

# Resistance developments in Estonia's population of pollen beetles (*Brassicogethes aeneus*)

Liina Kann<sup>1,2</sup>, Mati Koppel<sup>3</sup>, Tanel Kaart<sup>4</sup>, Bulat Islamov<sup>1</sup>, Pille Sooväli<sup>1</sup> and Andres Mäe<sup>1</sup>

<sup>1</sup>Department of Plant Protection, Estonian Crop Research Institute, J Aamisepa 1, 48309 Jõgeva vald, Estonia

<sup>2</sup>Department of Chemistry and Biotechnology, Tallinn University of Technology, Akadeemia tee 15, Tallinn, Estonia

<sup>3</sup>Chair of Plant Health, Institute of Agricultural and Environmental Sciences,

Estonian University of Life Sciences, Kreutzwaldi 5, 51006, Tartu, Estonia

<sup>4</sup>Chair of Animal Breeding and Biotechnology, Estonian University of Life Sciences, Kreutzwaldi 46, 51006 Tartu, Estonia

e-mail: liina.kann@etki.ee

A total of 111 local pollen beetle populations were collected from both winter and spring oilseed rape fields, in the main oilseed growing regions of Estonia between 2015–2019. The objective was to analyse the insecticide-susceptibility of the pollen beetle population (in the form of *Brassicogethes aeneus*). The pollen beetle samples were tested for sensitivity to lambda-cyhalothrin, thiacloprid, and chlorpyrifos. The efficacy of the tested insecticides varied considerably by region. We observed a clear decrease in susceptibility to lambda-cyhalothrin and thiacloprid, but sensitivity to chlorpyrifos remained stable throughout the period between 2015 and 2019. Amongst the tested samples in that period, a total of 3% were classified as susceptible to lambda-cyhalothrin, 18% as moderately resistant, 70% as resistant, and 7% as highly resistant. In the case of thiacloprid, 21% of the samples were highly susceptible to the insecticide, 39% were susceptible, and 41% had reduced levels of susceptibility to the insecticide. The information which was presented tended to confirm the ongoing evolution of insecticide resistance in the *B. aeneus* population in Estonia, while also highlighting the importance of data-based decisions when optimising insecticide resistance management in the field.

**Key words:** *Brassicogethes aeneus*, oilseed rape, pyrethroid resistance, neonicotinoid resistance, organophosphates resistance, susceptibility monitoring

## Introduction

Changes in climatic conditions are making it more conducive for the spreading of pathogens, pests, and weeds (in terms of milder and more suitable winter weather for the over-wintering of crops), which in turn will lead to more intensive use of pesticides (Hakala et al. 2011, Leger 2021). The pollen beetle, *Brassicogethes aeneus* F. syn. *Meligethes aeneus* F. (Coleoptera: Nitidulidae), is currently the most destructive pest across Europe in oilseed rape of the winter (WOSR) and spring (SOSR) varieties (*Brassica napus* L.) (Slater et al. 2011, Zimmer et al. 2011b). The pollen beetles cause significant yield losses in oilseed rape growing areas (Hansen 2004, Gagic et al. 2016). Severe damage is caused by beetles feeding during early budding stages, leaving podless stalks after flowering and reducing yields (Williams and Free 1978, Ekbohm and Borg 1996, Williams 2010). The long-term intensive use of insecticides has led to the development of resistance in many countries, such as Germany (Heimbach et al. 2006), Denmark (Hansen 2003, Hansen 2008, Kaiser et al. 2018), Czech Republic (Seidenglanz et al. 2017, Stará and Kocourek 2018, Spitzer et al. 2020), Poland (Wegorek and Zamoyska 2008, Węgorzek et al. 2009, Philippou et al. 2011), Finland (Tiilikainen and Hokkanen 2008), and Lithuania (Makūnas et al. 2011, Šmatas et al. 2012).

It is often the case that chemical insecticides are sprayed several times during the growing seasons. Richardson (2008) calculated that the number of applications was similar both for SOSR and WOSR, ranging from zero to four in various countries. In many European countries, pollen beetle populations had effectively been controlled by synthetic pyrethroids until 1999 (Hansen 2003, Zimmer et al. 2011b). The absence of insecticides with a different mode of action (MoA) has increased the problem of insecticide resistance in the pollen beetle population (Heimbach et al. 2006). This continuous selection pressure, starting at the beginning of the 1980s when pyrethroids were introduced into Europe, has over the years changed the susceptibility of pollen beetle sensitivity levels to pyrethroids in many European countries (Williams 2010, Zimmer et al. 2011b). Pyrethroids are contact insecticides which act as sodium channel modulators, causing hyperexcitation and, in some cases, nerve block. Sodium channels are involved in the propagation of action potentials along nerve axons (Palagacheva et al. 2020, Williams 2010, IRAC 2021b). Many resistance cases have been described in the form of a metabolic mechanism which is based on over-expression of cytochrome P450 monooxygenase CYP6BQ23, or on substitutions in the amino acid

sequence of the target protein (Zimmer et al. 2014a). The mutation in CYP6BQ23 plays a major role in resistance to lambda-cyhalothrin (Nauen et al. 2012). The decline in the efficacy of pyrethroids was discovered in 2007 in Denmark and Sweden, due to the appearance of the target-site mutation, L1014F (known as *knock-down resistance*, or *kdr*) (Nauen et al. 2012).

The neonicotinoid class of insecticides were introduced onto the market in Europe in 2007 (Seidenglanz et al. 2017). One of the most often-used systemic insecticides, thiacloprid, has been used in many European countries (Brandes et al. 2018, Williams 2010). Based on the 'Insecticide Resistance Action Committee' (IRAC), which produces the 'Coleoptera Working Group' reports, the majority of European populations were susceptible to neonicotinoid insecticides until 2014. After that, a continuous decrease was observed in susceptibility levels, with reporting in 2018 stating that there is a clear trend which shows an increasing number of pollen beetle populations with a lower level of sensitivity (<75% mortality) (IRAC 2021a). Kaiser et al. (2018) found reduced susceptibility levels against thiacloprid in German populations. The tolerance mechanism to neonicotinoids has not yet been well characterised (Kocourek et al. 2021).

Organophosphates are contact systemic insecticides which act as acetylcholinesterase inhibitors (AChE) (Williams 2010). Organophosphate chlorpyrifos resistance or tolerance in pollen beetles has not been documented (Seidenglanz et al. 2017, Spitzer et al. 2020).

In Estonia, ten years ago, Veromann and Toome (2011) carried out studies on pyrethroid resistance, finding that pollen beetle populations were highly susceptible. Some years later, Kovács et al. (2015) and Kortspärn et al. (2015) had already observed some levels of decrease in susceptibility to pyrethroids. Previous studies regarding resistance to neonicotinoids and organophosphates have not been carried out in the Estonian pollen beetle population.

The objective of this study was to describe and assess the development of resistance against lambda-cyhalothrin, chlorpyrifos, and thiacloprid insecticides in the pollen beetle population during a five-year period. The findings which are presented here also contain a cross-resistance study which compares the susceptibility levels of pollen beetles to lambda-cyhalothrin and thiacloprid.

