Research Article

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Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape

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Abstract

BACKGROUND: Pollen beetle, *Meligethes aeneus* F. (Coleoptera: Nitidulidae), is a major pest in European winter oilseed rape. Recently, control failures with pyrethroid insecticides commonly used to control this pest have been reported in many European countries. For resistance management purposes, the neonicotinoid insecticide thiacloprid was widely introduced as a new mode of action for pollen beetle control.

RESULTS: A number of pollen beetle populations collected in Germany, France, Austria, Great Britain, Sweden, Denmark, Finland, Poland, Czech Republic and Ukraine were tested for pyrethroid resistance using lambda-cyhalothrin-coated glass vials (adult vial test). Most of the populations tested exhibited substantial levels of resistance to lambda-cyhalothrin, and resistance ratios ranged from <10 to >2000. A similar resistance monitoring bioassay for the neonicotinoid insecticide thiacloprid was developed and validated by assessing baseline susceptibility data for 88 European pollen beetle populations. A variation of less than fivefold in response to thiacloprid was detected. The thiacloprid adult vial bioassay is based on glass vials coated with an oil-dispersion-based formulation of thiacloprid, resulting in a much better bioavailability compared with technical material. Analytical measurements revealed a >56 and 28 day stability of thiacloprid and lambda-cyhalothrin in coated glass vials at room temperature, respectively. No cross-resistance between thiacloprid and lambda-cyhalothrin based on log-dose probit–mortality data was detected.

CONCLUSION: Pyrethroid resistance in many European populations of *M. aeneus* was confirmed, whereas all populations are susceptible to thiacloprid when tested in a newly designed and validated monitoring bioassay based on glass vials coated with oil-dispersion-formulated thiacloprid. Based on the homogeneous results, it is concluded that thiacloprid could be an important chemical tool for pollen beetle resistance management strategies in European winter oilseed rape.

Keywords: pollen beetle; pyrethroid resistance; thiacloprid baseline; resistance monitoring; oilseed rape; *Meligethes aeneus*

1 INTRODUCTION

Winter oilseed rape is one of the most important crops in several European countries. The four main oilseed-rape-growing countries in Europe are France (1.58 million ha in 2009), Germany (1.55 million ha), Poland (0.8 million ha) and the United Kingdom (0.68 million ha).[1] The pollen beetle, *Meligethes aeneus* F. (Coleoptera: Nitidulidae), is one of the major pests in European oilseed rape and known to be quite destructive once infestation thresholds are exceeded and no chemical control measures are taken.[2] After emerging from overwintering sites, adults start to infest oilseed rape plants in mid-March until May and can damage the flowering parts by feeding and oviposition. In particular, feeding larvae cause bud abscission. The consequences of these infestations are podless stalks and dramatically reduced yields, so the farmers need to control pollen beetles to keep numbers low and to avoid economic damage. All over Europe, pyrethroid insecticides have a long history in pollen beetle control.[3] In many countries the common practice is more than one insecticide application against pollen beetle per season.[4] The requirement for control and the limited availability of compounds from other chemical classes have conspired, resulting in intense selection pressures being imposed by pyrethroid insecticides.[5] In 2005 almost 100% of all insecticide applications in oilseed rape in Germany involved pyrethroid insecticides.[3,6]

The first case of reduced susceptibility of pollen beetle to pyrethroids was reported in 1999 in the Champagne region in north-eastern France.[7,8] Confirmed cases of pyrethroid resistance in Germany were documented in 2002, and in 2006 more than 50% of the winter oilseed rape acreage in Germany was affected.[3] First cases of pyrethroid resistance in Denmark were described in 2000 and 2001 and confirmed in a larger study in 2003.[7,8] Since then, pyrethroid resistance data from several other countries

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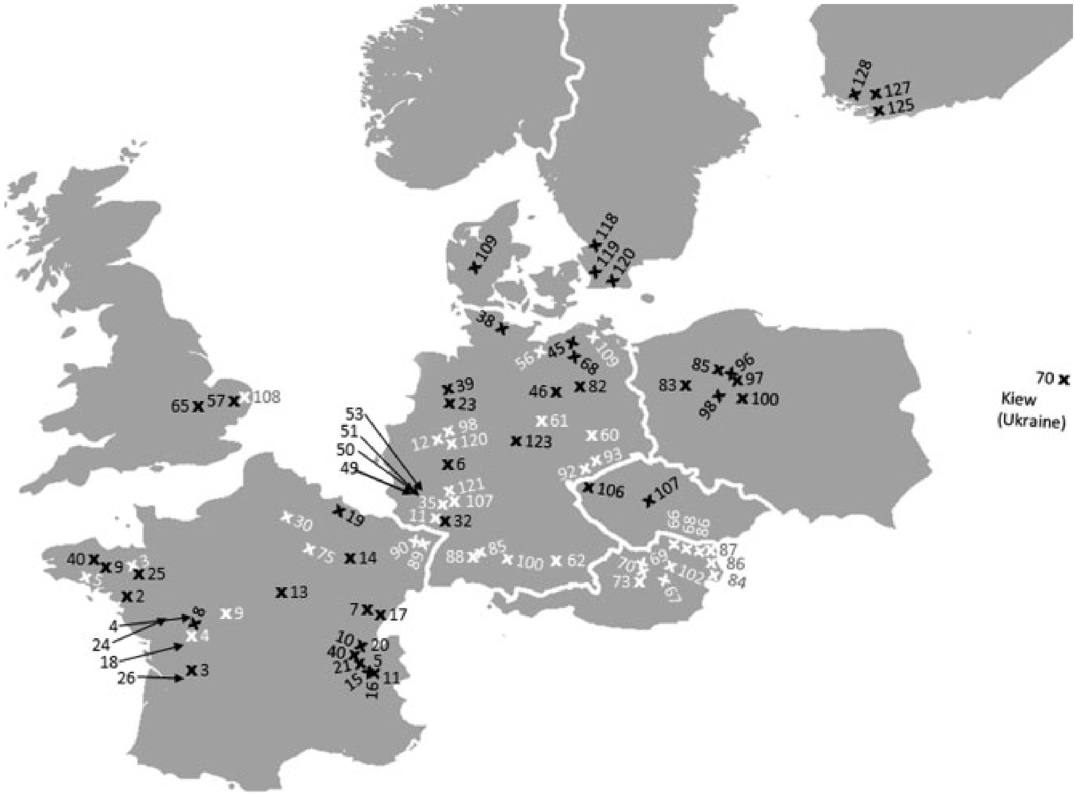

Figure 1. Pollen beetle sampling sites in Europe (white and black numbers mark collection sites in 2009 and 2010 respectively).

in Europe, such as Switzerland, France and Poland, have been published.[9–11] The United Kingdom is one of the major oilseed-rape-growing countries in the EU and seemed to be less affected by pyrethroid resistance until recently. Only in 2007 were the first resistant populations discovered.[12] Pollen beetle pyrethroid resistance monitoring carried out by the Insecticide Resistance Action Committee (IRAC) in 2008 confirmed that pyrethroid resistance is widespread in Europe, particularly in France, Germany and Poland.[13,14] Resistance to pyrethroids in pollen beetle is not limited to individual compounds, but affects the whole chemical class of pyrethroid insecticides, although some of them seem to show higher activity at recommended field rates than others.[3] The problem of pollen beetle resistance to pyrethroids in European winter oilseed rape was also covered in a recent workshop organised by the European and Mediterranean Plant Protection Organisation (EPPO).[15]

In 2007 the first resistance management strategy for pollen beetle in winter oilseed rape was recommended in Germany and is mainly based on alternations with thiacloprid, belonging to the chemical class of neonicotinoids, known to target insect nicotinic acetylcholine receptors.[16] Thiacloprid has been fully registered for pollen beetle control since 2007, and, since its introduction, other insecticides with different modes of action have been investigated for their potential against pollen beetle in order to increase diversity for resistance management purposes.[17,18] The pollen beetle resistance management strategy implemented in Germany includes a well-defined application scheme based on the occurrence of pollen beetle before and during flowering, and additionally taking into account other oilseed rape pests such as weevils.[19–21] The strategy also considers as an emergency exemption the use of organophosphate insecticides such as chlorpyrifos-methyl at high infestation levels before flowering.

