

RESEARCH ARTICLE

Cabbage stem flea beetle's (*Psylliodes chrysocephala* L.) susceptibility to pyrethroids and tolerance to thiacloprid in the Czech Republic

Jitka Stará¹*, František Kocourek¹

Department of Integrated Crop Protection against Insect Pests, Division of Crop Protection and Plant Health, Crop Research Institute, Prague, Czech Republic

* These authors contributed equally to this work.

* stara@vurv.cz



OPEN ACCESS

Citation: Stará J, Kocourek F (2019) Cabbage stem flea beetle's (*Psylliodes chrysocephala* L.) susceptibility to pyrethroids and tolerance to thiacloprid in the Czech Republic. PLoS ONE 14(9): e0214702. <https://doi.org/10.1371/journal.pone.0214702>

Editor: Carla A Ng, University of Pittsburgh, UNITED STATES

Received: March 18, 2019

Accepted: September 4, 2019

Published: September 20, 2019

Copyright: © 2019 Stará, Kocourek. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript.

Funding: The work was financially supported through a grant from the projects MZE-R00418 and NAZV QK 1820081 of the ministry of Agriculture of the Czech Republic.

Competing interests: The authors have declared that no competing interests exist.

Abstract

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae), has recently become a major pest species in winter oilseed rape in the Czech Republic. The susceptibility of CSFB populations from two localities to six pyrethroids, two neonicotinoids, one organophosphate and one oxadiazine was evaluated in 2015–2018 in glass vial experiments. The susceptibility of CSFB to thiacloprid and thiamethoxam was evaluated in feeding experiment in 2017 and 2018. High susceptibility of CSFB populations to *lambda*-cyhalothrin, cypermethrin, esfenvalerate, *tau*-fluvalinate, etofenprox, deltamethrin, chlorpyrifos, indoxacarb and acetamiprid was observed in the glass vial experiments. The LC50 and LC90 data obtained for pyrethroids in these experiments in 2015 represent baseline for CSFB resistance monitoring to pyrethroids in the Czech Republic. High tolerance of CSFB to thiacloprid of CSFB was demonstrated in glass vial and the feeding experiment, too.

Introduction

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* Linnaeus 1758 (Coleoptera: Chrysomelidae) is one of the major pest species of winter oilseed rape in Western Europe [1,2,3]. CSFB has one generation per year and undergoes summer diapause. The period of adult summer diapause is not influenced through constant or changing environmental temperatures or short or long daylight conditions [4]. In autumn, adults migrate into new fields of oilseed rape. Females lay eggs after mating on or close to the plants. After hatching, the larvae feed on leaf bases and terminal leaflets. All the developmental stages can overwinter and females can resume laying eggs in the spring.

Since 2009, the first evidence of CSFB resistance to pyrethroids has been demonstrated as a consequence of the long-term selective pressure of pyrethroids applied for the control of CSFB in oilseed rape [2]. The resistance factor according to the LC50 values for *lambda*-cyhalothrin

reached 80 when 28 local populations of CSFB from Germany were examined [1]. Failure to control CSFB has become a major issue in Germany and the United Kingdom [3]. Until now, no data were available about the susceptibility of CSFB to insecticides in the Czech Republic.

Since 2013, three active neonicotinoid ingredients, thiamethoxam, clothianidin and imidacloprid, have been restricted in Europe for seed treatments [5]. Until the start of the restriction, CSFB control in northern Germany and further regions of Western Europe has relied on seed dressings with neonicotinoids or spraying with pyrethroids in autumn [2,6]. Similarly, CSFB in the Czech Republic was effectively controlled until 2013 by seed dressings of winter oilseed rape with neonicotinoids. Using pyrethroids in oilseed rape in autumn was sporadic. In 2014, the first economically important injuries to oilseed rape caused by CSFB were recorded by farmers in the central part of the Czech Republic. Since 2015, CSFB has been controlled in most of the regions of the Czech Republic by spraying with pyrethroids.

The present study brings the results of i) evaluation of the susceptibility of local populations of CSFB from the Czech Republic to six pyrethroids, two neonicotinoids, one organophosphate and one oxadiazine in glass vial experiment, ii) evaluation of susceptibility of CSFB to two neonicotinoids in feeding experiments.

Materials and methods

Sample collection

The field populations of CSFB originated from winter oilseed rape fields located in Potěhy (49°52'9.695"N, 15°25'12.344"E) and Prague (50°5'15.437"N, 14°17'58.980"E). CSFB adults were collected by sweeping during June in 2015–2018. According to the CSFB population density, 500–1,500 adults per population were collected in particular years and populations. Beetles with some oilseed rape plant material in the net insulators were transported to the laboratory. Prior to initiating the bioassays, samples of the beetles were stored at +5°C. The transported and stored beetles were fed winter oilseed rape leaves. The beetles were tested for susceptibility within 48 h after collection. Only live, mobile insects were used for the bioassays. A total of 4,876 CSFB beetles were tested in bioassays in 2015–2018.

Bioassays

Insecticides. Six analytical standards (*lambda*-cyhalothrin, cypermethrin, esfenvalerate, tau-fluvalinate, etofenprox and deltamethrin), one organophosphate (chlorpyrifos), one oxadiazine (indoxacarb) and two neonicotinoids (thiacloprid and acetamiprid) were obtained from Sigma Aldrich Co. LLC., Prague, Czech Republic. Four to seven doses of insecticides diluted with acetone were used to generate dose-response curves. The rates recommended in the Czech Republic for the field application were calculated assuming that the highest recommended rate of a commercial product in 400 L of water is used per hectare (Table 1). To generate dose-response curves, the rates of the tested insecticides corresponding to 0.1%–1,000% of the field rates were tested (Table 1). Thiacloprid was tested in 2015–2017 as the analytical standard and in 2017–2018 as the formulated product Biscaya 240 OD. Stock solutions of Biscaya 240 OD were prepared by dissolving 140.4 mg of Biscaya 240 OD formulation (containing 32.4 mg of thiacloprid) in 2 mL of distilled water, and subsequently adjusted to 100 mL with acetone. All further dilutions were made in acetone.