## Material and methods

### A sample collection of pollen beetles

Pollen beetles were sampled from 111 different locations in Estonia, from both WOSR and SOSR fields in the growing season between 2015 and 2019 (Fig. 1 and Table 1). A total of eight Estonian counties were involved in the study: Lääne- Viru, Jõgeva, Tartu, Põlva, Võru, Valga, Viljandi, and Järva. Approximately 400–500 beetles per field were collected at the oilseed rape phenological growth stage, when between 10–50% of flowers on the main raceme were open (BBCH 61–65). Those beetles which were collected from an individual field were considered to be a single sample or a local population. The minimum distance between fields was 1.7 kilometres. Adult beetles were placed in perforated plastic bags along with some oilseed rape buds as a food source during transportation to the laboratory.



Fig. 1. Pollen beetle sampling sites in 2015–2019. Estonian counties: LÄ = Lääne-Viru; JÕ = Jõgeva; TA = Tartu; PÕ = Põlva; VÕ = Võru; VA = Valga; VI = Viljandi; JÄ = Järva

Table 1. Collection of pollen beetle samples from eight of the main oilseed rape growing counties, between 2015 and 2019

Year	Crop	County								Total <sup>1</sup>
		LÄ <sup>2</sup>	JÕ	TA	PÕ	VÕ	VA	VI	JÄ	
2015	WOSR <sup>3</sup>	2 <sup>5</sup>	5	3	1	1	1	2	– <sup>6</sup>	15
	SOSR <sup>4</sup>	4	6	1	1	–	1	1	1	15
2016	WOSR	–	3	1	–	1	–	1	1	7
	SOSR	–	6	–	–	–	–	–	–	6
2017	WOSR	2	6	–	–	–	–	–	–	8
	SOSR	1	8	–	–	–	–	–	–	9
2018	WOSR	4	5	1	1	1	–	–	1	13
	SOSR	–	3	1	2	3	–	1	2	12
2019	WOSR	2	3	4	–	1	1	1	3	15
	SOSR	3	5	2	–	–	–	1	–	11
	Total <sup>1</sup>	18	50	13	5	7	3	7	8	111

<sup>1</sup> total number of fields; <sup>2</sup> LÄ = Lääne-Viru; JÕ = Jõgeva; TA = Tartu; PÕ = Põlva; VÕ = Võru; VA = Valga; VI = Viljandi; JÄ = Järva; <sup>3</sup> WOSR = winter oilseed rape; <sup>4</sup> SOSR = spring oilseed rape; <sup>5</sup> number of fields; <sup>6</sup> not collected

### A determination of sensitivity levels to insecticides in pollen beetles

Bioassays were conducted by means of the IRAC test methods, No 011, No 021, and No 025 (IRAC 2015). The beetles were stored overnight at room temperature (18–20 °C), following transportation and prior to exposure in order to allow them to recover. About 15–20 adult beetles were placed in glass vials which had been coated with different concentrations of lambda-cyhalothrin, thiacloprid, and chlorpyrifos (Table 2), and these were stored in the laboratory for twenty-four hours, at room temperature, while also being protected from exposure to direct sunlight.

Table 2. Insecticide doses used in the bioassay

Percentage of recommended label application rate	Lambda-cyhalothrin <sup>a</sup> (µg cm <sup>-2</sup> )	Thiacloprid <sup>a</sup> (µg cm <sup>-2</sup> )	Chlorpyrifos <sup>a</sup> (µg cm <sup>-2</sup> )
0	Only acetone	Only acetone	Only acetone
16			0.3
20	0.015	0.144	
100	0.075	0.72	
200		1.44	

<sup>a</sup> The test concentrations of active ingredients being used are based on the IRAC recommendation for field applications of lambda-cyhalothrin (7.5g a.i. ha<sup>-1</sup>), thiacloprid (72g a.i. ha<sup>-1</sup>), and chlorpyrifos (187.5g a.i. ha<sup>-1</sup>)

After the twenty-four hours were up, the beetles were transferred out of glass vials at the centre of a circle which had a diameter of 15cm. Any beetles which did not leave the circle after a period of one minute were considered to have been affected and were classified as having expired. When more than 20% of the beetles in the control treatment were affected, the sample was left out of the data analysis.

Glass vials containing lambda-cyhalothrin (coated with an active ingredient) and thiacloprid (coated with a commercial product called Biscaya<sup>®</sup>) were prepared by Bayer AG (Germany). Glass vials with lambda-cyhalothrin were stored at room temperature and thiacloprid at 4 °C, and for no longer than between 4–6 weeks after preparation. Chlorpyrifos vials were made up according to the IRAC method, No 025 (IRAC 2015), using the Estonian Crop Research Institute laboratory with the addition of active ingredient chlorpyrifos (Sigma-Aldrich, Germany), subsequently being stored at a temperature of –20 °C for no more than one month.

## The use of insecticides by class and the active ingredient in Estonia

Those insecticides which had been registered in Estonia in 2015 for use in oilseed rape and their general use are described in Table 3. Chlorpyrifos was in use until 2019, and thiacloprid until 2020 (Republic of Estonia Agriculture and Food Board 2021).

Table 3. Insecticides which have been authorised in Estonia for use against pollen beetles in oilseed rape and turnip rape, and the utilisation of such insecticides in 2015

MoA <sup>1</sup>	Chemical subgroup <sup>1</sup>	AI <sup>4</sup>	Commercial product <sup>2</sup>	Field treatment <sup>2</sup>			Total use AI, kg <sup>3</sup>
			Name	AI, g l <sup>-1</sup>	l ha <sup>-1</sup>	AI, g ha <sup>-1</sup>	
3A	Pyrethroids Pyrethrins	lambda-cyhalothrin	Kaiso 50 EG	50	0.15	7.5	9.10
			Karate Zeon	50	0.10–0.15	5–7.5	
			Karis 10 CS <sup>6</sup>	100	0.05–0.75	5–75	
1B	Organophosphates	chlorpyrifos	Hel 250 CS <sup>6</sup>	250	0.5–0.75	125–187.5	16.01
			Pyrimex 250 CS <sup>6</sup>	250	0.5–0.75	125–187.5	
			Pyrimex Supreme <sup>6</sup>	262	0.75–1.25	196.5–327.5	
4A	Neonicotinoids	thiacloprid	Proteus OD <sup>5</sup>	100	0.6–0.75	60–75	2177.50
			Biscaya	240	0.3	72	

<sup>1</sup> According to IRAC; <sup>2</sup> Plant protection products which have been authorised in Estonia according to the Republic of Estonia Agriculture and Food Board; <sup>3</sup> The use of pesticides in agricultural holdings by active substance and crop, according to Statistics Estonia: <https://www.stat.ee/>;

<sup>4</sup> AI = active ingredient; <sup>5</sup> Active ingredients, thiacloprid and deltamethrin; <sup>6</sup> Deleted from the register by the end of the 2019 growing period

