

# THESIS

Author

**KIRUI DANIEL KWEMOI**

**GEORGIKON CAMPUS**

**2023**



**HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE  
SCIENCES**

**INSTITUTE OF PLANT PROTECTION**

**MSC. PLANT PROTECTION**

**TITLE: PYRETHROID RESISTANCE MONITORING OF  
POLLEN BEETLE (*Brassicogethes aeneus*) IN HUNGARIAN  
RAPE FIELDS**

**AUTHOR: KIRUI DANIEL KWEMOI**

**Primary Thesis Advisor: Dr ZSOLT MARCZALI (Associate  
professor)**

**GEORGIKON CAMPUS**

**2023**

## TABLE OF CONTENTS

CHAPTER 1.0 INTRODUCTION .....	1
1.1Background of the study .....	1
1.2 Problem statement of the research.....	2
1.3 Aim of the study.....	4
CHAPTER 2.0 LITERATURE REVIEW .....	5
2.1 Oilseed rape. ....	5
2.1.1.Basic requirements.....	6
2.1.2 Health benefits of rapeseed oil .....	7
2.1.3Taxonomy and morphology.....	8
2.1.4 Production .....	10
2.2 Pollen beetle.....	14
2.2.1Main Host plants.....	14
2.2.2Damage.....	15
2.2.3Symptoms .....	17
CHAPTER 3 MATERIALS AND METHODS .....	19
3.1 Materials .....	20
3.2 Methods.....	20
3.2.1 IRAC susceptibility test method No. 011-v3.....	21
3.3 Statistical analysis .....	25
4.1 Kruskal-Wallis mortality Tests .....	27
CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS .....	38
CHAPTER 6. SUMMERY. ....	41

ACKNOWLEDGEMENT.....	42
CHAPTER 7. REFERENCES.....	43

## List of Figures

Figure 1 Development of average yields of oilseed rape in main producing countries from 1961-2017 .....	2
Figure 2 Oilseed rape flowering raceme .....	10
Figure 3 Production of rape and turnip rape seed, sunflower seed, and soybean in million tonnes in the EU .....	12
Figure 4 Illustration of the life cycle of oilseed rape pollen beetle .....	16
Figure 5 Male adult of <i>Brassicogethes aeneus</i> .....	18
Figure 6 Pollen beetle on rape blossom .....	19
Figure 7 Sweep net for collection.....	21
Figure 8 Collected adult beetles. ....	21
Figure 9 Technical grade lambda-cyhalothrin .....	22
Figure 10 Glass vials with 2.27 cm diameter, 8.6cm length, 20ml glass vial (inner surface; 58.218 cm <sup>2</sup> .....	23
Figure 11 Rotation of the vials in a fume hood to vaporise acetone. ....	24
Figure 12 Mortality assessment on a white paper with a 15cm circle .....	24
Figure 13 Graph of mortality for Vése village .....	35
Figure 14 Graph of mortality for Szécsény village .....	35

## List of Tables

Table 1 Harvested production and cultivation area in 2015. ....	13
Table 2 Susceptibility categories utilised for determining pollen beetle susceptibility to pyrethroids.....	25
Table 3 Kruskal–Wallis mortality test for Vése 2018.....	29
Table 4 Kruskal–Wallis mortality test for Vése 2019.....	30
Table 5 Kruskal–Wallis mortality test for Szécsény 2018.....	31
Table 6 Kruskal–Wallis test mortality for Szécsény 2019.....	32

## CHAPTER 1.0 INTRODUCTION

### 1.1 Background of the study

*Brassica napus*, also known as oilseed rape, is a plant in the Brassicaceae family (AHUJA et al.,2011). It is the world's third-largest source of vegetable oil and is mainly grown for its oil-rich seed. The oil is obtained by crushing the seeds. Rapeseed meal, a by-product of the crush, is frequently used in animal feed. Rapeseed oilseed is the second-largest source of protein meal in the world.

The crop has grown to play a significant role in many arable rotations. It is a good substitute for a cereal crop and is frequently called a “break crop.” Growing oilseed rape has several agronomic advantages, such as enhancing soil tilth and serving as an entry crop for first wheat, which can therefore aid in maximising the first wheat yield opportunity. Oilseed rape is a healthy high-margin break crop in the rotation but given the high prices for seeds brought on by the current global demand, it has the potential to be a cash crop if it can be planted and managed till harvest.