The objectives of the present study were to develop a robust, reliable, rapid and validated method for effective assessment of pollen beetle susceptibility to thiacloprid, and to establish baseline data with populations collected in several European countries that could be used in future monitoring campaigns to detect early shifts in susceptibility. Furthermore, the pyrethoid resistance status of all collected populations was tested in parallel to check for cross-resistance issues.

2 MATERIALS AND METHODS

2.1 Insects

In April/May 2009 and 2010, pollen beetle populations were collected in winter oilseed rape fields from different European countries, including the most important oilseed-rape-producing countries France, Germany, Poland and Great Britain (Fig. 1). The adult insects were packed in plastic bags with some rape buds and foliage and shipped to Bayer CropScience in Monheim, Germany. After arrival in the laboratory, beetles were stored for 24 h at 4 °C. Two hours before bioassay, the insects were removed from the refrigerator and equilibrated to room temperature (20 ± 2 °C). Those beetles of lower fitness remained on the bottom of the bag and were not used for the bioassays.

2.2 Pyrethroid resistance monitoring bioassay

All pyrethroid resistance monitoring bioassays were conducted according to instructions outlined in the IRAC's susceptibility method No. 11 'Pollen beetle susceptibility monitoring bioassay – synthetic pyrethroids'.[22] The method is based on glass vials

Table 1. IRAC pyrethroid resistance classification scheme for pollen beetles

Concentration (% of label rate)	Affected beetles (%)	Classification	Code
100	100	Highly susceptible	1
20	100		
100	100	Susceptible	2
20	$<$100		
100	$<$100 to $\geq$90	Moderately resistant	3
100	$<$90 to $\geq$50	Resistant	4
100	$<$50	Highly resistant	5

(Zinsser Analytics, Germany) coated with defined concentrations of lambda-cyhalothrin. Beetles confined to glass vials were assessed for mortality after 24 h. The IRAC method was slightly modified in two points: (1) the assessment was done by directly scoring affected beetles in the vials rather than using the recommended filter disc assessment arena; (2) instead of the two pyrethroid concentrations, five concentrations were used to generate dose–response curves, i.e. 0.375 µg AI cm^{-2} inner glass surface (500% of the common field application rate of 7.5 g ha^{-1}), 0.075 µg cm^{-2} (100%), 0.015 µg cm^{-2} (20%), 0.003 µg cm^{-2} (4%) and 0.0006 µg cm^{-2} (0.8%). For one highly sensitive population obtained from Ukraine (strain 70-10), two more concentrations were added, i.e. 0.00012 and 0.000024 µg cm^{-2}. Two of the tested concentrations (100 and 20% of the field rate) were used to classify the degree of pyrethroid resistance in tested populations by using the IRAC-recommended rating scheme (Table 1).[22]

2.3 Thiacloprid adult vial bioassay

In order to check pollen beetle populations for thiacloprid baseline susceptibility, the above-mentioned adult vial bioassay method for pyrethroids was slightly modified. Vials were coated using a commercially available 240 g thiacloprid L^{-1} OD formulation (Biscaya® OD 240; Bayer CropScience), as preliminary trials revealed that technical material is not appropriate, even when applied with adjuvants (results not shown). Stock solutions were prepared by dissolving 140.4 mg of OD 240 formulation (containing 32.4 mg thiacloprid) in 2 mL of distilled water, subsequently adjusted to 100 mL with acetone. All further dilutions were made in acetone. For coating purposes, glass vials (20 mL volume, 45 cm^2 internal surface) were filled with 500 µL of solution. For all bioassays, five thiacloprid concentrations were used: 1.44 µg cm^{-2} internal surface area (corresponding to 200% of the field recommended rate of 72 g ha^{-1}), 0.72 µg cm^{-2} (100%), 0.144 µg cm^{-2} (20%), 0.0288 µg cm^{-2} (4%) and 0.00576 µg cm^{-2} (0.8%). The vials were rotated for a minimum of 2 h and subjected to a further obligatory evaporation phase without rotation for a minimum of 2 h (or overnight) before being capped and stored. The prepared vials could be stored at room temperature (dark) for a minimum of 4 weeks without a significant loss of thiacloprid (see Sections 2.4 and 3.2).

Some trials using an identical procedure to that described above were done with blank formulation in order to check for mortality possibly caused by exceeding a maximum level of oil formulation in coated vials.

For testing purposes, ten pollen beetles were placed in each vial, using three replicates per concentration and population (plus an acetone control). Capped vials were then stored upright at 20 ± 2 °C for 24 h. Prior to assessment, vials were briefly shaken to differentiate live and affected beetles more easily.

2.4 Storage stability tests and analytics

In order to investigate the storage stability of lambda-cyhalothrin and thiacloprid in coated glass vials, three replicates per concentration were analysed for active ingredient after 0, 14, 28 and 56 days. Coated glass vials were stored in the dark at both 4 and 20 °C to check for temperature effects. Stored vials were washed 2 times with 500 µL acetonitrile, and the volumes were combined and subjected to quantitative HPLC-MS/MS measurements. The samples were measured on an Applied Biosystems API4000 QTrap MS/MS system running in positive electrospray MRM mode with a capillary voltage of 4 kV and a Turbo V gas temperature of 500 °C. The HPLC system was a Waters Acquity UPLC consisting of a binary solvent manager, a column manager and a sample manager. The samples were run on a Waters Acquity HSS T3 1.8 µm column (size 50 × 2.1 mm) running in reverse-phase gradient mode. For the determination of thiacloprid, acetonitrile/water/0.1% formic acid was used as eluent, whereas methanol/2 mM NH_4OAc/1% acetic acid was used for the determination of lambda-cyhalothrin.

For quantitation, the MRM transitions 253.1 > 126.0 (thiacloprid) and 467.1 > 225.1 (lambda-cyhalothrin) were monitored. The peak integrals were calibrated externally against a standard calibration curve with a correlation coefficient $r > 0.99$. The limits of quantitation (S/N > 10) are 10 pg mL^{-1} for thiacloprid and 100 pg mL^{-1} for lambda-cyhalothrin.

2.5 Data analysis

The lethal concentration (LC) values were calculated by probit analysis using Polo Plus software v.1 (LeOra Software, Berkeley, CA). All mortality figures were corrected for control mortality using Abbott's formula.[23] Further statistical analyses were performed with Graphpad Prism 5 software (GraphPad Software Inc., La Jolla, CA). Analysis of variance (ANOVA) procedures, Student *t*-tests and appropriate post-tests (e.g. the Tukey–Kramer test) were performed to test for significant differences between strains, resistance classes, treatments and insecticides.