## Statistical analysis

Statistical analyses were carried out, with figures being constructed using the statistical package, R 3.5.3 (R Core Team 2019). The information was analysed in order to find the levels for beetles, after having considered a total of 13569 beetles in the lambda-cyhalothrin analysis, and 17401 beetles in the thiacloprid analysis. In both analyses, the response variable was the mortality of pollen beetles, with this being presented in the form of the number of affected and non-affected beetles per dose and population combination. The logistic models were fitted out so that they could be used to consider the fixed effects of dose, year, and host crop, and all pairwise and three-wise interaction effects. The mortality of pollen beetles at different combinations of the various involved factors was estimated in the form of marginal means (*alias* the least-square means), on a scale of the response variable (ie. in terms of probability scale), using the package, *emmeans* (Lenth et al. 2021). In pairwise comparisons of different years, and with SOSR and WOSR in the same year and at the same dosage rate combination, the Tukey adjustment was applied for multiple testing. In order to be able to study the relationships between susceptibility to different insecticides in the same populations, use was made of average mortalities per insecticide, doses, and population. With this population-level detail in place, a linear correlation analysis was carried out in order to study the relationships between mortalities in pollen beetles at different levels of dosage of lambda-cyhalothrin and thiacloprid in the same populations. Those average mortalities were also used to determine population resistance levels against lambda-cyhalothrin, according to the IRAC method, No 011 (Table 4, IRAC 2015). In the case of thiacloprid, method No 021 was used (IRAC 2015), and this was classified according to Kaiser et al. (2018). Thiacloprid susceptibility was defined based on mortality at a dosage rate of 200%, being classified as highly susceptible with a mortality rate of >95%, susceptible with mortality between 94% and 75%, and reduced susceptibility with mortality levels between 74% and 50%, and reduced susceptibility with a mortality rate at <50%. The concordance between resistance levels against lambda-cyhalothrin and thiacloprid was tested with the Fisher exact test. All of the results were considered to be statistically significant at  $p \leq 0.05$ .

Table 4. IRAC classification for pyrethroids according to IRAC No 011 (IRAC 2015)

Concentration (% of label rate)	Affected (%)	Classification
100%	100%	Highly susceptible
20%	100%	
100%	100%	Susceptible
20%	<100%	
100%	<100% to ≥ 90%	Moderately resistant
100%	<90% to ≥ 50%	Resistant
100%	< 50%	Highly resistant

## Results

### The susceptibility of pollen beetles to lambda-cyhalothrin

There were significant effects in terms of dose and year, and in host crop by year, host crop by dose, dose by year, and host crop by year by dose interactions, in terms of mortality levels of lambda-cyhalothrin (all  $p < 0.001$ ). However, there was no significant difference in general between SOSR and WOSR ( $p = 0.077$ ). This indicates that, in addition to the effect of dose rate, there were also differences between years which depended both upon dose rate and host crop, but on average mortality rates were no different for pollen beetles which had been collected from SOSR and WOSR fields. However, in terms of susceptibility to lambda-cyhalothrin, a clear decrease was discovered in terms of mortality over time. In 2015, in terms of the mortality levels of pollen beetles in relation to lambda-cyhalothrin at the recommended dose of 100% (7.5g a.i ha<sup>-1</sup>), the rate was approximately 90% both in SOSR and WOSR populations. By 2017 that mortality rate had significantly decreased, down to between 65–69% (for both of the host crops,  $p < 0.001$ ), remaining within the same range or slightly lower in subsequent years (Table 5).

Table 5. Marginal means of pollen beetle mortality (%; ±standard error) at different lambda-cyhalothrin doses

Dose rate	Host crop	Year				
		2015 (n=30) <sup>3</sup>	2016 (n=13)	2017 (n=17)	2018 (n=25)	2019 (n=26)
0%	SOSR <sup>1</sup>	0.3±0.3 <sup>a</sup>	8.5±1.4 <sup>b</sup>	2.7±0.8 <sup>a</sup>	1.9±0.6 <sup>a</sup>	1.0±0.5 <sup>a</sup>
	WOSR <sup>2</sup>	0.2±0.2 <sup>a</sup>	0.0±0.0 <sup>ab</sup>	5.4±1.2 <sup>b</sup>	1.7±0.6 <sup>a</sup>	1.3±0.5 <sup>a</sup>
20%	SOSR	73.7±2.2 <sup>a,A</sup>	65.2±2.5 <sup>a</sup>	33.9±2.4 <sup>b</sup>	48.8±2.0 <sup>c</sup>	30.1±1.9 <sup>b,A</sup>
	WOSR	81.3±1.6 <sup>a,B</sup>	65.2±2.9 <sup>b</sup>	32.5±2.4 <sup>c</sup>	45.2±2.2 <sup>d</sup>	40.9±2.0 <sup>cd,B</sup>
100%	SOSR	90.1±1.4 <sup>a</sup>	96.7±0.9 <sup>b,A</sup>	69.1±2.3 <sup>c</sup>	63.4±1.9 <sup>c</sup>	65.6±2.2 <sup>c</sup>
	WOSR	89.9±1.2 <sup>a</sup>	78.3±2.5 <sup>b,B</sup>	65.2±2.5 <sup>c</sup>	58.1±2.1 <sup>c</sup>	61.2±2.0 <sup>c</sup>

<sup>1</sup>SOSR: spring oilseed rape; <sup>2</sup>WOSR: winter oilseed rape; <sup>3</sup>Number of populations tested. Those means without a common small superscript letter in the same row per year and dose rate combination, and means with different capital superscript letter are statistically significantly different (pairwise comparisons followed by Tukey adjustment for multiple testing). The absence of capital superscript letters indicates an absence of any significant difference between SOSR and WOSR in the same year and at the same dosage rate combinations.

### The susceptibility of pollen beetles to thiacloprid

Similarly to the case with lambda-cyhalothrin, there were significant effects in terms of dosage and year, and in terms of host crop by year, host crop by dose, dose by year, and host crop by year by dose interactions in terms of mortality in the use of thiacloprid (all  $p < 0.001$ ). There was no significant difference in general between SOSR and WOSR ( $p = 0.523$ ). In the use of thiacloprid, changes in the mortality rates for pollen beetles were partially similar: the mortality rate decreased in time, in pollen beetles from both SOSR and WOSR fields. The decrease in mortality was faster in pollen beetles from WOSR fields in comparison to the situation in SOSR fields. The mortality rates with a thiacloprid dose of 200% (72g a.i. ha<sup>-1</sup>) were significantly lower in pollen beetles from SOSR fields in 2015 when compared to the results for those from WOSR fields (90.2% and 97.8% respectively,  $p < 0.001$ ). A significant decrease (in both of the host crops  $p < 0.001$ ) in mortality rates for pollen beetles was observed between 2016 and 2018. In 2018 the mortality rates for pollen beetles from SOSR fields decreased, and was significantly higher when compared to that of pollen beetles from WOSR fields (67.8% and 56.7% respectively,  $p < 0.001$ ). However, mortality rates increased significantly in pollen beetles from both host crops (going up by 9.6% in SOSR fields and by 21.6% in WOSR fields, both  $p < 0.001$ ), and reaching a comparable level of between 77–78% in 2019 (Table 6).