Glass vial experiment. IRAC adult vial tests [7] no. 31 (pyrethroids), no. 21 (neonicotinoids), no. 25 (organophosphates) and no. 27 (oxadiazines) were used to conduct the bioassay. The glass vials (P-lab, CZ, internal surface 32.4 cm²) were filled with 520 µL of insecticide and rotated on a hotdog roller at room temperature until the acetone was completely evaporated. Subsequently, the beetles were transferred into each vial. Three replicates were used, and 10

Table 1. The recommended field rates and tested rates of insecticides used to evaluate CSFB susceptibility from the Prague and Potěhy localities in 2015–2018.

Tested insecticides	Field rates (µg/cm ²)	Tested rates (µg/cm ²)
cypermethrin	0.25	0.0025, 0.025, 0.05, 0.25
deltamethrin	0.075	0.000075, 0.00075, 0.0075, 0.015, 0.075, 0.75
esfenvalerate	0.075	0.0075, 0.015, 0.075, 0.75
etofenprox	0.6	0.06, 0.12, 0.60, 6.00
lambda-cyhalothrin	0.075	0.00075, 0.0075, 0.015, 0.075, 0.75
tau-fluvalinate	0.48	0.00048, 0.0048, 0.0192, 0.048, 0.096, 0.48, 4.80
chlorpyrifos	3.00	0.03, 0.0752, 0.15, 0.30, 0.60, 3.00
indoxacarb	0.255	0.024, 0.048, 0.24, 2.40
acetamiprid	0.20	0.002, 0.02, 0.04, 0.20
thiacloprid	0.72	0.072, 0.144, 0.72, 1.44, 7.20

<https://doi.org/10.1371/journal.pone.0214702.t001>

beetles were used for each insecticide dose in each replication. The control vials were treated with pure acetone. The vials were incubated under controlled conditions at 20°C and 60% relative humidity with a 16:8 h light:dark photoperiod. The number of severely affected beetles (i.e., dead and moribund) was counted after 48 h.

Feeding experiment. Oilseed rape of the Exssence variety was sown at the rate of 400,000 seeds per ha with a plot seeder Wintersteiger (Wintersteiger Sägen GmbH, Arnstadt, Germany) on small plots (0.2 ha) in the field in Prague locality with three variants: (1) without seed dressing, (2) seed dressing with Sonido (thiacloprid), and (3) seed dressing with Cruiser (thiamethoxam). The plots were left without treatment with herbicides or insecticides. The conditions of the experiment permitted the natural infestation by CSFB adults. The incidences of adults in the plots were monitored by Moericke yellow traps. The traps were inspected in 2–3 day intervals. In 2017, the plants from the experiment were collected in two periods, 24 days and 35 days after sowing of the oilseed rape seeds. 5.5 and 6.2 adults were caught per trap from the plant emergence to the first and second term of plant collection, respectively. Growth stage of oilseed rape plants according to BBCH-scale corresponding to dates of plant sampling are presented in Table 2. In 2018, the plants were collected 29 days after sowing of the oilseed

Table 2. Content of pesticide residues in oilseed rape plants (values ± standard deviation), average weight of plants before analysis and average injury to plants caused by CSFB. Values with different letters within a column in each experiment are significantly different. LOQ—limit of quantification of the analytical method.

Terms of seeding	days from seeding	variant	average content of pesticides residues (mg/kg)	average weigh of plant (g)	average injury of plant (%)	BBCH-scale
12 th September 2017	24	control	<LOQ	0.34 ± 0.04 ^a	34.7 ± 3.27 ^a	12
		thiacloprid	185 ± 96.6 ^a	0.28 ± 0.02 ^a	46.3 ± 10.6 ^a	12
		thiamethoxam	3,213 ± 122 ^b	0.57 ± 0.03 ^b	0.97 ± 0.91 ^b	12
12 th September 2017	35	control	<LOQ	0.83 ± 0.11 ^a	74.7 ± 12.1 ^a	13
		thiacloprid	43.7 ± 12.2 ^a	0.91 ± 0.12 ^a	86.8 ± 4.15 ^a	13
		thiamethoxam	208 ± 47.8 ^b	1.09 ± 0.18 ^a	9.08 ± 3.49 ^b	13
13 th September 2018	29	control	<LOQ	0.84 ± 0.32 ^a	37.0 ± 21.6 ^a	13
		thiacloprid	44.8 ± 0	1.80 ± 0.90 ^b	37.0 ± 16.4 ^a	13
		thiamethoxam	101 ± 0	1.69 ± 0.39 ^b	15.0 ± 9.72 ^b	13

<https://doi.org/10.1371/journal.pone.0214702.t002>

rape seeds. 1.8 adults were caught per trap from the plant emergence to the term of plant collection. Regarding to the intentional late seeding of oilseed rape in experimental plots, the plant injuries caused by ECB adults were high (Table 2). On each plant collection day, the plants were weighed on a Kern electronic scale EMB200-2 (Kern & Sohn, Balingen, Germany) and the injury caused by CSFB adults to the plants in percentage of leaf loss was evaluated. From 10 to 15 plants in three repetitions from each variant were analysed for their content of thiacloprid and thiamethoxam residues.