3 RESULTS

3.1 Validation of the adult vial test based on thiacloprid OD 240 formulation

The trials with oil dispersion blank formulation in glass vials revealed an upper limit of 200% of the field recommended rate based on thiacloprid content (absent in the blank formulation) not affecting pollen beetle after 24 h (Fig. 2). The high percentage of affected beetles at rates above 200% is probably a consequence of the oil film on the internal surface area of the vials. In all cases the observed mortality is linked to pollen beetles that stuck to the internal glass vial surface, rather than symptoms of poisoning as observed with thiacloprid.

3.2 Stability of insecticides in coated vials

The concentration of thiacloprid in glass vials coated for resistance monitoring purposes remains stable at both 4 and 20 °C, and, even after a storage period of 56 days, no significant differences compared with directly analysed samples (0 days) were observed ($P > 0.05$) (Fig. 3). Although the analytical results revealed a stable and unchanged concentration of thiacloprid over a period of 56 days, a somewhat lower efficacy against pollen beetle was

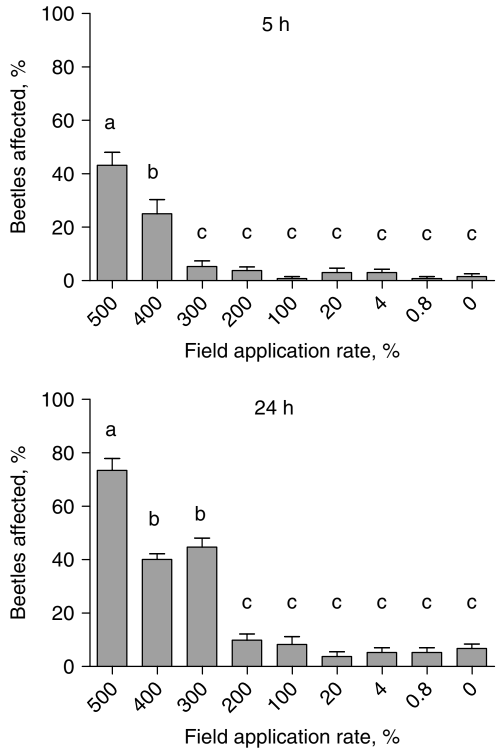

Figure 2. Effect of blank oil-dispersion formulation of 'Biscaya' on pollen beetles in an adult vial test after 5 and 24 h. Data are mean values ± SEM ($n = 4$), and different letters indicate significant differences ($P < 0.05$, *t*-test).

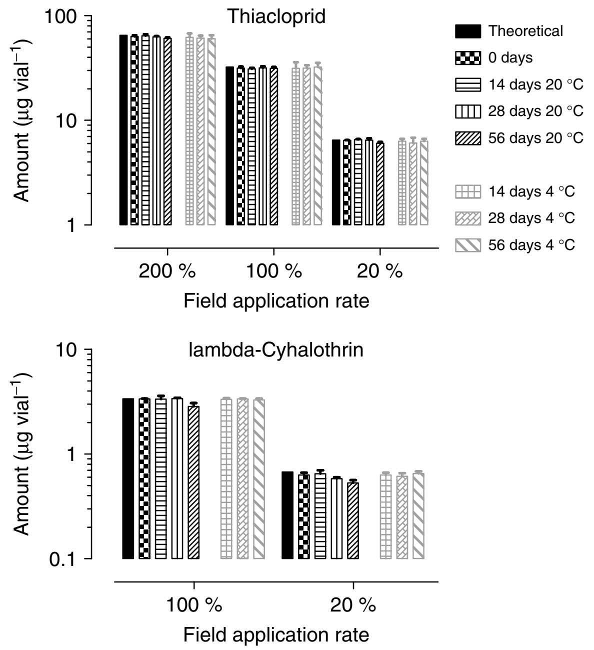

Figure 3. Stability of active ingredients in coated glass vials stored at different temperatures and analysed after different elapsed time intervals. Data are mean values ± SD ($n = 3$).

observed in such vials (data not shown), and therefore it is suggested that vials be stored for no longer than 28 days.

The concentration of lamda-cyhalothrin also did not change significantly up to 4 weeks after storage at both 4 and 21 °C. However, after 8 weeks of storage at 20 °C, a slight but significant decrease in the concentration of lambda-cyhalothrin was observed ($P < 0.05$) (Fig. 3).

3.3 Pyrethroid resistance monitoring

Susceptibility to lambda-cyhalothrin of 25 European pollen beetle populations collected in 2009 and 2010 was tested, and calculated LC_{50} values ranged from as low as 0.0001 µg cm^{-2} (0.1% of field rate) to 0.051 µg cm^{-2} (67% of field rate), leading to resistance ratios of up to 500-fold (Table 2). However, based on extrapolated LC_{95} values, resistance factors even exceeded 1000-fold in some populations collected in Germany (5) and France (2). Several populations showed less than 95% mortality at fivefold the recommended field rate (Table 2). The samples collected in 2010 included one highly susceptible population (LC_{95} 0.00042 µg cm^{-2}) collected in central Ukraine that was taken as the reference to calculate all resistance ratios given in Table 2. All dose–response bioassays included both concentrations (i.e. 100 and 20% of the field dose) recommended by the IRAC for resistance class determination, and all classes from 1 to 5 were present in the European populations collected, at least in 2010 (Table 2). Fifteen out of 50 tested populations (i.e. 30%) could be classified as pyrethroid susceptible. All IRAC resistance classes determined and based on two concentrations per population could be well separated by their dose–response relationship when including all tested concentrations, thus supporting the IRAC-proposed classification (indicated by arrows and dotted lines in Fig. 4). However, dose–response relationships for populations assigned to classes 3 to 5 (moderately to highly resistant) were not as well separated as those belonging to classes 1 to 3 (highly susceptible to moderately resistant) (Fig. 4).

3.4 Baseline susceptibility of thiacloprid

Baseline susceptibility to thiacloprid was determined based on log-dose probit–mortality results of 33 and 55 field populations of pollen beetle collected in 2009 and 2010 respectively. The calculated LC_{50} values ranged from 0.038 to 0.122 µg cm^{-2} and from 0.04 to 0.196 µg cm^{-2} in 2009 and 2010 respectively (Table 3). Thus, the LC_{50} values for both years show a maximum variation of fivefold between all 88 populations tested, compared with a maximum variation of approximately 500-fold determined for the pyrethroid lambda-cyhalothrin. The LC_{95} values ranged from 0.47 to 1.48 µg cm^{-2} and from 0.3 to 2.22 µg cm^{-2} in 2009 and 2010 respectively (Table 3). Again, the variation in response is quite low, so the method will be considered to provide reliable data in future resistance monitoring campaigns. Combining all data from 2009 and 2010 revealed non-significantly differing composite LC_{95} values based on field rates of 134–146% (Table 3).

The very low variation in pollen beetle response to thiacloprid in both years 2009 and 2010 resulted in overlapping dose–response curves, indicating no shift in susceptibility from one year to the other (Fig. 4). Based on the results obtained, it is suggested that 200, 100 and 20% of the field rate be used as discriminating doses in adult vial tests for future monitoring purposes (see the arrows in Fig. 4). The mean mortalities obtained in adult vial tests for the 200, 100 and 20% rates were 98.5 ± 2.9%, 95.7 ± 7.2% and 54.8 ± 12% respectively.