Table 6. Marginal means of pollen beetle mortality (%;  $\pm$ standard error) at different thiacloprid doses

Dose rate	Host crop	Year				
		2015 (n=30) <sup>3</sup>	2016 (n=13)	2017 (n=17)	2018 (n=25)	2019 (n=26)
0%	SOSR <sup>1</sup>	0.3 $\pm$ 0.3 <sup>a</sup>	8.5 $\pm$ 1.4 <sup>b</sup>	2.7 $\pm$ 0.8 <sup>a</sup>	1.9 $\pm$ 0.6 <sup>a</sup>	1.0 $\pm$ 0.5 <sup>a</sup>
	WOSR <sup>2</sup>	0.2 $\pm$ 0.2 <sup>a</sup>	0.0 $\pm$ 0.0 <sup>ab</sup>	5.4 $\pm$ 1.2 <sup>b</sup>	1.7 $\pm$ 0.6 <sup>a</sup>	1.3 $\pm$ 0.5 <sup>a</sup>
20%	SOSR	64.9 $\pm$ 2.4 <sup>a,A</sup>	53.5 $\pm$ 2.6 <sup>b</sup>	29.0 $\pm$ 2.6 <sup>c</sup>	42.9 $\pm$ 2.2 <sup>d</sup>	29.4 $\pm$ 2.1 <sup>c,A</sup>
	WOSR	80.9 $\pm$ 1.6 <sup>a,B</sup>	51.0 $\pm$ 3.1 <sup>b</sup>	26.7 $\pm$ 2.4 <sup>c</sup>	40.8 $\pm$ 2.1 <sup>bd</sup>	37.9 $\pm$ 2.2 <sup>d,B</sup>
100%	SOSR	85.4 $\pm$ 1.8 <sup>a,A</sup>	75.3 $\pm$ 2.2 <sup>b</sup>	54.5 $\pm$ 2.9 <sup>c</sup>	64.2 $\pm$ 2.0 <sup>d,A</sup>	58.7 $\pm$ 2.2 <sup>cd,A</sup>
	WOSR	95.7 $\pm$ 0.8 <sup>a,B</sup>	74.3 $\pm$ 2.5 <sup>b</sup>	50.1 $\pm$ 2.6 <sup>c</sup>	55.0 $\pm$ 2.2 <sup>c,B</sup>	65.3 $\pm$ 2.0 <sup>d,B</sup>
200%	SOSR	90.2 $\pm$ 1.5 <sup>a,A</sup>	96.9 $\pm$ 0.8 <sup>b,A</sup>	81.5 $\pm$ 2.3 <sup>c,A</sup>	67.8 $\pm$ 1.9 <sup>d,A</sup>	77.2 $\pm$ 1.9 <sup>c</sup>
	WOSR	97.8 $\pm$ 0.6 <sup>a,B</sup>	81.7 $\pm$ 2.5 <sup>b,B</sup>	65.4 $\pm$ 2.5 <sup>c,B</sup>	56.7 $\pm$ 2.1 <sup>c,B</sup>	78.3 $\pm$ 1.8 <sup>b</sup>

<sup>1</sup>SOSR: spring oilseed rape; <sup>2</sup>WOSR: winter oilseed rape; <sup>3</sup>number of populations tested. Those means without a common small superscript letter in the same row and means with a different capital superscript letter for each year and dose rate combination are statistically significantly different (pairwise comparisons followed by Tukey adjustment for multiple testing). The absence of any capital superscript letters indicates an absence of significant difference between SOSR and WOSR in the same year and with the same dose rate combination.

### The susceptibility of pollen beetles to chlorpyrifos

The pollen beetle's mortality rates with a test of chlorpyrifos of 16% of the recommended field dose rate (187.5 g a.i. ha<sup>-1</sup>) came out constantly at 100% in all fields, years, and host crops, and no further statistical analyses were carried out in terms of this particular area of information.

### Monitoring resistance levels to lambda-cyhalothrin in pollen beetle populations

The analysis of resistance levels in pollen beetle populations revealed similar differences and changes as for the analysis of mortalities. While in 2015 pollen beetle samples were susceptible or moderately resistant against lambda-cyhalothrin in more than 60% of fields, only resistant or highly resistant samples were found in 2017. The increase in the proportion of resistant populations was faster in WOSR fields in comparison to SOSR fields (Fig. 2).

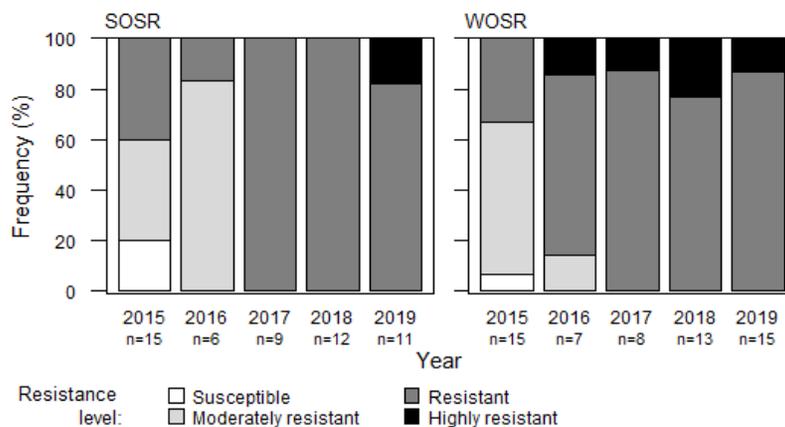


Fig. 2. The distribution of pollen beetle populations according to resistance levels against lambda-cyhalothrin by years and host crops, classified according to IRAC No 011 (IRAC 2015) and Table 2. (SOSR: spring oilseed rape; WOSR: winter oilseed rape)

### Monitoring resistance levels to thiacloprid in pollen beetle populations

In addition, resistance levels in pollen beetles against thiacloprid were low in 2015, with mortality rates at a dosage of 200% being  $\geq$ 75% in all of the tested fields. The samples were highly susceptible, at 86.6% in WOSR fields and at 40.0% in SOSR fields. Even just two years later there were no highly susceptible samples to be found. SOSR fields samples in 2018 were classified as having a reduced susceptibility (mortality rate 50–74%) of 91.7%, and in WOSR fields the samples were found to be of reduced susceptibility of 92.3%, respectively (38.5% of samples from WOSR fields found a mortality rate which was below 50%). The pollen beetles population's susceptibility rates increased again in both types of host crop in the last study year. Among the tested pollen beetles 45.5% of the samples from SOSR fields and 46.7% of the samples from WOSR fields were susceptible to thiacloprid (Fig. 3).