Analysis of residues of neonicotinoids in oilseed rape plants

The residues of thiacloprid and thiamethoxam as active substances of the seed dressings Sonido and Cruiser, respectively, were analysed in samples of oilseed rape plants by the QuE-ChERS method followed by LC-MS/MS. From each variant and repetition, a composite sample of 2.50 g of plants was used for the analyses. The analyses were carried out in the accredited laboratory at the University of Chemistry and Technology in Prague as a service.

Data analysis

The bioassay data were analysed using a probit analysis via a dose-effect analysis in XLSTAT 2015 (Addinsoft USA, New York, NY, USA). The doses of insecticides were ln-transformed. The lethal concentrations (LC50s) were fitted with 95% confidence limits. The LC50 values in particular bioassays were considered significantly different when the respective 95% confidence limits did not overlap. A Pearson correlation was used to analyse the correlation between years and LC50s of insecticides *lambda*-cyhalothrin, *tau*-fluvalinate and thiacloprid. Pearson correlation was used also to analyse the correlation between years and slopes of the probit models calculated for insecticides *lambda*-cyhalothrin, *tau*-fluvalinate and thiacloprid.

The mortality data of the CSFB after application of thiacloprid in particular years and localities in the glass vial experiments and the injury of plants, weight of the oilseed rape plants and content of active substances of pesticides in the seed-dressing experiments were compared using a one-way analysis of variance (ANOVA) after testing their normality by the Shapiro-Wilk test using the XLSTAT 2015 statistical software package (Addinsoft Inc., New York, USA). The means were separated using the Tukey HSD analysis.

Results

Susceptibility to pyrethroids in the glass vial experiment

The susceptibilities of CSFB populations from Prague and Potěhy localities in 2015–2018 to 6 active substances of pyrethroids expressed as LC50s are given in Table 3. The probit data (N, LC50, slope, *chi*-square and *R*²) for particularly years are presented in S1–S4 Tables. In relation to data from Mecklenburg-Western Pomerania in Germany [1], the LC50 values demonstrated that both tested local CSFB populations were highly susceptible to the pyrethroids cypermethrin, deltamethrin, esfenvalerate, etofenprox, *lambda*-cyhalothrin and *tau*-fluvalinate. The LC50s for the pyrethroids evaluated after 48 h ranged from 0.0002 µg/cm² (deltamethrin, Prague 2016) to 0.13 µg/cm² (*tau*-fluvalinate, Prague 2018). The LC50 values for *lambda*-cyhalothrin didn't differ between years 2015–2018 in CSFB populations from both the localities (Table 3). The LC50 values for *tau*-fluvalinate for CSFB from Potěhy in 2018 was significantly higher than the values in 2015 (Table 3). Similarly, the LC50 values for *tau*-fluvalinate in Prague in 2017 and 2018 were higher than the values in 2015 (Table 3). However, the slope of the bioassay with *tau*-fluvalinate on CSFB samples from Prague locality in 2015 was steep and calculation of confidence limits was not possible. Hence, comparisons of the CSFB

Table 3. Lethal concentrations LC50 ($\mu\text{g}/\text{cm}^2$) of active substances of insecticides and confidence limits (CL) calculated for populations of CSFB collected in Prague locality in 2015–2018. Values with different numbers for the same letters in the same line are significantly different (the CLs overlap). nd—no CL defined. x—no data available.

population	active substance	2015	2016	2017	2018
Prague	<i>lambda</i> -cyhalotrin	0.0004 (0.0002/0.0006) ^a	x	0.001 (0.0004/0.002) ^a	0.001 (0.0005/0.003) ^a
	<i>tau</i> -fluvalinate	0.008 (nd)	x	0.09 (0.06/0.13) ^a	0.13 (0.09/0.20) ^a
	deltamethrin	0.0006 (0.0004/0.001) ^b	0.0002 (0.0001/0.0003) ^a	x	x
	chlorpyrifos	0.67 (0.55/0.98) ^b	0.07 (0.05/0.08) ^a	0.03 (nd)	0.14 (nd)
	indoxacarb	0.03 (0.01/0.06) ^a	0.02 (0.02/0.03) ^a	x	x
	acetamiprid	0.03 (0.03/0.04) ^b	0.002 (0.0005/0.003) ^a	x	x
	thiacloprid	0.63 (0.32/7.54) ^a	7.92 (2.77/89.7) ^{ab}	139 (67.8/534) ^b	x
	Biscaya	x	x	0.79 (0.52/1.36) ^a	1.48 (0.54/33.7) ^a
	Potěhy	<i>lambda</i> -cyhalotrin	0.002 (0.001/0.003) ^a	x	x
<i>tau</i> -fluvalinate		0.02 (0.01/0.03) ^a	x	x	0.08 (0.05/0.12) ^b
thiacloprid		8.10 (2.60/152) ^a	14.1 (3.79/686) ^a	67.8 (42.9/127) ^a	x
Biscaya		x	x	0.67 (0.50/0.89)	3.47 (nd)

<https://doi.org/10.1371/journal.pone.0214702.t003>

susceptibility between 2015 and following years was not possible. The LC50 values for *tau*-fluvalinate in 2017 and 2018 in Prague locality were not significantly different (Table 3).

The mortalities of CSFB from Prague and Potěhy localities after application of deltamethrin, cypermethrin, esfenvalerate, etofenprox, *lambda*-cyhalothrin and *tau*-fluvalinate in concentrations corresponding to 100% of the recommended field concentration were 100% in 2015. In 2017 and 2018, the mortality of CSFB from Prague locality after application of *tau*-fluvalinate in concentration corresponding to 100% of the recommended field concentration decreased to 86.7% and 79.2%, respectively (Table 4). However, the difference in CSFB mortalities from Prague locality between years was not statistically significant. Similarly, the mortality of CSFB from Potěhy decreased from 2015 to 2018 to 86.9% and the difference in mortalities was statistically significant (Table 4). No decrease of CSFB mortality in 2017 or 2018 was observed after application of *lambda*-cyhalothrin in both the localities (Table 4).