3.5 Cross-resistance investigations

In all populations tested in both 2009 and 2010, no trends of cross-resistance were observed between lambda-cyhalothrin

Table 2. Log-dose probit–mortality data for lambda-cyhalothrin against pollen beetle populations collected in 2009 and 2010 (adult vial test)

Strain	Country	P-RC[a]	LC_{50} ($\mu g\ cm^{-2}$) (field rate %)	95% CL ($\mu g\ cm^{-2}$)	LC_{95} ($\mu g\ cm^{-2}$) (field rate %)	95% CL ($\mu g\ cm^{-2}$)	Slope ($\pm$ SE)	RR[b] LC_{50}	RR[b] LC_{95}
67-09	Austria	1	0.001 (1.4)	0.0008–0.0014	0.005 (7.2)	0.004–0.011	2.27 (±0.2)	10	13
84-09	Austria	1	0.0008 (1.1)	0.0006–0.0012	0.004 (5.6)	0.003–0.009	2.36 (±0.21)	8	10
109-09	Germany	1	0.0008 (1)	0.0006–0.0009	0.003 (4.3)	0.003–0.005	1.53 (±0.1)	8	8
102-09	Germany	2	0.0011 (1.5)	0.0004–0.0028	0.013 (17.4)	0.004–0.105	1.46 (±0.11)	11	33
68-09[c]	Austria	2	0.003 (3.9)	0.0015–0.0057	0.02 (26.3)	0.009–0.140	1.99 (±0.16)	30	50
87-09	Austria	2	0.0008 (1.1)	0.0004–0.0018	0.006 (8.1)	0.003–0.06	1.93 (±0.16)	8	15
90-09	France	2	0.0011 (1.5)	0.0007–0.0017	0.007 (9.1)	0.004–0.018	2.10 (±0.17)	11	18
108-09	Great Britain	2	0.0013 (1.7)	0.0009–0.0017	0.009 (12)	0.006–0.018	1.94 (±0.16)	13	23
56-09	Germany	3	0.005 (6.7)	0.0017–0.0141	0.177 (236)	0.046–4.109	1.03 (±0.07)	50	443
61-09	Germany	3	0.009 (12.1)	0.0058–0.0147	0.186 (248.5)	0.089–0.580	1.25 (±0.09)	90	465
66-09	Austria	3	0.0048 (6.4)	0.0053–0.0078	0.192 (256)	0.011–0.066	2.72 (±0.24)	48	480
69-09[c]	Austria	3	0.0067 (8.9)	0.0053–0.0084	0.075 (99.9)	0.052–0.119	1.57 (±0.11)	67	188
70-09[c]	Austria	3	0.0061 (8.1)	0.0048–0.0078	0.104 (138.5)	0.069–0.173	1.33 (±0.09)	61	260
73-09	Austria	3	0.0053 (7.1)	0.0031–0.0093	0.108 (144.7)	0.048–0.409	1.26 (±0.09)	53	270
86-09	Austria	3	0.0037 (4.9)	0.0023–0.0059	0.074 (98.3)	0.037–0.215	1.09 (±0.08)	37	185
88-09	Germany	3	0.0084 (11.2)	0.0047–0.0153	0.189 (251.9)	0.079–0.794	1.22 (±0.08)	84	473
107-09	Germany	3	0.0087 (11.6)	0.0032–0.0251	0.147 (196.2)	0.043–3.365	1.34 (±0.09)	87	368
120-09	Germany	3	0.0056 (7.5)	0.0027–0.0114	0.144 (191.9)	0.053–0.903	1.17 (±0.08)	56	360
35-09	Germany	4	0.0088 (11.7)	0.002–0.0419	0.559 (745.2)	0.086–176.01	0.92 (±0.06)	88	1398
30-09	France	4	0.0149 (19.8)	0.0056–0.0441	0.254 (338.7)	0.073–6.15	1.34 (±0.09)	149	635
60-09	Germany	4	0.0104 (13.8)	0.0022–0.0566	0.355 (473.4)	0.063–181.13	1.07 (±0.07)	104	888
62-09	Germany	4	0.0123 (16.4)	0.0058–0.0274	0.29 (387.3)	0.097–2.43	1.2 (±0.081)	123	725
85-09	Germany	4	0.0167 (22.3)	0.0057–0.0586	0.562 (749.4)	0.125–28.46	1.08 (±0.08)	167	1405
100-09	Germany	4	0.0181 (24.1)	0.0144–0.0229	0.227 (302.6)	0.16–0.367	1.5 (±0.10)	181	568
121-09	Germany	4	0.0173 (23)	0.0077–0.042	0.454 (604.9)	0.138–5.482	1.16 (±0.08)	173	1135
70-10	Ukraine	1	0.0001 (0.1)	0.0001–0.0001	0.0004 (0.5)	0.0003–0.0008	2.93 (±0.29)	1	1
2-10	France	2	0.0035 (4.7)	0.0017–0.0073	0.062 (83.3)	0.024–0.393	1.31 (±0.09)	35	155
4-10	France	2	0.0053 (7.1)	0.002–0.0139	0.092 (122.5)	0.029–1.193	1.34 (±0.09)	53	230
3-10	France	2	0.0034 (4.5)	0.0023–0.0051	0.032 (42.4)	0.018–0.079	1.69 (±0.13)	34	80
125-10	Finland	2	0.0012 (1.6)	0.0004–0.0028	0.019 (24.9)	0.006–0.253	1.36 (±0.12)	12	48
128-10	Finland	2	0.0012 (1.6)	0.0004–0.0028	0.02 (26.1)	0.007–0.237	1.36 (±0.1)	12	50
127-10	Finland	2	0.0008 (1.1)	0.0004–0.0014	0.006 (7.6)	0.003–0.024	1.92 (±0.16)	8	15
5-10	France	3	0.0047 (6.2)	0.0032–0.0066	0.064 (85.4)	0.037–0.139	1.4 (±0.1)	47	160
109-10	Denmark	3	0.017 (22.7)	0.0095–0.0294	0.135 (180.3)	0.068–0.458	1.83 (±0.12)	170	338
119-10	Sweden	3	0.016 (21.3)	0.0104–0.0246	0.114 (151.5)	0.064–0.297	1.9 (±0.14)	160	285
107-10	Czech Republic	3	0.0037 (5)	0.0023–0.0061	0.053 (70.5)	0.026–0.158	1.43 (±0.1)	37	133
118-10	Sweden	3	0.0281 (37.5)	0.0195–0.0401	0.265 (353.2)	0.161–0.539	1.69 (±0.11)	281	663
120-10	Sweden	3	0.0162 (21.6)	0.0119–0.0219	0.121 (161.7)	0.078–0.226	1.88 (±0.14)	162	303
7-10	France	4	0.0177 (23.6)	0.0073–0.0407	0.517 (689.6)	0.169–4.487	1.12 (±0.07)	177	1293
32-10	Germany	4	0.0284 (37.8)	0.0125–0.063	0.58 (773)	0.204–4.489	1.26 (±0.11)	284	1450
96-10	Poland	4	0.0231 (30.8)	0.0088–0.0575	0.237 (316)	0.086–2.491	1.63 (±0.13)	231	593
98-10	Poland	4	0.021 (28.1)	0.0104–0.0414	0.214 (285)	0.093–1.055	1.63 (±0.11)	210	535
8-10	France	4	0.0155 (20.6)	0.0049–0.045	0.896 (1194.2)	0.204–248.2	0.93 (±0.06)	155	2240
39-10	Germany	4	0.0046 (6.1)	0.0007–0.0209	0.1 (133.3)	0.022–1.842	1.23 (±0.08)	46	250
85-10	Poland	4	0.0299 (39.9)	0.0251–0.0355	0.123 (164)	0.091–0.173	1.96 (±0.09)	299	308
97-10	Poland	4	0.0212 (28.2)	0.0064–0.0669	0.152 (203.2)	0.053–5.225	1.91 (±0.13)	212	380
100-10	Poland	4	0.0148 (19.8)	0.0072–0.0292	0.245 (326.8)	0.1–1.261	1.35 (±0.09)	148	613
106-10	Czech Republic	4	0.0369 (49.2)	0.0188–0.0694	0.232 (309.3)	0.111–1.059	2.04 (±0.14)	369	580
68-10	Germany	5	0.0383 (51.1)	0.0131–0.1088	0.578 (770)	0.18–12.086	1.3 (±0.16)	383	1445
82-10	Germany	5	0.0506 (67.4)	0.0255–0.0938	0.315 (420)	0.152–1.493	1.99 (±0.16)	506	788
Composite 2009			**0.0045 (6)**	**0.0026–0.0075**	**0.113 (150.1)**	**0.053–0.356**	**1.17(±0.075)**		
Composite 2010			**0.0098 (13.1)**	**0.0063–0.0153**	**0.237 (315.8)**	**0.119–0.639**	**1.19(±0.08)**		