Since 1981, the introduction of high-yielding cultivars with an increasing proportion of hybrids has been chiefly responsible for the growth in the oilseed rape crop's average yield. Rapeseed oil for human consumption and meal as a good animal feed now have new markets thanks to the improved yield potential and the development of double-low varieties (2% erucic acid and 25 mol/g glucosinolates in seeds) (DIMOV AND MÖLLERS, 2010). This has increased the crop's profitability and increased the area under cultivation. However, the yearly growth rate of average yields in Europe (Germany, France, and the United Kingdom) and Australia has decreased since 1990, for example, in Germany from 2.1% in 1961-1990 to 0.5% in 1991-2018. In contrast, it continues to rise in China (2.0%) and Canada (1.5%). (Figure 1). The decline in average yields in

Europe and Australia has been linked to increased pest and disease stress, warm temperatures, low precipitation, and a decrease in the availability of registered active ingredients for chemical control, such as the ban on neonicotinoids (KUTCHER et al., 2010; ASSEFA et al., 2018). (NOLEPPA, 2017).

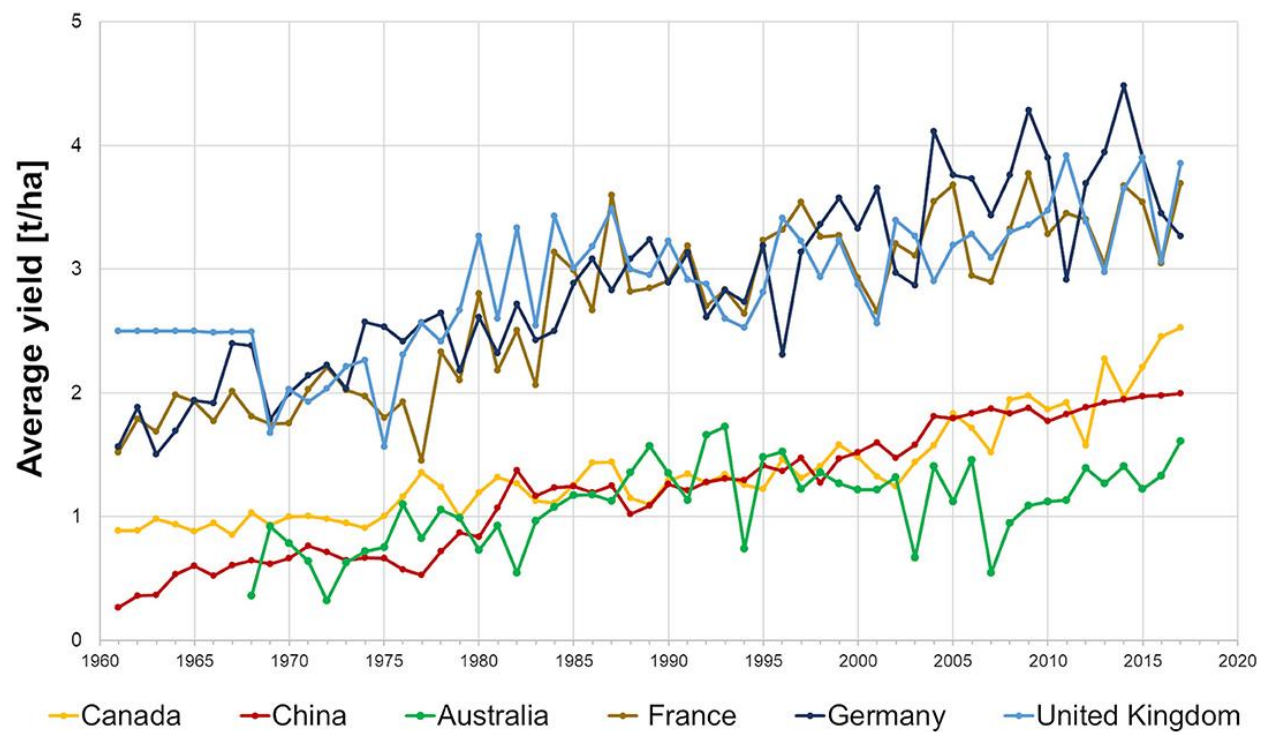


Figure 1 Development of average yields of oilseed rape in main producing countries from 1961-2017