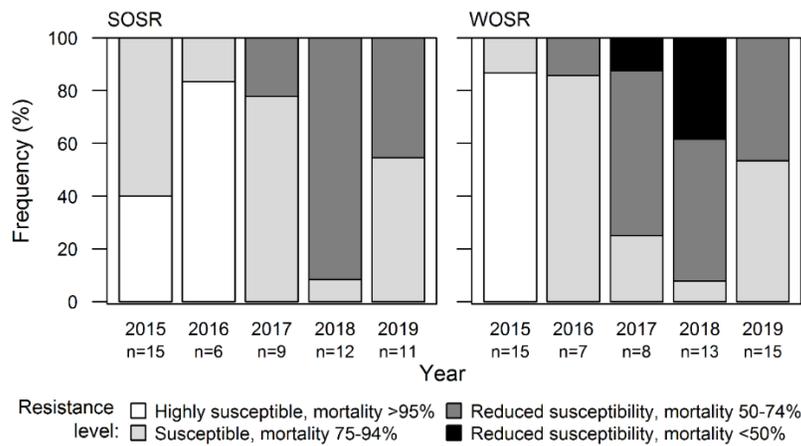


Fig. 3. The distribution of pollen beetle populations against thiacloprid according to resistance levels by years and host crops, classified according to IRAC No 021 (IRAC 2015), and Kaiser et al. (2018). Mortality rates at a dose level of 1.44  $\mu\text{g cm}^{-2}$  (SOSR: spring oilseed rape; WOSR: winter oilseed rape)

### The correlation between lambda-cyhalothrin resistance and thiacloprid resistance

A correlation analysis revealed strong positive relationships between mortalities in pollen beetles at lambda-cyhalothrin dosage rates of 20% and 100%, both from SOSR and WOSR fields ( $r=0.70$  and  $r=0.74$ , both  $p<0.001$ , respectively). The results were also similar in the case of thiacloprid: there existed intermediate positive relationships between mortalities in pollen beetles at dosage rates of 20%, 100%, and 200% in SOSR fields ( $r=0.62$ ,  $r=0.55$ , and  $r=0.57$ , all  $p<0.001$ , for dosage pairs of 20% to 100%, 20% to 200%, and 100% to 200% respectively), and there are strong positive relationships in WOSR fields ( $r=0.80$ ,  $r=0.68$ , and  $r=0.81$ , all  $p<0.001$ , for dosage pairs of 20% to 100%, 20% to 200%, and 100% to 200% respectively).

Also discovered was a relatively strong correlation in terms of resistance against lambda-cyhalothrin and also resistance against thiacloprid. In the fields and across those years which had lower mortality rates for a 100% dosage rate of lambda-cyhalothrin, lower mortality rates were also measured, at a 200% dosage rate of thiacloprid (Fig. 4a), and this relationship did not differ remarkably between SOSR and WOSR (the correlation coefficients were at 0.74 and 0.66, both  $p<0.001$ , respectively). A similar relationship was revealed in the analysis of resistance levels of populations. Populations which were highly resistant against lambda-cyhalothrin were also not susceptible to thiacloprid, and vice versa, while at the same time entire populations which were indeed susceptible to thiacloprid were also susceptible to lambda-cyhalothrin (Fig. 4b).

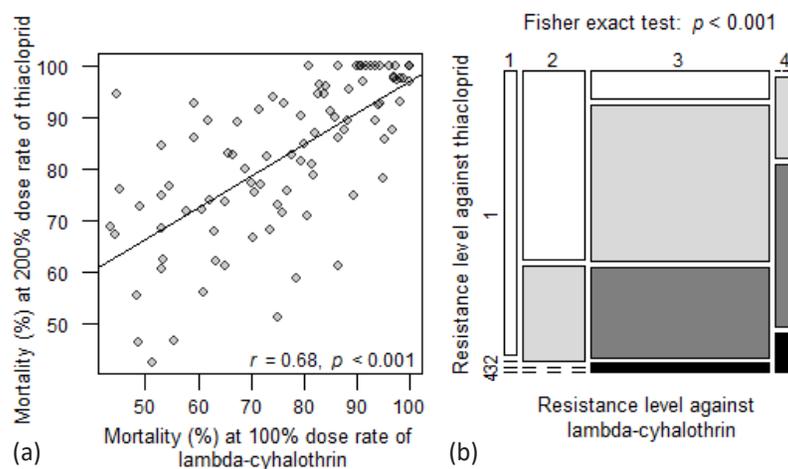


Fig. 4a. The relationship between mortalities at a 100% dosage rate of lambda-cyhalothrin and a 200% dosage rate of thiacloprid; presented numerically is a linear correlation coefficient with  $p$ -value. Fig. 4b. The concordance between resistance levels in populations against lambda-cyhalothrin and thiacloprid; level 4 denotes highly resistant populations and level 1 denotes susceptible, according to IRAC No 011, while level 4 shows reduced susceptibility populations and level 1 shows highly susceptible populations according to IRAC No 021 (IRAC 2015) and Kaiser et al. (2018).

## Discussion

The increasing cultivation of oilseed rape and prevailing milder climatic conditions have created favourable conditions for the spread of pollen beetles which, in turn, leads to the increased use of insecticides in Estonia (Skellern et al. 2017, Leger 2021, Statistics Estonia 2021b).

A retrospective study has been carried out for this paper, covering the years 2015–2019 in order to evaluate changes in insecticide sensitivity in Estonian pollen beetle populations, with collections from those populations having been made from commercial oilseed rape fields. The information which has been provided in this paper has revealed that, after continuous treatment with lambda-cyhalothrin, beetle sensitivity levels to this ingredient have gradually decreased. This is in accordance with details which were presented by Slater et al. (2011), who demonstrated that, in Estonia in the years 2008–2009 pollen beetle populations which had been collected were shown to be mainly susceptible or highly susceptible to lambda-cyhalothrin, but even then there were a few moderately resistant beetles present in the populations. The development of lambda-cyhalothrin resistance has evolved somewhat later in Estonia when compared to other parts of Europe, and this is probably partially due to the less intensive cultivation of oilseed rape and the later authorisation and less intensive use of the insecticides in question (Statistics Estonia 2021a,b). Although the results of this study have revealed fluctuations in mortality rates for pollen beetles which have been treated with lambda-cyhalothrin, a decrease in mortality has been observed across the years 2015–2016 (except SOSR susceptibility increased in the year 2016). Between 2017–2019, those mortality rates stabilised and remained almost at the same level (Suppl. Fig. S1). Similar fluctuation has been detected in Sweden also, where resistance to pyrethroids began in 2001 and peaked in the year 2010, caused by increased usage of different MoA of insecticides and reduced usage of other pyrethroids (cypermethrin, deltamethrin) (Riggi et al. 2016). It indicates that the adaptation of pollen beetles to lambda-cyhalothrin is a time-consuming and complicated process.