[a] P-RC = pyrethroid resistance index after IRAC susceptibility method No.11.
[b] RR = resistance ratio based on strain 70-10.
[c] Including ≥20% *Meligethes viridescens*; in all other strains, ≤5% *M. viridescens*.

Table 3. Log-dose probit–mortality data for thiacloprid against pollen beetle populations collected in 2009 and 2010 (adult vial test)

Strain	Country	P-RC[a]	LC_{50} (μg cm^{-2}) (field rate %)	95% CL (μg cm^{-2})	LC_{95} (μg cm^{-2}) (field rate %)	95% CL (μg cm^{-2})	Slope (± SE)	RR[b] LC_{50}	RR[b] LC_{95}
67-09	Austria	1	0.05 (7)	0.02–0.1	0.92 (127.8)	0.38–4.39	1.3 (±0.08)	1	2
84-09	Austria	1	0.05 (6.9)	0.03–0.09	0.73 (101.5)	0.34–1.91	1.41 (±0.09)	1	2
5-09	France	2	0.07 (9.2)	0.05–0.09	0.59 (81.5)	0.36–1.17	1.74 (±0.13)	2	1
102-09	Germany	2	0.09 (12)	0.04–0.17	0.83 (115.5)	0.36–4.28	1.67 (±0.11)	2	2
68-09[c]	Austria	2	0.04 (5.5)	0.02–0.07	0.51 (70.9)	0.24–1.81	1.48 (±0.1)	1	1
87-09	Austria	2	0.07 (10.3)	0.04–0.14	0.88 (122.5)	0.4–3.57	1.53 (±0.1)	2	2
98-09	Germany	2	0.09 (12.3)	0.03–0.25	1.19 (165.3)	0.38–20.65	1.46 (±0.9)	2	3
89-09	France	2	0.04 (5.6)	0.02–0.07	0.51 (70.1)	0.26–1.73	1.7 (±0.13)	1	1
108-09	Great Britain	3	0.1 (13.8)	0.05–0.19	1.1 (152.5)	0.49–4.72	1.58 (±0.1)	3	3
13-09	Germany	3	0.04 (5.3)	0.02–0.07	0.47 (65.8)	0.22–1.66	1.51 (±0.1)	1	1
56-09	Germany	3	0.08 (11.1)	0.04–0.14	0.66 (91.6)	0.32–2.46	1.79 (±0.12)	2	2
61-09	Austria	3	0.06 (8.3)	0.04–0.1	0.71 (99.2)	0.36–2.12	1.53 (±0.1)	2	2
66-09	Austria	3	0.06 (7.6)	0.03–0.1	0.87 (121.2)	0.4–3.68	1.37 (±0.08)	2	2
69-09[c]	Austria	3	0.06 (8.7)	0.03–0.13	0.86 (118.9)	0.34–4.99	1.45 (±0.09)	2	2
70-09[c]	Austria	3	0.04 (6)	0.02–0.09	0.67 (92.5)	0.25–4.51	1.38 (±0.09)	1	2
73-09	France	3	0.05 (7.1)	0.02–0.11	1.01 (140.1)	0.37–6.74	1.27 (±0.08)	1	2
75-09	Austria	3	0.09 (11.9)	0.05–0.13	0.87 (120.8)	0.47–2.27	1.63 (±0.11)	2	2
86-09	Germany	3	0.09 (12.7)	0.05–0.16	0.68 (93.7)	0.34–2.3	1.9 (±0.13)	2	2
88-09	Germany	3	0.08 (11.3)	0.02–0.22	1.23 (170.8)	0.4–17.17	1.39 (±0.09)	2	3
92-09	Germany	3	0.07 (10.2)	0.04–0.12	0.98 (135.5)	0.48–3.05	1.46 (±0.09)	2	2
93-09	Germany	3	0.05 (6.9)	0.03–0.09	0.76 (105.9)	0.33–3.16	1.39 (±0.09)	1	2
107-09	Germany	3	0.10 (14.5)	0.06–0.17	0.85 (117.8)	0.44–2.62	1.81 (±0.12)	3	2
120-09	France	4	0.1 (13.9)	0.04–0.23	1.26 (175.6)	0.47–10.47	1.49 (±0.09)	3	3
3-09	France	4	0.10 (14.2)	0.06–0.18	1.1 (152.2)	0.53–3.78	1.59 (±0.1)	3	3
4-09	France	4	0.08 (12.1)	0.04–0.17	1.01 (140.9)	0.43–5.14	1.54 (±0.1)	2	2
9-09	Germany	4	0.07 (10)	0.02–0.19	1.22 (169.1)	0.38–18.11	1.34 (±0.08)	2	3
11-09	Germany	4	0.12 (16.9)	0.05–0.3	1.01 (139.6)	0.39–10.51	1.62 (±0.1)	3	2
12-09	France	4	0.05 (7.3)	0.03–0.1	0.68 (95)	0.28–3.61	1.47 (±0.09)	1	2
30-09	Germany	4	0.07 (9.3)	0.04–0.12	0.84 (116.5)	0.39–3.05	1.5 (±0.1)	2	2
35-09	Germany	4	0.12 (16.1)	0.05–0.25	1.49 (206.1)	0.59–9.44	1.49 (±0.09)	3	3
62-09	Germany	4	0.05 (6.4)	0.02–0.09	0.76 (105.7)	0.31–3.98	1.35 (±0.08)	1	2
85-09	Germany	4	0.08 (11.1)	0.06–0.11	0.58 (74.7)	0.35–0.99	1.98 (±0.01)	2	1
100-09	Germany	4	0.11 (15.3)	0.05–0.24	1.02 (141.2)	0.41–7.32	1.71 (±0.11)	3	2
70-10	Ukraine	1	0.12 (16.4)	0.1–0.14	1.17 (162.8)	0.86–1.7	1.65 (±0.11)	3	3
127-10	Finland	2	0.04 (6)	0.04–0.05	0.57 (79)	0.42–0.71	1.7 (±0.13)	1	1
125-10	Finland	2	0.07 (10.1)	0.04–0.12	0.71 (98.7)	0.41–1.43	1.56 (±0.09)	2	2
128-10	Finland	2	0.11 (14.8)	0.06–0.15	1.28 (178)	0.7–3.26	1.78 (±0.14)	3	3
2-10	France	2	0.11 (15.5)	0.07–0.18	1.61 (223.3)	0.87–4.05	1.42 (±0.09)	3	4
4-10	France	2	0.05 (7.3)	0.03–0.1	0.45 (62.5)	0.2–2.31	1.77 (±0.12)	1	1
25-10	France	2	0.06 (8.6)	0.04–0.1	1 (138.6)	0.48–3.08	1.36 (±0.08)	2	2
3-10	France	2	0.11 (15)	0.05–0.22	1.04 (144.2)	0.45–5.27	1.67 (±0.11)	3	2
15-10	France	2	0.06 (8.8)	0.04–0.11	0.65 (90.5)	0.31–2.41	1.63 (±0.11)	2	2
24-10	France	2	0.06 (8.6)	0.05–0.08	0.76 (105.1)	0.49–1.34	1.51 (±0.1)	2	2
26-10	France	2	0.12 (16.2)	0.08–0.16	1 (138.7)	0.62–1.94	1.76 (±0.12)	3	2
107-10	Czech Republic	3	0.13 (17.3)	0.07–0.2	1.74 (242.1)	0.9–4.83	1.43 (±0.09)	3	4
109-10	Denmark	3	0.06 (7.9)	0.05–0.07	0.59 (81.3)	0.42–0.87	1.62 (±0.11)	2	1
12-10	France	3	0.06 (8.4)	0.03–0.13	0.95 (131.5)	0.36–6	1.38 (±0.09)	2	2
14-10	France	3	0.09 (12.9)	0.04–0.2	1.02 (142.1)	0.41–6.71	1.58 (±0.1)	2	2
5-10	France	3	0.04 (5.3)	0.02–0.07	0.43 (60.1)	0.23–1.8	1.48 (±0.1)	1	1
13-10	France	3	0.10 (14.4)	0.07–0.15	0.97 (134)	0.56–2.2	1.7 (±0.11)	3	2
19-10	France	3	0.05 (6.3)	0.04–0.06	0.45 (62.3)	0.26–0.55	1.82 (±0.13)	1	1
23-10	Germany	3	0.10 (14.2)	0.05–0.2	1.29 (178.8)	0.54–6.54	1.5 (±0.09)	3	3
38-10	Germany	3	0.08 (10.5)	0.04–0.15	0.68 (93.9)	0.29–3.8	1.73 (±0.12)	2	2
40-10	France	3	0.07 (9.4)	0.05–0.09	0.47 (65.8)	0.3–0.92	1.95 (±0.14)	2	1