The increased LC50 values for *tau*-fluvalinate in 2017 and 2018 together with the decreased mortality after application of *tau*-fluvalinate in 2017 and 2018 indicates a shift in CSFB susceptibility to *tau*-fluvalinate after three years of CSFB control by spraying *tau*-fluvalinate in the spring period onto oilseed rape.

Pearson analysis found statistically significant correlation between years and LC50 values for *tau*-fluvalinate ($r_p = 0.924$, $p = 0.025$, $R^2 = 0.854$) and thiacloprid ($r_p = 0.813$, $p = 0.045$, $R^2 = 0.661$) while the correlation between years and LC50 values for *lambda*-cyhalothrin was not significant ($r_p = 0.414$, $p = 0.489$, $R^2 = 0.171$). In opposite to this, the correlation between years and slope of the probit model for *lambda*-cyhalothrin was significant ($r_p = -0.944$, $p = 0.016$, $R^2 = 0.891$) but was not significant for *tau*-fluvalinate ($r_p = -0.651$, $p = 0.234$, $R^2 = 0.424$) and thiacloprid ($r_p = 0.644$, $p = 0.167$, $R^2 = 0.415$).

Susceptibility to organophosphates in the glass vial experiment

Sufficient susceptibility of CSFB from two localities to chlorpyrifos was demonstrated in 2015–2018 (Table 3, S1–S4 Tables). The LC50 values ranged from 0.01 $\mu\text{g}/\text{cm}^2$ (Prague 2016) to 0.67 $\mu\text{g}/\text{cm}^2$ (Prague 2015). The slopes of the bioassays from Potěhy in 2015 and Prague in 2017 and 2018 were steep and calculation of confidence limits were not possible (S1, S3 and S4 Tables).

Table 4. Mean mortality (%) of CSFB from Prague and Potěhy localities at 100% of the recommended field rate of insecticides. Standard deviations are given in brackets. Values with different letters within a row are significantly different. x—no data available. F, df, R²—ANOVA data.

population	active substance	2015	2016	2017	2018	F	df	R ²
Prague	lambda-cyhalotrin	100 (0)	x	100 (0)	100 (0)	x	x	x
	tau-fluvalinate	100 (0) ^a	x	86.7 (18.9) ^a	79.2 (19.1) ^a	1.27	6	0.30
	deltamethrin	100 (0)	100 (0)	x	x	x	x	x
	chlorpyrifos	100 (0)	100 (0)	100 (0)	100 (0)	x	x	x
	indoxacarb	100 (0) ^a	97.5 (4.71) ^a	x	x	0.71	5	0.13
	acetamiprid	100 (0)	100 (0)	x	x	x	x	x
	thiacloprid	50 (16.3) ^a	20 (8.16) ^a	0 (0) ^a	x	23.7	6	0.89
	Biscaya	x	x	53.3 (4.71) ^b	27.4 (4.49) ^a	36.1	4	0.90
Potěhy	lambda-cyhalotrin	100 (0)	x	x	100 (0)	x	x	x
	tau-fluvalinate	100 (0) ^a	x	x	86.9 (4.44) ^b	98.9	4	0.96
	thiacloprid	43.3 (9.43) ^a	26.7 (23.5) ^a	0 (0) ^a	x	5.23	5	0.68
	Biscaya	x	x	33.3 (5.77) ^a	16.3 (15.5) ^a	1.80	4	0.31

<https://doi.org/10.1371/journal.pone.0214702.t004>

The CSFB mortality after application of chlorpyrifos in concentration corresponding to 100% of the recommended field concentration was 100% in Prague locality (Table 4). The same CSFB mortality was obtained in Potěhy locality in 2015 (data not presented). Data obtained for chlorpyrifos in 2015–2018 demonstrate stable high CSFB susceptibility from Prague to organophosphates.

Susceptibility to oxadiazine in the glass vial experiment

The LC50 values for CSFB from two localities in 2015–2016 showed a high susceptibility to indoxacarb (Table 3, S1 and S2 Tables). The LC50s for indoxacarb ranged from 0.02 µg/cm² (Prague 2016) to 0.03 µg/cm² (Prague 2015, Potěhy 2015) and did not differ significantly between populations. The CSFB mortality after application of indoxacarb in concentrations corresponding to 100% of the field concentration was 100% in 2015 in both the Prague (Table 4) and Potěhy (data not presented) localities. In 2016, the mortality of CSFB from Prague decreased to 97.5% (Table 4). However, the difference in mortalities between 2015 and 2016 was not significantly different.

Susceptibility to neonicotinoids in the glass vial experiment

The LC50 values indicate that the CSFB populations from two localities were susceptible to acetamiprid. The LC50s ranged from 0.002 µg/cm² (Prague 2016) to 0.03 µg/cm² (Prague 2015) (Table 3, S1 and S2 Tables). The CSFB mortalities after application of acetamiprid in concentrations corresponding to 100% of the recommended field concentration was 100% in both the Prague (Table 4) and Potěhy (data not presented) localities.

