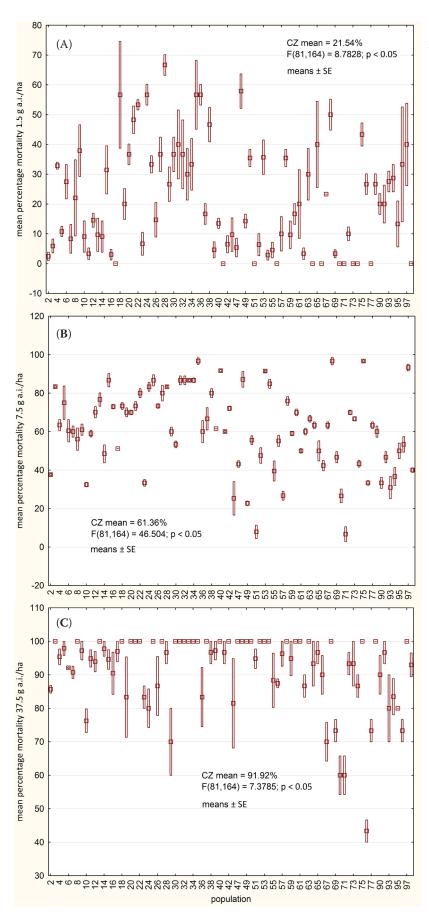


Figure 4. Mean percentage mortalities of *Meligethes* (collected in 2013) induced by 1.5 g (A), 7.5 g (B) and 37.5 g (C) of lambda-cyhalothrin per ha

of the assemblage 10% of populations exceeded the RR LC $_{50}$ value of 50 and no population exceeded the RR LC $_{50}$ value of 100. Only in 10.47% of populations in the assemblage the LC $_{90}$ values were below the European field rate and 44.19% of populations exceeded the RR LC $_{90}$ value of 50 (Table 1).

In 2013 only Czech populations were tested. Mainly resistant (PRI 4; 65.85%) and highly resistant (PRI 5; 26.83%) populations were recorded. The collection also contained a relatively small proportion of moderately resistant populations (PRI 3). Highly susceptible (PRI 1) and susceptible (PRI 2) populations were absent (Tables 2 and 3). Effects of a concentration equivalent to the common European field rate (7.5 g a.i./ha) on the individual populations varied significantly ($F_{81,164}$ = 46.504, P = 0.000). Mean percentage mortalities induced by this concentration ranged from 6.67% to 96.67% in the assemblage. The total average stated for all Czech populations in 2013 (61.36%) was a bit higher than it was in 2012 (57.24%) (Figures 4B and 3B). The effects of the concentration equivalent to a fifth of the common field rate (1.5 g a.i./ha) also significantly varied in many cases ($F_{81, 164} = 8.7828$, P = 0.0000). Mean percentage mortalities ranged 0.00-66.7%. Total collection averages stated separately for the two years were very similar (2012 – 24.55%; 2013 – 21.54%) (Figures 3A and 4A). The concentration equivalent to the rate of 37.5 g a.i./ha induced higher and more stable effects in comparison with the lower concentrations tested. Surprisingly the frequency of highly insusceptible populations to this concentration decreased in the 2013 collection in comparison with the 2012 collection (Figures 4C and 3C). The LC_{50} values estimated for the populations exceeded the European field rate in many cases again (17.14% of populations in the collection). 95.71% of populations in the assemblage exceeded the RR LC₅₀ value of 15, 87.14% the value of 30, 65.71% the value of 50, 35.71% the value of 100, and 8.57% of populations exceeded the RR LC₅₀ value of 200. Five populations in the assemblage (7.14%) had LC_{90} values below the European field rate and in 41.43% of populations exceeded the RR LC $_{90}$ value of 50 (Table 2).

DISCUSSION

In general, the levels of resistance of Czech *Meligethes* populations to esteric pyrethroid lambda-cyhalothrin were very high in 2012 and 2013. Slovak populations seemed to be somewhat more susceptible (Figures