Table 3. (*Continued*)

46-10	Germany	3	0.12 (16)	0.07–0.18	1.67 (231.3)	0.87–4.48	1.42 (±0.09)	3	4
123-10	Germany	3	0.05 (6.8)	0.04–0.06	0.52 (72)	0.35–0.73	1.8 (±0.14)	1	1
119-10	Sweden	3	0.09 (12.4)	0.06–0.13	0.65 (90.7)	0.38–1.54	1.9 (±0.14)	2	2
118-10	Sweden	3	0.07 (10.3)	0.03–0.16	0.82 (114)	0.32–5.86	1.57 (±0.1)	2	2
120-10	Sweden	3	0.09 (12.7)	0.05–0.16	0.68 (93.7)	0.34–2.3	1.89 (±0.13)	2	2
106-10	Czech Republic	4	0.14 (19.3)	0.09–0.21	1.29 (179)	0.73–3.12	1.7 (±0.11)	4	3
8-10	France	4	0.05 (7.5)	0.02–0.12	1.77 (245.7)	0.61–12.14	1.09 (±0.07)	1	4
10-10	France	4	0.09 (12.5)	0.06–0.13	1.45 (200.9)	0.85–2.97	1.37 (±0.08)	2	3
16-10	France	4	0.09 (12.7)	0.05–0.16	1.11 (154)	0.52–4.06	1.52 (±0.1)	2	3
18-10	France	4	0.07 (10.1)	0.04–0.12	0.93 (128.9)	0.49–2.47	1.49 (±0.09)	2	2
20-10	France	4	0.10 (13.7)	0.06–0.15	1.06 (147.6)	0.58–2.65	1.59 (±0.01)	3	2
7-10	France	4	0.07 (9.8)	0.04–0.12	0.95 (132.2)	0.46–3.14	1.46 (±0.09)	2	2
9-10	France	4	0.08 (11.1)	0.04–0.15	1.49 (206.8)	0.65–5.91	1.29 (±0.08)	2	3
11-10	France	4	0.05 (6.7)	0.02–0.09	1.11 (154.5)	0.46–4.79	1.21 (±0.07)	1	3
17-10	France	4	0.08 (10.8)	0.04–0.14	0.67 (93.5)	0.33–2.45	1.75 (±0.12)	2	2
21-10	France	4	0.12 (16.7)	0.07–0.2	0.96 (133.3)	0.49–3.02	0.12 (±0.1)	3	2
6-10	Germany	4	0.10 (14.4)	0.06–0.16	1.27 (176.5)	0.69–3.19	1.51 (±0.1)	3	3
32-10	Germany	4	0.18 (24.9)	0.12–0.27	2.22 (308.6)	1.26–5.12	1.55 (±0.1)	5	5
39-10	Germany	4	0.08 (10.9)	0.05–0.12	0.60 (83.7)	0.33–1.69	1.86 (±0.13)	2	1
45-10	Germany	4	0.06 (8.5)	0.03–0.14	0.94 (130.9)	0.36–6.32	1.39 (±0.09)	2	2
50-10	Germany	4	0.2 (27.3)	0.16–0.24	1.35 (186.9)	1.03–1.87	1.97 (±0.14)	5	3
53-10	Germany	4	0.17 (23.7)	0.14–0.21	1.16 (161.2)	0.89–1.61	1.98 (±0.14)	4	3
83-10	Poland	4	0.11 (15.2)	0.06–0.21	0.94 (130)	0.43–4.33	1.77 (±0.12)	3	2
49-10	Germany	4	0.12 (17)	0.06–0.24	1.27 (176)	0.55–6.2	1.62 (±0.1)	3	3
51-10	Germany	4	0.13 (17.6)	0.07–0.22	1.84 (255.8)	0.79–5.32	1.49 (±0.1)	3	4
65-10	Great Britain	4	0.18 (24.3)	0.13–0.23	0.86 (119.7)	0.6–1.43	2.38 (±0.18)	5	2
57-10	Great Britain	4	0.15 (20.8)	0.12–0.19	2.04 (283.4)	1.47–3.05	1.45 (±0.09)	4	5
96-10	Poland	4	0.09 (11.8)	0.07–0.1	0.63 (87.4)	0.47–0.91	1.89 (±0.13)	2	1
98-10	Poland	4	0.09 (12.8)	0.08–0.11	0.52 (72)	0.39–0.74	2.2 (±0.17)	2	1
85-10	Poland	4	0.10 (14.5)	0.05–0.21	0.89 (124.1)	0.39–4.58	1.76 (±0.12)	3	2
97-10	Poland	4	0.11 (15.3)	0.07–0.16	1.28 (178)	0.74–2.86	1.55 (±0.1)	3	3
100-10	Poland	4	0.08 (11.2)	0.03–0.18	1.14 (157.7)	0.43–8.55	1.43 (±0.09)	2	3
68-10	Germany	5	0.07 (9.4)	0.05–0.1	0.66 (91.2)	0.39–1.38	1.67 (±0.12)	2	2
82-10	Germany	5	0.11 (15.2)	0.06–0.21	0.94 (130)	0.43–4.33	1.77 (±0.12)	3	2
Composite 2009			**0.07 (9.8)**	**0.04–0.13**	**0.96 (133.7)**	**0.44–3.71**	**1.45 (±0.09)**		
Composite 2010			**0.09 (12.3)**	**0.06–0.12**	**1.05 (145.6)**	**0.67–1.91**	**1.53 (±0.1)**		

[a] P-RC = pyrethroid resistance class after IRAC susceptibility method No.11.
[b] RR = resistance ratio based on strain 5–10.
[c] Including ≥20% *Meligethes viridescens*; in all others, ≤5% *M. viridescens*.

and thiacloprid. With regard to the IRAC pyrethroid resistance classification, which clearly and significantly separates populations based on their allocation to different resistance classes, it was demonstrated that thiacloprid does not follow the same trend (Tables 2 and 3). Even those populations classified as highly resistant to pyrethroids did not show any lower susceptibility to thiacloprid, suggesting the complete lack of cross-resistance. This was also statistically validated by regression analysis revealing no correlation between LC_{50} and LC_{95} values for the two compounds ($P > 0.05$).