In contrast to acetamiprid, the susceptibility of CSFB populations from both the localities to thiacloprid tested as analytical standard was insufficient. The LC50 values for thiacloprid ranged from 0.63 µg/cm² (Prague 2015) to 139 µg/cm² (Prague 2017) (Table 3, S1–S3 Tables). In both localities, comparison of the LC50 values between the years showed a decreasing susceptibility of CSFB to thiacloprid from 2015 to 2017 (Table 3). The LC50 values obtained for thiacloprid as a formulated product Biscaya 240 OD were much lower than the values for analytical standard of thiacloprid (Table 3).

The CSFB mortality after application of analytical standard of thiacloprid in concentrations corresponding to 100% of the recommended field concentration decreased from 50.0% in

Prague locality in 2015 to 0% in both the localities in 2017 (Table 4). However, the difference was not statistically significant. The mortality data obtained for formulated product Biscaya 240 OD showed decreasing mortalities of CSFB from 2017 to 2018 in both the localities.

Susceptibility of CSFB to neonicotinoids in the feeding experiment

The content of pesticide residues differed between the treatment variants in both plant collection periods in 2017. In the first period (24 days from seeding), the content of thiamethoxam in plants was 17 times higher than the content of thiacloprid. In the second treatment period (35 days from seeding), the content of thiamethoxam in plants was 5 times higher than the content of thiacloprid (Table 2). In 2018, 29 days from seeding, the content of thiamethoxam in plants was 2 times higher than the content of thiacloprid. The average weight of the plants was significantly higher in the thiamethoxam variant than in the thiacloprid and control variants in the first plant collection period in 2017. In the second plant collection period in 2017 and in 2018, the average weight of plants did not differ significantly between the variants thiacloprid and thiamethoxam. The average injury to plants in the thiacloprid variant did not differ significantly from the control variant without treatment and was significantly higher than in the thiamethoxam variant in both years and all periods of plant collection (Table 2).

Discussion

Susceptibility to pyrethroids in the glass vial experiment

The present study showed good efficacy of the pyrethroids *lambda*-cyhalothrin, deltamethrin, cypermethrin, *tau*-fluvalinate, esfenvalerate and etofenprox against local CSFB populations in the Czech Republic in 2015–2018. The LC50 for *lambda*-cyhalothrin in the Prague population in 2015 was 0.0004 $\mu\text{g}/\text{cm}^2$. Heimbach and Müller [1] recorded in Germany in 2008 the most sensitive population to *lambda*-cyhalothrin with an LC50 of 0.0002 $\mu\text{g}/\text{cm}^2$ (assessment after 5 h). Compared to the most susceptible CSFB population from Germany, resistance factor of 5 and 20 were calculated in 2018 in the samples from Prague and Potěhy, respectively. Similarly, Højland and Kristensen [8] recorded the most susceptible population to *lambda*-cyhalothrin from North Denmark with LC50 of 0.00012 $\mu\text{g}/\text{cm}^2$. Compared to this population, resistance factor of 8 and 33 were calculated in 2018 in the samples from Prague and Potěhy, respectively. The samples from Potěhy in 2018 were even more resistant than the most resistant population from South Denmark with resistance factor 28 [8]. However, 100% mortality of CSFB from both Prague and Potěhy populations after application of *lambda*-cyhalothrin in a concentration corresponding to 100% of the recommended field concentration still reflects a satisfactory efficacy of *lambda*-cyhalothrin. The mortality response of the samples from Potěhy to reduced rate of *lambda*-cyhalothrin showed slight decrease in sensitivity. The samples from Potěhy in 2018 showed 70.3% mortality at 0.015 $\mu\text{g}/\text{cm}^2$ *lambda*-cyhalothrin in the bioassay compared to 93.3% mortality of the samples from Prague (data not presented). The slight decrease in the susceptibility of CSFB to pyrethroids in the Potěhy population might reflect the repeated application of pyrethroids in the last four years in this locality.

The LC50 and LC90 values for pyrethroids in 2015 in the Prague population represent a susceptibility baseline of CSFB prior to the selective pressure of pyrethroids on resistance in the Czech Republic. These baseline data can be used to monitor the susceptibility of CSFB populations to pyrethroids in the Czech Republic. Wide scale monitoring of resistance in 2017 on 7 populations from the Czech Republic (unpublished data) showed high susceptibility to *lambda*-cyhalothrin and *tau*-fluvalinate and slight decrease of susceptibility of one population to *tau*-fluvalinate. In 2018, monitoring of resistance (unpublished data) showed high

susceptibility of 10 populations to *lambda*-cyhalothrin and decrease of susceptibility to *tau*-fluvalinate in 13 from the 14 tested populations.

High susceptibility to pyrethroids with very low resistance ratios were also observed in Danish CSFB samples [3]. In contrast to this, evidence for *kdr*-resistance in CSFB was published from Germany [2], where the pyrosequencing diagnostic assay showed a *kdr*-allele frequency of 90–100% in populations expressing high levels of resistance in adult vial tests. Cross-resistance to *lambda*-cyhalothrin, *tau*-fluvalinate, etofenprox and bifenthrin was also shown [2]. In contrast to the German samples, where the survival of CSFB at high concentrations of *lambda*-cyhalothrin correlated well with the presence of the *kdr*-allele, the presence of *kdr*-genotypes in samples from the United Kingdom did not always correlate well with resistant phenotypes. This indicates a possible incidence of an as yet undisclosed, metabolic-based mechanism [3]. Regarding an alarming trend in the spread of CSFB resistance to pyrethroids, including the *kdr*-mutation and metabolic-based mechanisms, it is not recommended to alternate the insecticides on the basis of pyrethroids in anti-resistance strategies. The introduction of pesticides with alternate modes of action is necessary.