Source FAOSTAT (<http://www.fao.org/faostat/en/#data/QC>)

## 1.2 Problem statement of the research

In Europe, oilseed rape is a valuable crop both commercially and politically. Since 2000, its significance has increased by about 60% in the area. However, the European oilseed rape Pollen



beetle, one of its main pests, has threatened production (*Brassicogethes spp*). When present in large populations, this insect can seriously harm the plant. Heavy beetle assaults can result in a significant loss in output, with some growers in northern Germany losing their entire harvest in 2006.

Broad-spectrum insecticides have historically been used to control pollen beetles. Still, until recently, pyrethroids were the only class of insecticides registered in many countries due to the removal of organophosphates as a control alternative.

The first reports of pesticide resistance in Polish populations of *Brassicogethes aeneus* Fabricius date back to 1967. However, the first instances of pyrethroid resistance were discovered in 1999 in North-Eastern France; however, pyrethroid-resistant beetles may have been widespread as early as 1997. Many studies have now located them using various techniques to evaluate pyrethroid susceptibility in many of the oilseed-rape growing regions of Europe.

The Insecticide Resistance Action Committee organised a Pollen Beetle Working Group in 2007. (IRAC). This action group brought together representatives from agrochemical companies, government regulators and advisors, academics, and research institutes to collaborate on providing essential information and advice that would allow for effective resistance and pest management of pollen beetle and other oilseed rape pests. As a result, the European resistance monitoring program, which employs a fundamental, uniform technique, was established to provide information on the current distribution and severity of pyrethroid-resistant beetles throughout Europe.

### **1.3 Aim of the study**

The study aimed to monitor the spread of pyrethroid-resistant pollen beetle populations because they require different control measures, and affected countries must develop strategies to control them in the future.

## CHAPTER 2.0 LITERATURE REVIEW

### 2.1 Oilseed rape

Oilseed rape is a vital crop in European Agriculture that rose notably to pre-eminence during the last part of the 20<sup>th</sup> century (ALLEN et al., 2001). *Brassica napus* dominate this European crop in the family of Brassicaceae, usually cited as *Brassica napus* spp, *oleifera*, and sometimes turnip rape (*Brassica campestris*). It is well-grown, especially in Finland and Sweden (BORISOVNA et al., 2021). Turnip rape, in some cases, is accorded subspecific status, as *B. campestris* ssp. *oleifera*. *Brassica napus* is widely grown outside Europe- especially in Australia and Canada and is commonly called canola. In Asian countries like Japan, China and India, the dominant rape crop is *B. campestris* (ALFORD et al., 2003).

Worldwide, the oilseed rape area is approximately 30 million ha. About 5 million ha is grown in Europe (ALFORD et al., 2003). Therefore, oilseed rape is significant, especially in central and Northern Europe, particularly in the Czech Republic, Denmark, France, Germany, Hungary, Poland, the UK, and Ukraine (OLESEN et al., 2011).

Seeds from oilseed rape are usually crushed to extract oil (MARTIN et al., 2015). The oil produced is used for culinary purposes, as fuel and as a lubricant, especially on machinery in the food processing industry. It is as well used in the production of synthetic rubber and soaps. The residue of the cake (seed cake) is usually used for fodder for livestock (MIRPOOR et al., 2021). The part that is left uncrushed is used for feeding birds. In some cases, the crop is as well grown as green salads and fodder for cattle.

Rapeseed *Brassica napus* is a biennial or annual herbaceous plant- grown for oil production, usually extracted from its seeds (DIXON et al., 2007). The oilseed plant has several erect which radiate

from a single base. The stems towards the base are purple. The plant's foliage is primarily smooth and bluish green. The highest leaves usually grow straight off the stem, while those at the base are stalked. The plant's inflorescence is pale, bright to yellow flowers whose diameter is about 11–15 mm after pollination pods contains a single row of 20–40 dark brown to black seeds. The growth height of the oilseed rape crop can reach 1.0–2.5m, mainly grown annually and usually harvested after one growing season (ULASET al., 2013)