4 DISCUSSION

4.1 Pyrethroid resistance monitoring

The present study confirmed earlier data showing widespread pyrethroid resistance in pollen beetles collected in winter oilseed rape in many European countries in 2009 and 2010. The data collected are based on an IRAC-recommended adult vial bioassay employing lambda-cyhalothrin as a reference pyrethroid.[22] The vials are usually coated with lambda-cyhalothrin and either stored in the laboratory until use or shipped to other laboratories or field stations, and the present studies have shown that they are stable for at least 8 weeks when stored at 4 °C in the dark, suggesting that a single production cycle before the season is sufficient. The use of a standard pyrethroid as suggested by the IRAC[22] was shown to be justified, as pollen beetle populations collected all over Europe ($n = 42$) were shown to be cross-resistant to other pyrethroids such as deltamethrin, alpha-cypermethrin, bifenthrin and etofenprox in an earlier study.[3] However, the extent of cross-resistance seems to differ between pyrethroids, with a tendency for some compounds to be less but still significantly affected, e.g. bifenthrin.[3] The high resistance factors reported resulted from a reference population taken from Ukraine (strain 70-10) (Table 2) and displaying the highest susceptibility to pyrethroids detected in 2009 and 2010. The population turned out to be 5–8-fold more susceptible than those usually assigned to IRAC pyrethroid

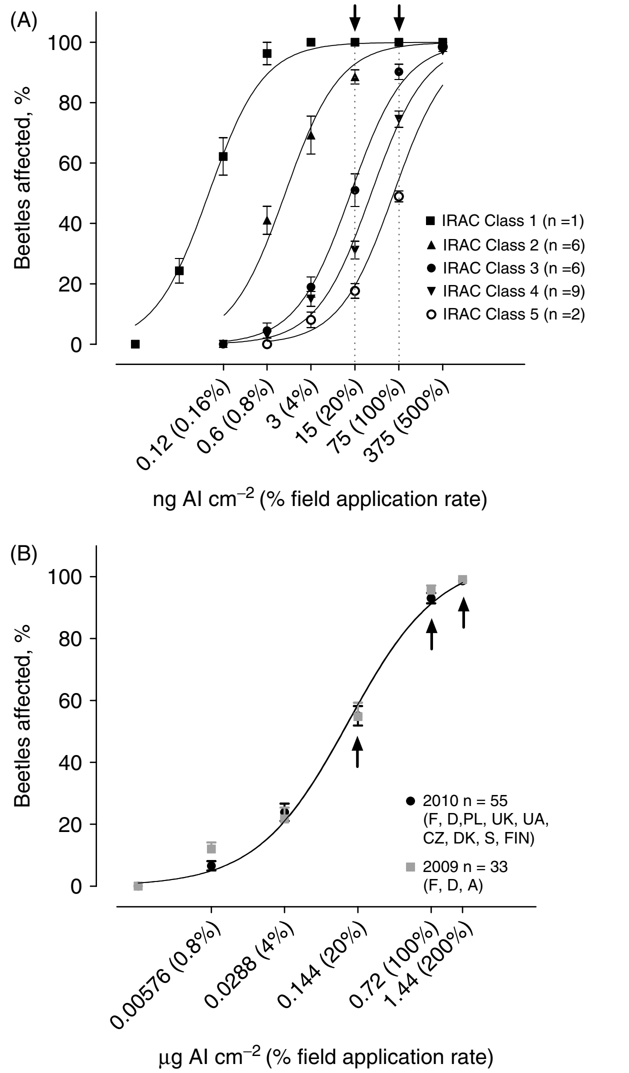

Figure 4. (A) Response of pollen beetle populations collected in 2010 to different concentrations of lambda-cyhalothrin and their classification to different pyrethroid resistance groups as proposed by the IRAC.[22] The arrows and dotted lines mark the IRAC-recommended discriminating rates. (B) Baseline susceptibility to thiacloprid of pollen beetle populations collected in 2009 and 2010. Dose–response curves represent mean values of combined data ± SEM. The arrows mark the proposed discriminating rates for future monitoring initiatives. Error bars = standard error mean; F = France, D = Germany, PL = Poland, UK = United Kingdom, UA = Ukraine, CZ = Czech Republic, DK = Denmark, S = Sweden, FIN = Finland, A = Austria.

susceptibility class 1, and therefore a number of individuals were subjected to a more detailed morphometric species determination based on leg morphology. The population indeed consisted up to 100% of *M. aeneus*, and additionally it showed no higher susceptibility to thiacloprid than the other populations (Table 3). The majority of the tested populations, i.e. 70%, turned out to be moderately to highly resistant to pyrethroids according to the IRAC rating scheme. Close to 90% of the randomly collected German populations ($n = 17$) exhibited pyrethroid resistance, and similar values have been reported earlier.[3] Reliable control of pollen beetle by pyrethroids is only possible in those regions where resistance monitoring data before application confirm susceptibility, i.e. LC_{95} values no greater than approximately 10% of the recommended field rate as shown in the present investigations (Table 2).

Apart from the lack of any resistance management considerations in oilseed rape, there are three main reasons among others that most likely contribute to the rapid spread of pollen beetle pyrethroid resistance in many European countries: (1) the dramatic expansion of winter oilseed rape cultivation in many countries, e.g. in Germany the cultivated winter oilseed rape acreage has doubled within the last 15 years, thus providing unlimited breeding sites and food sources for pollen beetles;[24] (2) low treatment thresholds result in an increased number of applications in some countries (the compensation ability of winter oilseed rape is often neglected), e.g. the threshold in Poland is reported to be 1–3 beetles per plant (BBCH 50–52) compared with 15 beetles per plant in the United Kingdom;[4] (3) a politically and environmentally driven ban of older classes of insecticides addressing other biochemical modes of action such as organophosphates, without having available appropriate alternatives other than pyrethroids.[3,6,18]

The evolution of insecticide resistance in pollen beetles is likely to be a rather old story considering reports that date back to 1921 and claim a disappointing performance of chemicals in pollen beetle control in Germany.[25] These early reports triggered the re-invention of other methods to control pollen beetle, mainly based on mechanical trapping devices.[26] However, such methods provided no means of control when high infestation levels were monitored, thus resulting in repeated spraying and dusting of natural insecticides such as nicotine, rotenone and also pyrethrum, the forerunner of the synthetic pyrethroids.[27] Later on, DDT and organophoshates were introduced and provided good control of pollen beetles.[28] DDT resistance development was first reported in Polish pollen beetle populations in 1967, and confirmed in trials in 1969.[29] DDT, as well as pyrethroids, acts on voltage-gated sodium channels, and it would have been interesting to know whether it selected for *kdr* (knockdown resistance, a well-known target site mutation)[30] in pollen beetles these days, as resistance ratios reported were quite high, i.e. exceeded factors of 1000-fold.[29]