Susceptibility to organophosphates and oxadiazine in the glass vial experiment

Evaluation of the susceptibility of CSFB to insecticides showed a high susceptibility of two local CSFB populations from the Czech Republic to chlorpyrifos and indoxacarb. Up to now, no resistance to chlorpyrifos has been recorded in CSFB. LC50 value 0.01 $\mu\text{g}/\text{cm}^2$ for chlorpyrifos was established in this paper for the most sensitive sample of CSFB population. Good efficacy of organophosphates was also published against *Brassicogethes aeneus* [9] and *Ceutorhynchus obstrictus* [10]. Chlorpyrifos has recently been recommended for the control of CSFB in the Czech Republic because of the need for alternation with pyrethroid-based insecticides in anti-resistance strategies.

Similarly, no resistance of CSFB or *B. aeneus* to indoxacarb was recorded up to now. LC50 value 0.02 $\mu\text{g}/\text{cm}^2$ for indoxacarb was established in this paper for the most sensitive sample of CSFB population. Till present, indoxacarb has not been used for control of CSFB in the Czech Republic and is a promising pesticide for anti-resistant strategies to slow down selection of CSFB resistance to pyrethroids. No cross-resistance between indoxacarb and pyrethroids and organophosphates was shown in insecticide-resistant *Spodoptera frugiperda* and *Plutella xylostella* [11]. However, a high resistance level to indoxacarb has been published for *C. obstrictus* from Poland [12].

Susceptibility to neonicotinoids in the glass vial experiment

This study showed different susceptibilities of both CSFB populations to the neonicotinoids acetamiprid and thiacloprid. Both tested populations of CSFB were highly susceptible to acetamiprid and simultaneously exhibited tolerance to thiacloprid. In the Czech Republic, thiacloprid is not registered for use against CSFB in oilseed rape crops. However, thiacloprid was frequently used in the Czech Republic against *Ceutorhynchus obstrictus* and *Dassineura brassicae* in flowering period of oilseed rape. These applications influence the CSFB larvae escaping the plants after termination of development. Hence, selection pressure of thiacloprid on CSFB larvae is not eliminated.

The slope values for probit models differed according to groups of insecticides. The lowest slopes with average value < 1.00 were calculated for thiacloprid, the highest slopes were calculated for chlorpyrifos with average value > 10.0 . The slopes for probit models calculated for the rest of the tested insecticides ranged from 1.00 to 10.0 (S1–S4 Tables).

Extensive surveys have shown that stronger resistance to neonicotinoids has been confirmed in some populations of *Bemisia tabaci* and *Leptinotarsa decemlineata* [13], *Trialeurodes vaporariorum* [14], *Nilaparvata lugens* [15] and *Myzus persicae* [16]. A study of *L. decemlineata* resistance to imidacloprid in populations from North America and Europe revealed an approximately 30-fold variation in the LC50 values from ingestion and contact bioassays against neonates [17]. After several years of imidacloprid application, 100-fold [18] and 309-fold [19] resistance ratios for imidacloprid were reported in populations of *L. decemlineata* from Long Island.

No shift to lower susceptibility to thiacloprid has been observed in populations of *B. aeneus* and *C. obstrictus* from several European countries in glass vial experiment with a commercially available thiacloprid formulation, Biscaya OD 240 [20]. According to Zimmer and Nauen [21], the adult vial test with an oil-dispersion-based formulation of thiacloprid resulted in a much better bioavailability compared with technical material.

Resistance to neonicotinoids has been slow to develop compared with other insecticide groups [13]. A target-site resistance has been documented from a selected neonicotinoid resistant laboratory strain of *N. lugens* [22]. However, this mutation has not been found in resistant strains of *N. lugens* collected from the field [23]. From recent evidence, it is clear that neonicotinoid resistance is at present based largely on P450-dependent monooxygenases [24]. It was found that up-regulation of CYP6CM1 in *B. tabaci* confers resistance not only to several neonicotinoids but also to pymetrozine [25]. Puinean et al. [16] published evidence that a P450 gene amplification event is associated with neonicotinoid resistance in *M. persicae*. Recently, R81T mutation conferring target-site resistance to neonicotinoids was recorded in *M. persicae* from southern Europe [26].

No cross-resistance between thiacloprid and acetamiprid or thiamethoxam was recorded for CSFB. Cross-resistance to neonicotinoids was described for several populations and biotypes of the whitefly (*B. tabaci*) [13]. Cross-resistance between imidacloprid and thiamethoxam and between acetamiprid and thiamethoxam was observed in *B. tabaci* [27]. Cross-resistance between imidacloprid, thiacloprid and acetamiprid was detected in *N. lugens* [1]. Similarly, cross-resistance between imidacloprid and acetamiprid was detected in *M. persicae* [28]. In the present study, the tolerance of CSFB to thiacloprid was detected with preserved susceptibility to acetamiprid and thiamethoxam. Tolerance to thiacloprid was also demonstrated in honeybees and bumble bees [29]. It was found, that the sensitivity of *Apis mellifera* and *Bombus terrestris* to neonicotinoids is determined by cytochrome P450s of the CYP9Q subfamily and that CYP9Q3 metabolizes thiacloprid with high efficiency but has little activity against imidacloprid. Further research is necessary to prove whether similar mechanism could explain the tolerance of CSFB to thiacloprid and sensitivity to thiamethoxam.

Little or no cross-resistance of neonicotinoids to older insecticide classes, such as pyrethroids, organophosphates and carbamates, has been observed [30]. One strain of the small brown planthopper (*Laodelphax striatellus*) maintained under laboratory conditions apparently acquired an 18-fold resistance to imidacloprid, without exposure to this insecticide, under strong selection from organophosphates and carbamates, while the unselected strains were fully susceptible to imidacloprid [31].