### **2.1.1 Basic requirements**

The oilseed rape is adapted to have extraordinary growth in cool, moist climates with a temperature range of 2-10 degrees Celsius; however, temperatures closer to 10 degrees accelerate the most rapid growth (HÅKANSSON et al.,2011). Therefore, the oilseed rape is an excellent season crop in subtropical regions and a winter crop in more temperate areas. The plant can grow on various soil types; however, soils of medium texture and well-drained are most preferred. Oilseed rape does well in a PH between 5.5 and 8.3 and never be seeded in the soil where other brassicas have grown within 3-4 years to avoid build-up and potential attack of pests and diseases (GUIDI et al., 2013).

#### **2.1.1.1 Propagation**

Oilseed rape is usually propagated by seeds primarily drilled in rows of a prepared field (MCDONALD et al., 2012). The tiny seeds are sown shallowly at approximately 2–3cm depth. Oilseed rape planted during spring is drilled in rows 18-23cm apart, while those seeded in winter require more space and are recommended to be drilled in rows approximately 40cm apart.

### **2.1.1.2 Crop management practices**

Weed control is vital in oilseed rape as it significantly affects crop growth and productivity (SKELLERN et al.,2018). Better seedbed preparation helps to reduce the growth of weeds. These optimal conditions are achieved by tilling the soil before planting during fall. A soil test is done before planting the oilseed rape to prevent nutrient deficiencies, as the crop has a high nutrient requirement (BARRACLOUGH et al.,1989). Fertiliser application is usually made to the seed furrows' sides to avoid crop damage. Nitrogenous fertiliser is applied at a recommended rate of 50-60 kg per hectare, particularly for oilseed rape planted during spring and 70 kg per hectare for winter sown crops (HOLMES et al.,1997). Besides nitrogenous fertiliser, oilseed rape may require adding other nutrients like phosphorus, potassium, sulphur, and magnesium to the soil. Soil testing determines the rate at which these nutrients are applied (ÖZTÜRK et al., 2010).

### **2.1.1.4 Harvesting**

Oilseed rape is a fast-ripening crop ready for harvesting when the seeds change from green to black (OSMATON et al.,2001). The oilseed rape crop is harvested mechanically by a combine harvester or swathing, while in some countries, it is usually cut by hand, for example, in China (PARI et al., 2020).

### **2.1.2 Health benefits of rapeseed oil**

Culinary and industrial are the two main types of rapeseed oil (GUNSTONE et al.,2004). However, the culinary, also called canola oil, is majorly used for kitchen cooking, while the latter is used in the chemical and automotive industries (PAHL, 2008). As a result, oil is used widely around the world.

Oilseed rape is widely known for its affordability and versatility as a cooking oil and is usually found in fried foods, salad dressings and baked goods (SUÁREZ et al., 2021). It is naturally

deficient in saturated fat while having the highest content of unsaturated fat, which is excellent for human health. In addition, it has an excellent source of E vitamin, the most vital antioxidant that usually improves eye and skin health.

Studies show that rapeseed oil is an excellent source of omega-3 fat called alpha-linolenic acid. Which is more beneficial to human health as it lowers blood pressure and eventually decreases the likelihood of heart attack (PUNIA et al., 2019).

### **2.1.3 Taxonomy and Morphology**

Around 3740 species in 325 genera comprise the Brassicaceae, also known as the mustard family (HOHMANN et al.,2015). It consists primarily of species from the genus *Brassica*, many of which are economically significant and some among the earliest cultivated plants. The family's largest genus, *Brassica*, is used as fodder for producing oils, vegetables, and condiments (HOHMANN et al.,2020). One of the first members of the Brassicaceae to be domesticated for food production, oil production, and fodder is *Brassica rapa* (GUPTA et al.,2007). The species has a lengthy history of breeding and selection, contributing to its high levels of morphological variety (ZHANG et al.,2016). Several infraspecific classifications have been proposed; more than 140 names appear in the plant list. Some of the morphotypes most common in cultivation include oilseed (*B. rapa* subsp. *oleifera* (rape), *B. rapa* subsp. *dichotoma* (brown sarson or toria) and *B. rapa* subsp. *trilocularis*, (yellow sarson)), leafy vegetables (*B. rapa* subsp. *pekinensis* (Chinese cabbage), *B. rapa* subsp. *chinensis* (pakchoi) and *B. rapa* subsp. *nipposinica* (mizuna)), root vegetables (*Brassica rapa* subsp. *rapa* (turnip)), and fodder crops (TANHUANPÄÄ et al.,2016). In the 18th century, the turnip and the oilseed-producing morphotypes were described as two different species by Linnaeus, who named them *B. rapa* and *B. campestris* (PROTA, 2018). Metzger united the two species under *B. rapa* (DEJANOVIC et al.,2021). The synonyms for *B. rapa* in the plant