A comprehensive study by Hansen (2003) showed that pollen beetle populations in Danish WOSR fields were more resistant than were pollen beetle populations in SOSR fields. No significant correlation was found in resistance between SOSR and WOSR fields in our results across the five year period being studied. This decreasing susceptibility loss in the years 2015–2016 within Estonian pollen beetle WOSR populations may be related to the fact that in countries in which both WOSR and SOSR are cultivated, pollen beetles first attack the winter variety for the purposes of reproduction and later migrate to spring-type host crop fields in order to oviposition (Hokkanen 1993, Ekbohm and Borg 1996, Hansen 2003). Pollen beetles often with the same generations are exposed to insecticides in both WOSR and SOSR fields, thereby increasing the selection pressure for resistance (Richardson 2008, Stratonovitch et al. 2014, Riggi et al. 2016).

Thiacloprid, an insecticide which belongs to the neonicotinoids group, was authorised in Europe in 2007, and has been a very effective insecticide with which to control pollen beetles until 2020, and were an important part of insect resistance management strategies (Republic of Estonia Agriculture and Food Board 2021). Zimmer et al. (2011a, 2014b) found that thiacloprid is effective, and no reduction in susceptibility has been recorded. Šmatas et al. (2012) demonstrated that in field conditions those insecticides which have different MoAs (pyrethroids, neonicotinoids, and organophosphates) revealed the same levels of efficacy against pollen beetles which were collected across the growing seasons for 2009–2010 in Lithuania. It should be noted that amongst those insecticides which were tested, the least effective was thiacloprid (Šmatas et al. 2012). According to the IRAC 'Coleoptera Working Group' and Kaiser et al. (2018), pollen beetle populations with lowered susceptibility to thiacloprid started to appear in Europe from 2018 (such as in Germany, Poland, and Sweden) (IRAC 2021a). Additionally, several subpopulations with reduced susceptibility to thiacloprid were identified in the Czech Republic two years later (Spitzer et al. 2020). And according to Hovorka et al. (2021) only 43.2 % of analysed Czechia populations were found to be highly susceptible to thiacloprid in the years 2017–2020. These findings are in accordance with our study which shows insensitivity loss for thiacloprid in Estonia. The sensitivity towards thiacloprid has gradually decreased between 2015 and 2018. All existing populations were either susceptible or highly susceptible in 2015, and 92% of the total number of populations had reduced susceptibility in 2018 (Fig. 3). Similar to our own findings, a decrease in susceptibility levels to thiacloprid has also been reported in Poland (Seidenglanz et al. 2017). Over a relatively long period now, the Estonian pollen beetle population has been exposed to insecticides, but a systematic resistance testing of the pollen beetle population had not been carried out prior to 2015.

As a result of the intensive use of thiacloprid, insecticide resistance has gradually evolved in Estonia's pest population. Thiacloprid, the active ingredient in Biscaya®, has been registered in Estonia for pollen beetle control in

oilseed rape since 2014, while another insecticide named Proteus OD which contains two classes of MoA (thiacloprid and deltamethrin) has been in use since 2006. This situation regarding oilseed rape fields explains a stepwise decrease in sensitivity levels to thiacloprid in Estonia (Suppl. Fig. S2). A proteogenomic study which was carried out by Kocourek et al. (2021) showed that the continuous presence of pyrethroid and/or neonicotinoid insecticide in the environment can be involved in pathogenesis-related protein 5 resemblances (PR5; up-regulated by insecticides) and RNA (DEAD-box) helicases (down-regulated by insecticides) in pollen beetles. Which may be employed to survive as a response to the presence of insecticides and has led to those changes which have been observed in the genome of the resistant populations.

There turned out to be quite a strong correlation in our study between resistance against lambda-cyhalothrin and thiacloprid. However, contrasting reports have emerged of cross-resistance between lambda-cyhalothrin and thiacloprid insecticides. Seidenglanz et al. (2017) referred to their observation that cross-resistance may exist, although Zimmer et al. (2011a) and Spitzer et al. (2020) could see no cross-resistance between lambda-cyhalothrin and thiacloprid. However, according to Hovorka et al. (2021) there is resistance between those active ingredients. These contrasting results could be caused by different environmental conditions during insecticide application, and different regional selective pressures in terms of insecticide application. Since we have not conducted any molecular or log dose probity mortality studies with our own data, we have to assume that there may be a high probability of pollen beetles being able to adapt to inappropriate conditions, especially since the action mechanisms are different for different insecticides. According to the available literature, it is possible that a metabolic mechanism which is based on enhanced oxidative detoxification due to the over-expression of different monooxygenases can confer cross-resistance to pyrethroids and neonicotinoids (Nauen and Denholm 2005).

We found a spatiotemporal pattern of evolution in regards to lambda-cyhalothrin and thiacloprid, spreading in time from northern Estonia to southern Estonia (Suppl. Figs. S3, S4). In the southern region, the cultivation of oilseed rape is less intensive, and accordingly insecticides are therefore less intensively used (Statistics Estonia 2021b). With the increased use of such insecticides, the further equalisation of resistance possibly occurs both against lambda-cyhalothrin and thiacloprid. Even though it is difficult to predict the emergence and the selection of insecticide resistance, it will occur and will compromise pest control activities.

The susceptibility of pollen beetles to chlorpyrifos was stable. Mortality rates for tested pollen beetles were at 100% throughout the test period. The results from other studies also suggest that chlorpyrifos was highly effective in the control of pollen beetles, and resistance to chlorpyrifos was not detected (Wegorek and Zamoyska 2008, Węgorok et al. 2009, Spitzer et al. 2020, Hovorka et al. 2021). Chlorpyrifos has been banned in Estonia since the year 2019.

Knowledge about the susceptibility of pollen beetles to insecticides would help farmers to adapt their spray programmes before insecticide resistance reaches a fixation level in the population, and insecticide field performance declines as a result. Better insecticide resistance management strategies with the use of insecticides which have different MoAs during the oilseed rape growing season may have resulted in the better control of insects, while integrated pest management with the promotion of beneficial insects into oilseed rape fields are both also necessary. Crop rotation and, whenever possible, growing WOSR are recommended in order to minimise the need for spraying in the cropping season. Furthermore, it is essential to continue with resistance monitoring in the pollen beetle population, ensuring the assessment of sensitivity/resistance dynamics as pollen beetles are major pests which can cause substantial yield losses, especially in SOSR fields.

## Conclusions

The resistance of pollen beetles to lambda-cyhalothrin has spread and increased rapidly across Estonia during the survey period of 2015–2019. A clear decrease of susceptibility in time has been discovered in 2015–2016, and this has remained within the same range across subsequent years (2017–2019). Amongst lambda-cyhalothrin treated samples, a total of 3% were classified as susceptible in the years 2015–2019, while 18% were moderately resistant, 70% were resistant, and 7% were highly resistant. There was no significant difference in susceptibility levels in beetle populations from SOSR and WOSR fields. A shift towards reduced susceptibility to thiacloprid was detected in the years 2015–2018, with 21% of samples being highly susceptible to the insecticide in those years being studied, 39% being susceptible, and 41% having reduced susceptibility. In contrast, the population remained highly susceptible to chlorpyrifos, with a pollen beetle mortality rate of 100% throughout the period. Since 2021 only eleven commercial insecticide products (pyrethroids and indoxacarb) have been available in Estonia. The expected restrictions on the number of insecticides available in the EU presents a challenge to future oilseed rape

production both in Europe in general and Estonia in particular. Resistant management strategies should be advocated in order to prolong the field efficacy of all MoAs which are being used against pollen beetles. The increasing and/or continuous adaptation of pollen beetles to those pesticides which are currently being used, highlights the need to include different IPM-related means of managing this pest.