Susceptibility to neonicotinoids in the feeding experiment

The feeding experiment showed different susceptibilities of the Prague CSFB population to thiacloprid and thiamethoxam. The samples were highly susceptible to thiamethoxam and tolerant to thiacloprid. The injury to plants in the thiacloprid variant was comparable to the control variant without seed dressing in the feeding experiment. In contrast to this, the seed

dressing with thiamethoxam importantly prevented CSFB injury in oilseed rape plants (Table 2). The increase in the average weight of plants in the thiamethoxam variant was observed as a result of the prevention of CSFB injury. A very low susceptibility of CSFB to thiacloprid and also to acetamiprid was reported by Dewar [32].

The experiments proved that seed dressing with thiamethoxam was highly effective for the control of oilseed rape plants in their early growth stages against CSFB. As a consequence of the restriction of seed dressing with three neonicotinoids by the European Commission [5], the injury to oilseed rape plants caused by CSFB increased. It resulted in the increased frequency of the foliar application of insecticides and increased costs for the control of oilseed rape in autumn. The increase in the foliar application of insecticides was also documented in the western part of the EU [6]. Seed dressing with thiacloprid had a very low efficacy due to the CSFB tolerance.

Conclusions

The study showed good efficacy of the pyrethroids *lambda*-cyhalothrin, deltamethrin, cypermethrin, *tau*-fluvalinate, esfenvalerate and etofenprox, organophosphate chlorpyrifos and oxadiazine indoxacarb against two local CSFB populations in the Czech Republic in 2015–2018. Both the CSFB populations were susceptible to neonicotinoids acetamiprid and thiamethoxam and tolerant to thiacloprid. The CSFB tolerance to thiacloprid was shown in both glass vial and feeding experiments.

Supporting information

S1 Table. Probit regression model parameters and fitted doses of active substances of insecticides describing the mortality of CSFB from Prague and Potěhy localities in 2015.

The model was $y = ax + b$, with a being log transformed, and the lower and upper confidence limits (CL) shown in parentheses. nd—no CL defined.

(PDF)

S2 Table. Probit regression model parameters and fitted doses of active substances of insecticides describing the mortality of CSFB from Prague and Potěhy localities in 2016.

The model was $y = ax + b$, with a being log transformed, and the lower and upper confidence limits (CL) shown in parentheses. nd—no CL defined.

(PDF)

S3 Table. Probit regression model parameters and fitted doses of active substances of insecticides describing the mortality of CSFB from Prague and Potěhy localities in 2017.

The model was $y = ax + b$, with a being log transformed, and the lower and upper confidence limits (CL) shown in parentheses. nd—no CL defined.

(PDF)

S4 Table. Probit regression model parameters and fitted doses of active substances of insecticides describing the mortality of CSFB from Prague and Potěhy localities in 2018.

The model was $y = ax + b$, with a being log transformed, and the lower and upper confidence limits (CL) shown in parentheses. nd—no CL defined.

(PDF)

Acknowledgments

We thank Anna Macáková and Jana Vincíková for excellent technical support.

Author Contributions

Conceptualization: František Kocourek.

Data curation: Jitka Stará.

Formal analysis: Jitka Stará.

Methodology: František Kocourek.

Supervision: František Kocourek.

Writing – original draft: Jitka Stará.

Writing – review & editing: Jitka Stará, František Kocourek.

References

1. Heimbach U, Müller A. Incidence of pyrethroid-resistant oilseed rape pests in Germany. *Pest Manag Sci*. 2013; 69: 209–216. <https://doi.org/10.1002/ps.3351> PMID: 23345124
2. Zimmer CT, Müller A, Heimbach U, Nauen R. Target-site resistance to pyrethroid insecticides in German populations of the cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). *Pestic Biochem Physiol*. 2014; 108: 1–7. <https://doi.org/10.1016/j.pestbp.2013.11.005> PMID: 24485308
3. Højland DH, Nauen R, Foster SP, Williamson MS, Kristensen M. Incidence, Spread and Mechanisms of Pyrethroid Resistance in European Populations of the Cabbage Stem Flea Beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). *PLoS ONE*. 2015; 10(12): e0146045. <https://doi.org/10.1371/journal.pone.0146045> PMID: 26717570
4. Sáringer G. Summer diapause of cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Col., Chrysomelidae). *Z Angew Entomol*. 1984; 98: 50–54.
5. EC (European Commission). Commission implementing regulation (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation (EU) No 540/2011, as regards the conditions of approval of the active substances clothianidin, thiamethoxam and imidacloprid, and prohibiting the use and sale of seeds treated with plant protection products containing those active substances. *OJ L*. 2013; 139: 12–26.
6. Zhang H, Breeze T, Bailey A, Garthwaite D, Harrington R, Potts SG. Arthropod pest control for UK oilseed rape—comparing insecticide efficacies, side effects and alternatives. *PLoS ONE*. 2017; 12(1): e0169475. <https://doi.org/10.1371/journal.pone.0169475> PMID: 28076392
7. IRAC Susceptibility test series. <http://www.irac-online.org/methods/> Cited 12 March 2019.
8. Højland D, Kristensen M. Target-site and metabolic resistance against λ -cyhalothrin cabbage stem flea beetles in Denmark. *Bull. Insectology* 2018; 71: 45–49.
9. Eickermann M, Delfosse P, Hoffmann L, Beyer M. A note on the insecticide sensitivity status of *Meligethes* species (Coleoptera: Nitidulidae) in Luxembourg. *J Plant Dis Protect*. 2011; 118: 134–140.
10. Zamojska J, Wegorek P. Preliminary Studies on the Susceptibility Level of *Ceutorhynchus assimilis* (Coleoptera: Curculionidae) to Acetamiprid and Chlorpyrifos in Poland and Resistance Mechanisms of the Pest to Acetamiprid. *J Insect Sci*. 2014; 14(1): <https://doi.org/10.1093/jisesa/ieu127> PMID: 25527578
11. Yu SJ, McCord E Jr. Lack of cross-resistance to indoxacarb in insecticide-resistant *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Pest Manag Sci*. 2007; 63: 63–67. <https://doi.org/10.1002/ps.1309> PMID: 17089332
12. Zamojska J. Differences in susceptibility of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) (Coleoptera: Curculionidae) and the pollen beetle (*Meligethes aeneus* F.) (Coleoptera: Nitidulidae) to indoxacarb and deltamethrin and resistance mechanisms of the cabbage seed weevil to indoxacarb. *Phytoparasitica*. 2017; 45: 407–418.
13. Nauen R, Denholm I. Resistance of Insect Pests to Neonicotinoid Insecticides: Current Status and Future Prospects. *Arch Insect Biochem*. 2005; 58: 200–215.
14. Gorman K, Devine G, Bennison J, Denholm I. Report of resistance to the neonicotinoid insecticide imidacloprid in *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Pest Manag Sci*. 2007; 63: 555–558. <https://doi.org/10.1002/ps.1364> PMID: 17437257