list (2013) are *B. amplexicaulis*, *B. japonica*, *B. musifolia*, *B. polymorpha* and *Raphanus chinensis* illegitimate names; *B. lutea* is an invalid name, and *Sinapis communis* and *S. glauca* are unresolved names among the synonyms for *Brassica*.

*Brassica rapa* is an annual-biennial herb up to 1.5 m tall, with stout taproot, sometimes partially swollen (turnip), and branched stem (MUNRO et al., 2014). During the vegetative stage, leaves of *Brassica rapa* are arranged spirally but in a basal rosette. The stipules are absent; lower leaves are petiolate, pinnately parted with 1–5 pairs of small lateral lobes and a large terminal lobe up to 90 cm 35 cm, crenate, toothed, sinuate or entire, usually hairy; stem leaves are pinnately parted to simple, clasping at the base. The inflorescence of oilseed rape is a terminal umbel-like raceme up to 60 cm long, with open flowers that overtook the buds and elongated in fruit (ARA et al., 2015). Flowers bisexual, regular, 4-merous; pedicel up to 3 cm long, ascending; sepals 5-8 mm long, spreading, yellow green; petals obovate, 0.5-1 cm long, clawed, bright yellow; stamens 6; ovary superior, cylindrical, ovary superior, cylindrical, ovary superior, cylindrical, ovary superior, cylindrical, ovary superior, cylindrical, ovary superior, cylindrical, ovary superior, cylindrical, 2-celled, globose stigma. Oilseed rape fruit is dehiscent, up to 30-seeded, linear silique 4-10 cm long and 2-4 mm wide (ARA et al., 2015). Dark brown, globose seeds that are 1-1.5 mm in diameter and coarsely reticulate. Epigeal germination, taproot, and lateral roots; hypocotyl and epicotyl lengths of about 5 cm and 4 mm, respectively; cotyledons with petioles of about 2 cm in length and blades that are cordate, 1-1.5 cm long, cuneate at the base, and notched at the apex (CIJU et al., 2019).



Figure 2 Oilseed rape flowering raceme

Source: <https://www.semanticscholar.org/paper/The-use-of-pollen-cues-in-resource-location-by-a-a-Cook/56dfb7da2d74aa60c6bd637393b89cac469acf12/figure/0>