## Acknowledgments

This research was supported by the Ministry of Rural Affairs of the Republic of Estonia with project no. 10.1-2/177 and by the Ministry of Education and Research of the Republic of Estonia with project “Sordiaretus” no. 2014-2020.4.01.16-0037. The authors would like to express their heartfelt thanks to employees of the Plant Protection Department at the Estonian Crop Research Institute, who helped to collect pollen beetles from the fields. We also appreciate the guidance of Jan Bílovský from Agrotest Fyto Ltd (Czech Republic).

## References

- Brandes, M., Heimbach, U. & Ulber, B. 2018. Impact of insecticides on oilseed rape bud infestation with eggs and larvae of pollen beetle (*Brassicogethes aeneus* (Fabricius)). *Arthropod-Plant Interactions* 12: 811–821. <https://doi.org/10.1007/s11829-018-9616-y>
- Ekbohm, B. & Borg, A. 1996. Pollen beetle (*Meligethes aeneus*) oviposition and feeding preference on different host plant species. *Entomologia Experimentalis et Applicata* 78: 291–299. <https://doi.org/10.1111/j.1570-7458.1996.tb00793.x>
- Gagic, V., Riggi, L.G., Ekbohm, B., Malsher, G., Rusch, A. & Bommarco, R. 2016. Interactive effects of pests increase seed yield. *Ecology and Evolution*: 2149–2157. <https://doi.org/10.1002/ece3.2003>
- Hakala, K., Hannukkala, A.O., Huusela-Veistola, E., Jalli, M. & Peltonen-Sainio, P. 2011. Pests and diseases in a changing climate: A major challenge for Finnish crop production. *Agricultural and Food Science* 20: 3–14. <https://doi.org/10.2137/145960611795163042>
- Hansen, L.M. 2003. Insecticide-resistant pollen beetles (*Meligethes aeneus* F.) found in Danish oilseed rape (*Brassica napus* L.) fields. *Pest Management Science* 59: 1057–1059. <https://doi.org/10.1002/ps.737>
- Hansen, L.M. 2004. Economic damage threshold model for pollen beetles (*Meligethes aeneus* F.) in spring oilseed rape (*Brassica napus* L.) crops. *Crop Protection* 23: 43–46. [https://doi.org/10.1016/S0261-2194\(03\)00167-4](https://doi.org/10.1016/S0261-2194(03)00167-4)
- Hansen, L.M. 2008. Occurrence of insecticide resistant pollen beetles (*Meligethes aeneus* F.) in Danish oilseed rape (*Brassica napus* L.) crops. *Bulletin OEPP/EPP Bulletin* 38: 95–98. <https://doi.org/10.1111/j.1365-2338.2008.01189.x>
- Heimbach, U., Müller, A. & Thieme, T. 2006. First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 58: 1–5.
- Hokkanen, H.M.T. 1993. Overwintering survival and spring emergence in *Meligethes aeneus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. *Entomologia Experimentalis et Applicata* 67: 241–246. <https://doi.org/10.1111/j.1570-7458.1993.tb01674.x>
- Hovorka, T., Kocourek, F., Horská, T. & Stará, J. 2021. Widespread resistance of pollen beetles to pyrethroids in Czechia with no evidence for *kdr* mutation. *Crop Protection* 145: 105648. <https://doi.org/10.1016/j.cropro.2021.105648>
- IRAC 2021a. Insecticide Resistance Action Committee Pollen beetle resistance monitoring posters 2012-2018. <https://irac-online.org/pests/meligethes-aeneus/posters/>. Accessed 24 February 2021.
- IRAC 2021b. The IRAC mode of action classification online. <https://irac-online.org/modes-of-action/>. Accessed 8 October 2021.
- IRAC 2015. Susceptibility Test Methods Series Method No: 011 Version: 3 (June 2009), Method No: 021 Version: 3.4, Method No: 025 Version: 2 (March 2014) <https://irac-online.org/methods/meligethes-aeneus-adults/>. Accessed 2 April 2015.
- Kaiser, C., Jensen, K.M.V., Nauen, R. & Kristensen, M. 2018. Susceptibility of Danish pollen beetle populations against  $\lambda$ -cyhalothrin and thiacloprid. *Journal of Pest Science* 91: 447–458. <https://doi.org/10.1007/s10340-017-0856-x>
- Kocourek, F., Stara, J., Sopko, B., Talacko, P., Harant, K., Hovorka, T. & Erban, T. 2021. Proteogenomic Insight into the Basis of the Insecticide Tolerance/Resistance of the Pollen Beetle *Brassicogethes (Meligethes) Aeneus*. *Journal of Proteomics* 233: 104086. <https://doi.org/10.1016/j.jpro.2020.104086>
- Kortspärn, K., Metspalu, L., Veromann, E., Kaasik, R. & Kovács, G. 2015. The attractiveness of cruciferous oilseed crops to pollen beetles (*Meligethes* spp.); resistance monitoring in pollen beetle populations. Master’s thesis. (in Estonian)
- Kovács G., Kaasik, R., Kortspärn, K., Metspalu, L., Luik, A. & Veromann, E. 2015. The pyrethroid resistance of the pollen beetle is increasing in Estonia. *Agonomy* 2015: 138–141. (in Estonian).
- Leger, R. 2021. Insects and their pathogenes in a changing climate. *Journal of Invertebrate Pathology* 184: 107644. <https://doi.org/10.1016/j.jip.2021.107644>
- Lenth, R.V., Buerkner, P., Herve, M., Love, J., Riebl, H. & Singmann, H. 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.4. <https://cran.r-project.org/web/packages/emmeans/index.html>
- Makūnas, V., Brazauskienė, I. & Šmatas, R. 2011. Resistance of *Meligethes aeneus* to pyrethroids in Lithuania. *Žemdirbystė-Agriculture* 98: 431–438.
- Nauen, R. & Denholm, I. 2005. Resistance of Insect Pests to Neonicotinoid Insecticides: Current Status and Future Prospects. *Archives of Insect Biochemistry and Physiology* 58: 200–215.