3A–C). Because in Germany the proportions of winter oilseed rape on arable land and intensity of the crop growing are similar to those in the Czech Republic and also because the development of Meligethes resistance is detailedly documented in Germany, it is easier to compare the situation in the two countries. On the basis of published data, which were obtained using the same methodology (ZIMMER & NAUEN 2011a; Heimbach & Müller 2013) it is possible to carefully conclude that the levels of Meligethes resistance to esteric pyrethroids were at a similar level in both countries in 2012 and 2013. However, the problem leading to the current situation progressed markedly faster in Germany. The results of pollen beetle monitoring showed a decline in susceptible populations in Germany since 2005 (HEIMBACH & MÜLLER 2013). At that time no monitoring was carried out and neither farmers nor field researchers had any suspicion of the existence of pollen beetle resistance in the Czech Republic (Seidenglanz et al. 2012). In Germany, the resistance increased very rapidly from year to year (Heimbach & Müller 2013). Since 2009, pollen beetle samples classified as highly resistant have been dominant there. The last populations classified as sensitive were detected in the central part of Germany in 2009 (HEIMBACH & MÜLLER 2013). In the Czech Republic the main changes occurred several years later (Table 3). The last sensitive populations were recorded here in 2012 (Table 3). In Germany resistant and highly resistant populations increased to 98.5% in 2011. There were 93.98 and 92.68% of resistant and highly resistant populations in the Czech Republic in 2012 and 2013, respectively (Table 3). The gradual rates of increase in frequencies of very insensitive individuals present in particular populations were apparently different during the period in both countries, but generally relatively fast. The current situation (2013) is highly unsatisfactory in both countries. According to a comprehensive European study of ZIMMER and NAUEN (2011a), which was also based on the same methodology, the mean LC₅₀ value estimated for included German *Meligethes* populations was 1.44 g a.i./ha in 2009/10 (17 German populations were compared, they were sampled in 2009 and 2010). Unfortunately, their collection included only two Czech Meligethes populations. Both of them were sampled in 2010 and showed significantly different levels of susceptibility to lambda-cyhalothrin (LC $_{50}$ = 0.37 and 3.69 g a.i./ha respectively). So, when we compare the current levels of resistance of the Czech Meligethes populations to esteric pyrethroids (stated

for the years 2012 and 2013 and presented in this paper) with the data published in ZIMMER and NAUEN (2011a) it is clear that the rate of deterioration is very fast.

Significant differences among many Czech populations in their susceptibility to the insecticide (Tables 1 and 2; Figures 3 and 4) were found although the sampling area was not so large when we work on the assumption that the resistance is a serious problem afflicting a substantial part of Europe (RICHARDSON 2008; ZLOF 2008). So not only great insensitivity to lambdacyhalothrin but also great variability in resistance levels to the insecticide were important characteristics of the Czech *Meligethes* populations in 2012 and 2013.

Even though the LC₉₀ means estimated for Czech Meligethes populations increased between 2012 and 2013, it is not possible to conclude that the situation in 2013 was worse than in 2012 because the dramatic increase was caused mainly by one population (No. 76). The situation changes when we exclude the most resistant extreme from the 2013 collection. In reality the frequency of very insensitive populations was higher in the assemblage sampled in 2012 compared to that of 2013. So, taking into account the overall unsatisfactory situation regarding Meligethes resistance to pyrethroids in the Czech Republic, the results from 2013 discussed here are probably the first ones since 2008 (Seidenglanz et al. 2011), when an interruption in the very fast continual decline in *Meligethes* susceptibility to pyrethroids was recorded.

In view of the current situation, essential changes to the whole system of pest management used in the Czech winter oil-seed rape fields are needed. Contemporary practices can result in the gradual loss of pollen beetle susceptibility to other insecticides (neonicotinoids are especially under threat). Other common insect pests (Ceutorhynchus spp., Phyllotreta spp.) can also acquire resistance (not only to pyrethroids) over the next few years, even though in 2013 the Czech populations of C. pallidactylus, C. obstrictus, and Phyllotreta spp. seemed to be fully susceptible to esteric pyrethroids (Seidenglanz et al. 2014). However, the first indications of reduced sensitivity of Ceutorhynchus obstrictus to acetamiprid (KOCOUREK et al. 2013b) and Phyllotreta spp. to thiacloprid (Seidenglanz et al. 2014) were recorded in the Czech Republic in 2013. In 2011 HEIMBACH and Müller (2013) encountered several C. obstrictus samples (all of them originated from the same locality of Birkenmoor, Schleswig-Holstein, northern Germany) which were significantly less sensitive to pyrethroids (not only to lambda-cyhalothrin, but also to etofenprox and tau-fluvalinate) than other samples from different parts of Germany. The same authors also recorded significant differences among the sensitivities of several C. pallidactylus samples. Recently, pyrethroid resistance (target site insensitivity) in cabbage stem flea beetles (Psylliodes chrysocephala) from northern Germany has also been confirmed (ZIMMER et al. 2014). According to HEIMBACH and MÜLLER (2013), the first resistant populations of the insect pest appeared in 2008 in a region near Schwerin (Mecklenburg-Western Pomerania). The resistance is currently limited to an area not far from the Baltic Sea coast in the middle of northern Germany, but major control problems can be supposed once the resistance expands.

Acknowledgement. The authors would like to thank to Eoghan O'Reilly for revision of the manuscript.

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 $\label{eq:Received May 7, 2014} Received May 7, 2014 \\ Accepted after corrections January 10, 2015 \\$

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