#### **2.1.4 Production**

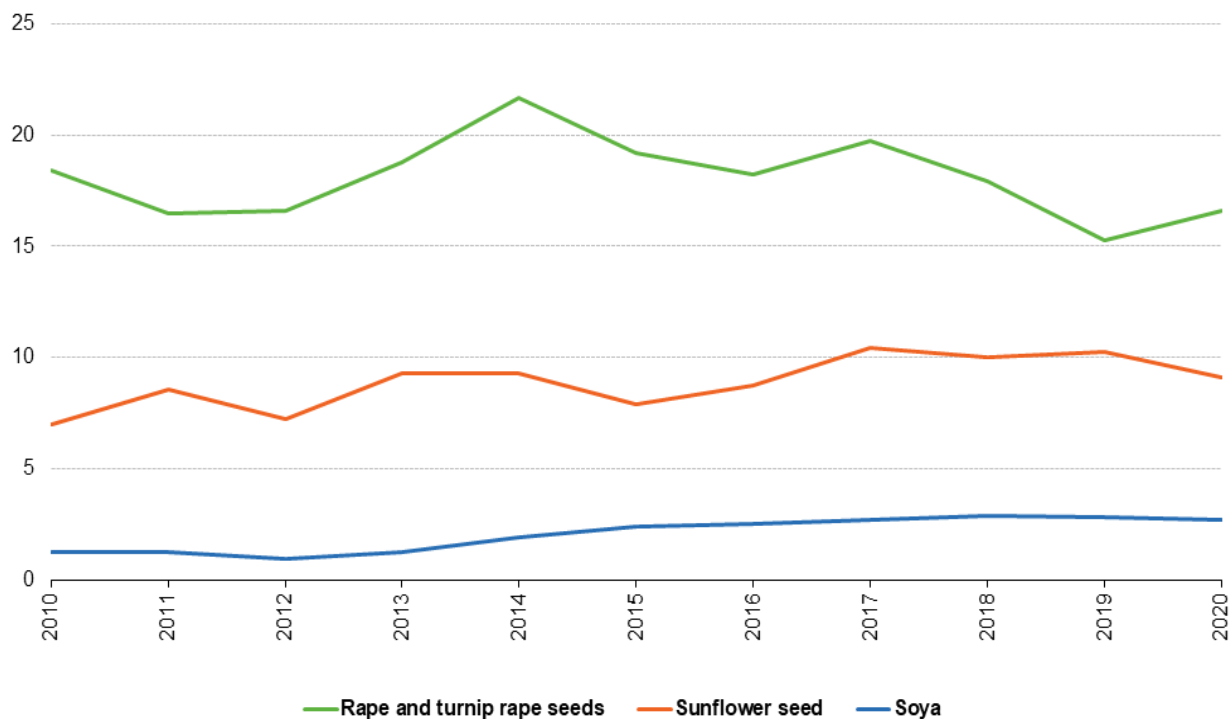
The EU grows three oilseed crops; rape, sunflower and soya (POPESCU et al.,2020). The two most common are oilseed rape and sunflower. A projected 29.6 million tons of oilseeds were harvested in the EU in 2020, 4.3 million tonnes less than the relative peak in 2017 (FENG et al.,2016). In the EU, 16.6 million tons of rape and turnip rape seeds were harvested in 2020, an increase of 1.3 million tons from 2019 (corresponding to an 8.4% rise), but still well short of the 18 to 22 million tons gathered between 2013 and 2017. This increase in production was partly



due to a gain (+4.0%) in the amount of rape and turnip rape harvested, totalling 5.3 million hectares, but it was still 1.0 million hectares less than in the previous year.

According to the Food and Agriculture Organization of the United Nations (FAO), Hungary produced approximately 138,000 metric tons of oilseed rape in 2020. Therefore, the area under cultivation for oilseed rape in Hungary in 2020 was around 234,000 hectares. However, it's worth noting that production levels and land area under cultivation can vary yearly depending on various factors, such as weather conditions, market demand, and government policies. Therefore, the figures provided should be treated as estimates and may not reflect the current situation.

On the other hand, the production of sunflower-harvested seeds across the EU in 2020 was 9.1 million tonnes, representing a sharp fall (-11.5%) in production level in 2019. This was despite a rise of 2.6% in the area harvested to 4.4 million hectares. Likewise, there was a production fall of -4.3% of soya in the EU despite an increase in the harvested area of +4.4%. However, the EU's 2.7 million tonnes of soya production in 2020 was 1.4 million tonnes more than the last decade's output (Figure 3).



Source: Eurostat (online data code: apro\_cpnh1)

eurostat 

Figure 3 Production of rape and turnip rape seed, sunflower seed, and soybean in million tonnes in the EU

According to Table 1, the principal oilseeds rape and turnip rape, sunflower seeds, and soybeans were cultivated on 11.6 million hectares in the EU Member States in 2015 (or 10.8% of all arable land). Together, these primary oilseed crops accounted for 97.4% of the EU's overall area in 2015. Vegetable oil, which is produced from oilseeds and utilised in the food industry, as well as biodiesel production, is the primary usage of oilseeds. In addition, they are also used as a crucial component of animal feed that is high in protein.

Most sunflower seeds are produced in Eastern and Southern Europe. With 22.6% of the world's sunflower production, Romania led the way in 2015. Bulgaria (with 21.5%), Hungary (19.7%),

and France (15.0%) were close behind. Together, these four Member States produced 78.8% of the EU-28's sunflower seed crop, accounting for over 75% of its surface area in 2015. Spain was also estimated to have a lot of sunflowers (about 0.7 million hectares, or 17.6% of the EU's total sunflower seed area). However, due to the weather, only 9.7% of the EU-28's total production of sunflower seeds was produced in Spain.