- Nauen, R., Zimmer, C.T., Andrews, M., Slater, R., Bass, C., Ekbom, B., Gustafsson, G., Hansen, L.M., Kristensen, M., Zebitz, C.P.W., & Williamson, M.S. 2012. Target-site resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus* F. *Pesticide Biochemistry and Physiology* 103: 173–180. <https://doi.org/10.1016/j.pestbp.2012.04.012>
- Palagacheva, N., Koleva, L., Dimitrov, Y. & Dospatliev, L. 2020. Bioassays resistance of pollen beetle (*Meligethes aeneus* F.) to synthetic pyrethroid cypermethrin. *Scientific Papers. Series A. Agronomy LXIII*: 300–305.
- Philippou, D., Field, L.M., Węgorzek, P., Zamojska, J., Andrews, M.C., Slater, R. & Moores, G.D. 2011. Characterising metabolic resistance in pyrethroid-insensitive pollen beetle (*Meligethes aeneus* F.) from Poland and Switzerland. *Pest Management Science* 67: 239–243. <https://doi.org/10.1002/ps.2061>
- Republic of Estonia Agriculture and Food Board 2021. Plant protection products authorized in Estonia. <https://portaal.agri.ee/avalik/#/taimekaitse/taimekaitsevahendid-otsing/et>. Accessed 28 February 2021.
- Richardson, D.M. 2008. Summary of findings from a participant country pollen beetle questionnaire. *Bulletin OEPP/EPPO Bulletin* 38: 68–72. <https://doi.org/10.1111/j.1365-2338.2008.01183.x>
- Riggi, L.G.A., Gagic, V., Bommarco, R. & Ekbom, B. 2016. Insecticide resistance in pollen beetles over 7 years - a landscape approach. *Pest Management Science* 72: 780–786. <https://doi.org/10.1002/ps.4052>
- Seidenglanz, M., Poslušná, J., Kolařík, P., Rotrekl, J., Hrudová, E., Tóth, P., Havel, J., Plachká, E., Tánčík, J. & Hudec, K. 2017. Negative correlations between the susceptibilities of Czech and Slovak pollen beetle populations to lambda-cyhalothrin and Chlorpyrifos-ethyl in 2014 and 2015. *Plant Protection Science* 53: 108–117. <https://doi.org/10.17221/187/2015-PPS>
- Skellern, M.P., Welham, S.J., Watts, N.P. & Cook, S.M. 2017. Meteorological and landscape influences on pollen beetle immigration into oilseed rape crops. *Agriculture, Ecosystems and Environment* 241: 150–159. <https://doi.org/10.1016/j.agee.2017.03.008>
- Slater, R., Ellis, S., Genay, J.P., Heimbach, U., Huart, G., Sarazin, M., Longhurst, C., Müller, A., Nauen, R., Rison, J.L. & Robin, F. 2011. Pyrethroid resistance monitoring in European populations of pollen beetle (*Meligethes* spp.): A coordinated approach through the Insecticide Resistance Action Committee (IRAC). *Pest Management Science* 67: 633–638. <https://doi.org/10.1002/ps.2101>
- Šmatas, R., Petraitiene, E., Makūnas, V., Brazauskienė, I. & Petraitiene, E. 2012. Sensitivity of pollen beetle (*Meligethes aeneus* F.) to insecticides with different modes of action and their efficacy in the field conditions. *Žemdirbystė-Agriculture* 99: 197–202 p.
- Spitzer, T., Bílovský, J. & Matušinsky, P. 2020. Changes in resistance development in pollen beetle (*Brassicogethes aeneus* F.) to lambda-cyhalothrin, etofenprox, chlorpyrifos-ethyl, and thiacloprid in the Czech Republic during 2013–2017. *Crop Protection* 135: 105224. <https://doi.org/10.1016/j.cropro.2020.105224>
- Stratonovitch, P., Elias, J., Denholm, I., Slater, R. & Semenov, M.A. 2014. An individual-based model of the evolution of pesticide resistance in heterogeneous environments: control of *Meligethes aeneus* population in oilseed rape crops. *PLoS ONE* 9: 1–24. <https://doi.org/10.1371/journal.pone.0115631>
- Stará, J. & Kocourek, F. 2018. Seven-year monitoring of pyrethroid resistance in the pollen beetle (*Brassicogethes aeneus* F.) during implementation of insect resistance management. *Pest Management Science* 74: 200–209. <https://doi.org/10.1002/ps.4695>
- Statistics Estonia 2021a. PM0281: Agricultural land and crops by county <https://www.stat.ee/>. Accessed 19 February 2021
- Statistics Estonia 2021b. KK2085: Quantity of active substance in sales of pesticides. <https://www.stat.ee/>. Accessed 1 March 2021.
- Tiilikainen, T.M. & Hokkanen, H.M.T. 2008. Pyrethroid resistance in Finnish pollen beetle (*Meligethes aeneus*) populations-is it around the corner? *Bulletin OEPP/EPPO Bulletin* 38: 99–103. <https://doi.org/10.1111/j.1365-2338.2008.01190.x>
- Veromann, E. & Toome, M. 2011. Pollen beetle (*Meligethes aeneus* Fab) susceptibility to synthetic pyrethroids-pilot study in Estonia. *Agronomy Research* 9: 365–369.
- Węgorzek, P., Mrówczyński, M. & Zamojska, J. 2009. Resistance of pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. *Journal of Plant Protection Research* 49: 119–127. <https://doi.org/10.2478/v10045-009-0016-2>
- Węgorzek, P. & Zamojska, J. 2008. Current status of resistance in pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. *Bulletin OEPP/EPPO Bulletin* 38: 91–94. <https://doi.org/10.1111/j.1365-2338.2008.01188.x>
- Williams, I.H. 2010. The major insect pests of oilseed rape in Europe and their management: An overview, in: *Biocontrol-Based Integrated Management of Oilseed Rape Pests*. Springer Netherlands. p. 1–43. [https://doi.org/10.1007/978-90-481-3983-5\\_1](https://doi.org/10.1007/978-90-481-3983-5_1)
- Williams, H.I. & Free, J.B. 1978. The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). *The Journal of Agricultural Science* 91: 453–459. <https://doi.org/10.1017/S0021859600046554>
- Zimmer, C.T., Bass, C., Williamson, M.S., Kaussmann, M., Wölfel, K., Gutbrod, O. & Nauen, R. 2014a. Molecular and functional characterization of CYP6BQ23, a cytochrome P450 conferring resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus*. *Insect Biochemistry and Molecular Biology* 45: 18–29. <https://doi.org/10.1016/j.ibmb.2013.11.008>
- Zimmer, C.T., Köhler, H. & Nauen, R. 2014b. Baseline susceptibility and insecticide resistance monitoring in European populations of *Meligethes aeneus* and *Ceutorhynchus assimilis* collected in winter oilseed rape. *Entomologia Experimentalis et Applicata* 150: 279–288. <https://doi.org/10.1111/eea.12162>
- Zimmer, C.T. & Nauen, R. 2011a. Cytochrome P450 mediated pyrethroid resistance in European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae). *Pesticide Biochemistry and Physiology* 100: 264–272. <https://doi.org/10.1016/j.pestbp.2011.04.011>
- Zimmer, C.T. & Nauen, R. 2011b. Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. *Pest Management Science* 67: 599–608. <https://doi.org/10.1002/ps.2137>