15. Wang YH, Wu SG, Zhu YCh, Chen J, Liu FY, Zhao XP, Wang Q, Li Z, Bo XP, Shen JL. Dynamics of imidacloprid resistance and cross-resistance in the brown planthopper, *Nilaparvata lugens*, Entomol Exp Appl. 2009; 131: 20–29.
16. Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS, Williamson MS, Bass C. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. PLoS Genet; 2010; 6: 1–11.
17. Olsen ER, Dively GP, Nelson JO. Baseline susceptibility to imidacloprid and cross resistance patterns in Colorado potato beetle (Coleoptera: Chrysomelidae) populations. J Econ Entomol. 2000; 93: 447–458. <https://doi.org/10.1603/0022-0493-93.2.447> PMID: 10826199
18. Zhao JZ, Bishop BA, Graphius EJ. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol. 2000; 93: 1508–1514. <https://doi.org/10.1603/0022-0493-93.5.1508> PMID: 11057725
19. Mota-Sanchez D, Hollingworth RM, Grafius EJ, Moyer DD. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Pest Manag Sci. 2006; 62: 30–37. <https://doi.org/10.1002/ps.1120> PMID: 16206238
20. Zimmer CT, Köhler H, Nauen R. Baseline susceptibility and insecticide resistance monitoring in European populations of *Meligethes aeneus* and *Ceutorhynchus assimilis* collected in winter oilseed rape. Entomol Exp Appl. 2014; 150: 279–288.
21. Zimmer CT, Nauen R. Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. Pest Manag Sci. 2011; 67: 599–608. <https://doi.org/10.1002/ps.2137> PMID: 21394884
22. Liu ZW, Williamson MS, Lansdell SJ, Han ZJ, Denholm I, Millar NS. A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides. J Neurochem. 2006; 99: 1273–1281. <https://doi.org/10.1111/j.1471-4159.2006.04167.x> PMID: 16981889
23. Puinean AM, Denholm I, Millar NS, Nauen R, Williamson MS. Characterisation of imidacloprid resistance mechanisms in brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). Pestic Biochem Physiol. 2010; 97: 129–132.
24. Jones CM, Daniels M, Andrews M, Slater R, Lind RJ, Gorman K, Williamson MS, Denholm I. Age-specific expression of a P450 monooxygenase (CYP6CM1) correlates with neonicotinoid resistance in *Bemisia tabaci*. Pestic Biochem Physiol. 2011; 101: 53–58.
25. Gorman K, Slater R, Blande JD, Clarke A, Wren J, McCaffery A, Denholm I. Cross-resistance relationship between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag Sci. 2010; 66: 1186–1190. <https://doi.org/10.1002/ps.1989> PMID: 20632380
26. Charaabi K, Boukhris-Bouhachem S, Makni M, Denholm I. Occurrence of target-site resistance to neonicotinoids in the aphid *Myzus persicae* in Tunisia, and its status on different host plants. Pest Manag Sci. 2018; 74: 1297–1301. <https://doi.org/10.1002/ps.4833> PMID: 29266681
27. Elbert A, Nauen R. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Manag Sci. 2000; 56: 60–64.
28. Foster S, Denholm I, Thompson R. Variation in response to neonicotinoid insecticides in peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae). Pest Manag Sci. 2003; 59: 166–173. <https://doi.org/10.1002/ps.570> PMID: 12587870
29. Manjon C, Troczka BJ, Zawora M, Field LM, Bass Ch, Nauen R. Unravelling the Molecular Determinants of Bee Sensitivity to Neonicotinoid Insecticides. Current Biology 2018; 28: 1137–1143. <https://doi.org/10.1016/j.cub.2018.02.045> PMID: 29576476
30. Denholm I, Devine G, Foster S, Gorman K, Nauen R. Incidence and management of insecticide resistance to neonicotinoids. Proc Brighton Crop Prot Conf Pest Dis. 2002; 1: 161–168.
31. Sone S, Hattori Y, Tsuboi S, Otsu Y. Difference in susceptibility to imidacloprid of the population of the small brown planthopper, *Laodelphax striatellus* Fallen, from various localities in Japan. J Pestic Sci. 1995; 20: 541–543.
32. Dewar AM. The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom. Pest Manag Sci. 2017; 73: 1305–1309. <https://doi.org/10.1002/ps.4511> PMID: 28019077