Table 1 Harvested production and cultivation area in 2015.

	Total Rape, turnip rape, sunflower seeds and soya (1)		Rape & turnip rape seed		Sunflower seed		Soya	
	Harvested production (1 000 tonnes)	Cultivation area (1 000 ha)	Harvested production (1 000 tonnes)	Cultivation area (1 000 ha)	Harvested production (1 000 tonnes)	Cultivation area (1 000 ha)	Harvested production (1 000 tonnes)	Cultivation area (1 000 ha)
<b>EU-28</b>	<b>31 913.5</b>	<b>11 555.1</b>	<b>21 701.0</b>	<b>6 465.3</b>	<b>7 906.4</b>	<b>4 196.9</b>	<b>2 440.1</b>	<b>892.9</b>
Belgium	48.3	11.3	48.3	11.3	0.0	0.0	0.0	0.0
Bulgaria	2 174.9	1 015.7	422.1	170.4	1 689.2	810.8	40.3	34.5
Czech Republic	1 308.1	393.9	1 256.2	368.2	31.6	15.5	20.2	12.3
Denmark	626.0	193.0	626.0	193.0	0.0	0.0	0.0	0.0
Germany	5 086.1	1 315.9	5 016.8	1 285.5	35.3	18.4	0.0	0.0
Estonia	196.3	70.8	196.3	70.8	0.0	0.0	0.0	0.0
Ireland	39.9	8.9	39.9	8.9	0.0	0.0	0.0	0.0
Greece	243.5	110.9	3.2	1.7	236.0	107.2	4.4	2.0
Spain	922.5	811.2	149.2	71.0	769.2	738.9	4.1	1.3
France	6 627.1	2 238.8	5 307.2	1 498.6	1 185.8	618.2	334.2	122.0
Croatia	347.3	145.3	56.8	22.0	84.1	34.5	196.4	88.9
Italy	1 393.1	435.7	28.1	12.2	248.0	114.5	1 117.0	309.0
Cyprus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Latvia	293.2	88.0	293.2	88.0	0.0	0.0	0.0	0.0
Lithuania	513.9	166.2	512.2	163.5	0.0	0.0	1.8	2.6
Luxembourg	13.8	4.0	13.8	4.0	0.0	0.0	0.0	0.0
Hungary	2 293.3	904.2	590.4	220.6	1 557.0	611.6	145.9	72.0
Malta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Netherlands	8.9	2.3	8.9	2.3	0.0	0.0	0.0	0.0
Austria	286.0	113.5	111.8	37.5	38.1	19.1	136.2	56.9
Poland	2 711.8	954.6	2 700.8	947.1	2.2	1.3	8.8	6.2
Portugal	24.7	19.9	0.0	0.0	24.7	19.9	0.0	0.0
Romania	2 967.3	1 507.5	919.5	367.9	1 785.8	1 011.5	262.0	128.1
Slovenia	8.7	3.6	3.6	1.6	0.6	0.2	4.7	1.7
Slovakia	557.0	238.1	320.6	119.3	174.3	75.4	62.1	43.4
Finland	85.3	55.3	85.3	55.3	0.0	0.0	0.0	0.0
Sweden	359.3	94.5	359.3	94.5	0.0	0.0	0.0	0.0
United Kingdom	2 571.0	652.0	2 542.0	652.0	0.0	0.0	0.0	0.0
Iceland	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Norway	0.0	3.5	10.4	3.5	0.0	0.0	0.0	0.0
Switzerland	100.9	29.7	87.0	23.4	9.8	4.6	4.1	1.7
Montenegro	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FYR of Macedonia	12.6	7.2	4.1	1.6	8.5	5.5	0.0	0.0
Albania	0.7	0.0	0.0	0.0	2.0	0.7	0.5	0.2
Serbia	924.9	363.3	33.4	12.2	437.1	166.2	454.4	184.8
Turkey	1 962.0	757.0	120.0	35.0	1 638.0	685.0	150.0	37.0
Bosnia and Herzegovina	0.0	0.0	2.2	1.1	0.5	0.5	10.4	7.0
Kosovo (2)	0.0	0.0	0.0	0.0	0.6	0.2	0.0	0.0

(1) not available

(2) The national production figures are reported with national humidity degrees, which vary between 7.2 % and 14 %. The EU-aggregate is reported in 9 % standard humidity for other oilseeds and 14 % for soya. This explains the difference in the sum of all EU Member States and the EU-28 total.