4.2 Thiacloprid method validation and baseline susceptibility

With the introduction of the neonicotinoid insecticide thiacloprid in 2006, the first new mode of action for decades was introduced for pollen beetle control in Germany. In the first year of its introduction, thiacloprid received only an emergency registration in Germany, and its use was limited to 100 000 ha, but since 2007 it has had full registration, and also in other European countries. The compound is well known and considered to be safe to honey bees, so it can be applied during flowering.[31,32]

The adult vial test developed by the authors is based on glass vials coated with a commercial oil-dispersion formulation of thiacloprid, Biscaya® OD 240. The bioassay system required considerable fine-tuning and many experiments before validation owing to the OD formulation used for coating. The highest possible rate that could be applied to the inner glass vial surface equalled 200% of the field recommended rate (1.44 µg cm^{-2}). Higher concentrations resulted in physical trapping of beetles on the oily surface and impractically long evaporation times. The shortest estimated evaporation time producing reliable and repeatable results is at least 4 h (including 2 h rotation time). Shorter intervals result in high beetle mortality as a consequence of a combination of physical and biological action, which even at rather low doses definitely represented unrealisitic exposure scenarios. The mean thiacloprid concentration resulting in 50% pollen beetle mortality is around 0.080 µg cm^{-2} when combining composite data of 2009 and 2010 (Table 3). This is in strong contrast to very low LC_{50}

Table 4. Relationships between field recommended rates and log-dose probit–mortality data for different insecticides against pyrethroid-susceptible pollen beetles (strain 84-09; adult vial test)

Compound	100% Field rate (g AI ha^{-1})	LC_{50} (g AI ha^{-1}) (field rate %)	95% CL	Quotient 100% field rate/LC_{50}	LC_{95} (g AI ha^{-1}) (field rate %)	95% CL	Quotient 100% field rate/LC_{95}
Thiacloprid	72	4.9 (6.8)	2.98–9.03	15	73.1 (101.5)	34.1–191.1	0.98
lambda-Cyhalothrin	7.5	0.08 (1.1)	0.06–0.12	94	0.41 (5.5)	0.26–0.92	18
Chlorpyrifos-methyl[a]	337.5	0.41 (0.1)	0.18–0.92	823	6.26 (1.9)	2.23–58.78	54

[a] Data obtained by conducting an adult vial bioassay according to IRAC method No.11.

values of approximately 0.0001 µg cm^{-2} recently published for thiacloprid using a similar methodology.[33] Such a low value is difficult to explain considering the much lower intrinsic toxicity of thiacloprid compared with a pyrethroid insecticide (ca 60-fold), and therefore the present authors think it is likely that evaporation times were too short (no checks with blank formulation were included), although the method itself is considered to be very practical and similar to the present method. Furthermore, the analytical results presented here suggest a good stability of the active ingredient in coated glass vials, so their production in advance of a resistance monitoring campaign is not considered to be problematic.

The very homogeneous efficacy results obtained with thiacloprid against pollen beetle populations collected in 2009 and 2010 revealed no shift as yet, and importantly no cross-resistance to pyrethroids. However, the latter is possibly not surprising considering the fact that, so far, in none of the neonicotinoid-targeted agricultural pest insects has cross-resistance to pyrethroids been described.[31] Neonicotinoid insecticides such as thiacloprid and acetamiprid are intrinsically less active than lambda-cyhalothrin and chlorpyrifos-methyl, which is reflected by the recommended rates not consistently providing a 100% mortality of pollen beetles. The variation in response to thiacloprid that was detected in pollen beetles was less than fivefold, and comparable with baseline studies conducted on other invertebrate pest species such as aphids and whiteflies.[31,32] Resistance monitoring studies with acetamiprid in 2004 in three pollen beetle populations collected in Poland suggested low resistance to neonicotinoid insecticides, and were based on the fact that mortality figures at recommended field rates were below 100%.[34] With reference to the lower intrinsic activity mentioned above and the lack of any presented baseline data, it is rather unlikely that the investigated Polish populations indeed show resistance. This is confirmed by another study by the same authors showing only slight variation in LC_{50} values, with mostly overlapping 95% fiducial limits, for acetamiprid in pollen beetle populations, again collected in Poland, but in 2005–2007.[11] However, the present authors definitely support the view of Wegorek *et al*.[11] that monitoring should continue for the development of resistance against different classes of insecticides in pollen beetles, particularly neonicotinoids. Neonicotinoids such as thiacloprid and acetamiprid show a much lower intrinsic activity and are used at relatively low rates compared with the organophosphate chlorpyrifos-methyl (Table 4). Once resistance occurs, these facts are likely to affect their field performance much faster than that of pyrethroids or organophosphates, i.e. based on LC_{95} values even resistance ratios as low as 5–10-fold are suggested to convert into neonicotinoid field failure against pollen beetle, whereas such low resistance ratios would never affect the efficacy of lambda-cyhalothrin at recommended rates. Considering the data presented in this study, it is presumed that a tenfold and 50-fold resistance to a pyrethroid such as lambda-cyhalothrin and an organophosphate such as chlorpyrifos-methyl, respectively, is unlikely to be of practical significance at recommended rates under field conditions (Table 4). Therefore, it is strongly suggested that thiacloprid be used in alternation with other pollen beetle insecticides of different modes of action in order to sustain its efficacy as a valuable tool in resistance management strategies. The fragile use rate/efficacy relationship implies the need for annual rather than biannual monitoring for neonicotinoid susceptibility in pollen beetle populations, particularly in those regions where this class of chemistry is heavily used owing to the lack of appropriate alternative chemical means.

5 CONCLUSION

Pyrethroid resistance in *Meligethes* ssp. is at moderate to high levels in many European countries, and therefore resistance management strategies based on mode-of-action rotation need to be implemented and have already been recommended.[19–21,35] In order to implement such a strategy, several chemical options not affected by resistance or representing new modes of action are necessary. Taken together, and summarising all results presented, it can be concluded that thiacloprid among other new chemical classes of insecticides is a valuable option for future pollen beetle control without any signs of resistance yet being detected. Thiacloprid belongs to the chemical class of neonicotinoid insecticides, which have only systematically been used for pollen beetle control for a few years. Neonicotinoid insecticides such as thiacloprid form an essential part of resistance management strategies.[21,35] Therefore, their performance should be carefully monitored in the future in order to detect early shifts in pollen beetle susceptibility. For this purpose, the adult vial bioassay based on a thiacloprid 240 g L^{-1} OD formulation (Biscaya®) was developed and validated using pollen beetle populations collected in several European countries in 2009 and 2010. The variation in response to thiacloprid of all populations tested is less than fivefold, and not related to pyrethroid resistance, suggesting full thiacloprid baseline susceptibility of all populations tested by the proposed monitoring method. The method was also considered by the IRAC for inclusion in their methods list.[36] For future neonicotinoid resistance monitoring initiatives with thiacloprid, it is suggested that 200% (1.44 µg cm^{-2}), 100% (0.72 µg cm^{-2}) and 20% (0.144 µg cm^{-2}) of the field recommended rate be employed as diagnostic doses providing a mean mortality of 98.5 ± 2.9%, 95.7 ± 7.2% and 54.8 ± 12% respectively. It has been taken into account that thiacloprid is intrinsically less active than pyrethroids and organophosphates, so the mean mortalities mentioned above indeed represent the real baseline activity, even though they do not consistently provide 100% mortality.

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