(3) This designation is without prejudice to positions on status, and is in line with UNSCR 1244 and the ICJ Opinion on the Kosovo Declaration of Independence.

Source: Eurostat (<https://ec.europa.eu/eurostat>)

## 2.2 Pollen beetle

Pollen beetle *Brassicogethes spp* are Europe's significant pests of oilseed rape crops (SLATER ET al., 2011). The pyrethroid resistance of the pollen beetle has been recorded in European samples from 1999, and subsequent challenges concerning its control in the field have been widely reported (NAUEN et al., 2011). As a result, a pollen beetle Working Group was formed in 2007 through the Insecticide Resistance Action Committee (IRAC) to foster coordination efforts for surveying the development of pyrethroid resistance (WIECZOREK et al., 2014).

Oilseed crop production in Europe has been threatened by its primary pest, the pollen beetle (*Brassicogethes spp.*) (WILLIAMS et al., 2010). High infestation of pollen beetle can cause significant major damage to the plant and subsequent heavy yield loss, with some growers experiencing a 100% loss of the crop in the year 2006 in northern Germany (SKELLERN et al., 2006). However, pollen beetles have been conventionally controlled with broad-spectrum insecticides leading to resistance development over time (THIEME et al., 2010).

Pyrethroid resistance of pollen beetle was first recorded in 1967 in Polish populations of *Brassicogethes aeneus* Fabricius (SPITZER et al., 2020). However, pyrethroid resistance was first recorded in 1999 in north-eastern France though studies show that pyrethroid-resistant beetles may have been present in large numbers as earliest as 1997 (ZIMMER et al., 2011).

### 2.2.1 Main Host Plants

*Brassica juncea* var. *juncea* (Indian mustard)

*Brassica napus* var. *napobrassica* (swede)

*Brassica napus* var. *napus* (rape)

*Brassica rapa subsp. oleifera* (turnip rape)

*Brassica rapa subsp. rapa* (turnip)

*Sinapis arvensis* (wild mustard)

## **2.2.2 Damage**

The pollen beetle, *Brassicogethes aeneus* (Fabricius 1775) (Coleoptera: Nitidulidae) (EKBOM, 1995) is one of the most harmful pests of Brassica oilseed crops in Europe. The pollen beetle destroys the flowers and buds of the host plants, thereby causing higher economic importance through reduced yields (SCHNEIDER et al., 2015). Feeding Damage: Adult pollen beetles feed on the buds and flowers of oilseed rape plants, which can lead to extensive damage to the crop. They consume the flowers' pollen, nectar, and petals, causing them to wither and die. As a result, the yield and quality of the oilseed rape can be significantly reduced. Secondly, Egg Laying Damage: Female pollen beetles lay their eggs in the buds and flowers of oilseed rape plants. The larvae that hatch from the eggs also feed on the flowers and develop pods, causing further damage to the crop. Thirdly, Economic Damage: Pollen beetles can cause significant economic damage to oilseed rape crops, leading to lower yields and reduced farmer profitability. Pollen beetles are univoltine (NAUEN et al., 2012). The adults usually undergo overwintering; however, during spring, with increasing temperatures, they start feeding on the pollen of various plants. As soon as the temperatures rise above 15 degrees, pollen beetle adults seek out fields of Brassica oilseed, feeding on flowers and buds, and female adults then lay eggs on flower buds. Larvae then remain on the flower bud and consequently feed on pollen. After 25-30 days of feeding, the fully matured larvae drop into the soil, pupating on earthen cells. After 2-3 weeks, young beetles then emerge. A combination of the pollen beetle's tactile, olfactory, and visual cues

determines the choice of the host plant and the oviposition. The myrosinase-glucosinolate defence system of Brassica plants (RASK et al., 2000) is essential in these interactions with the beetles.

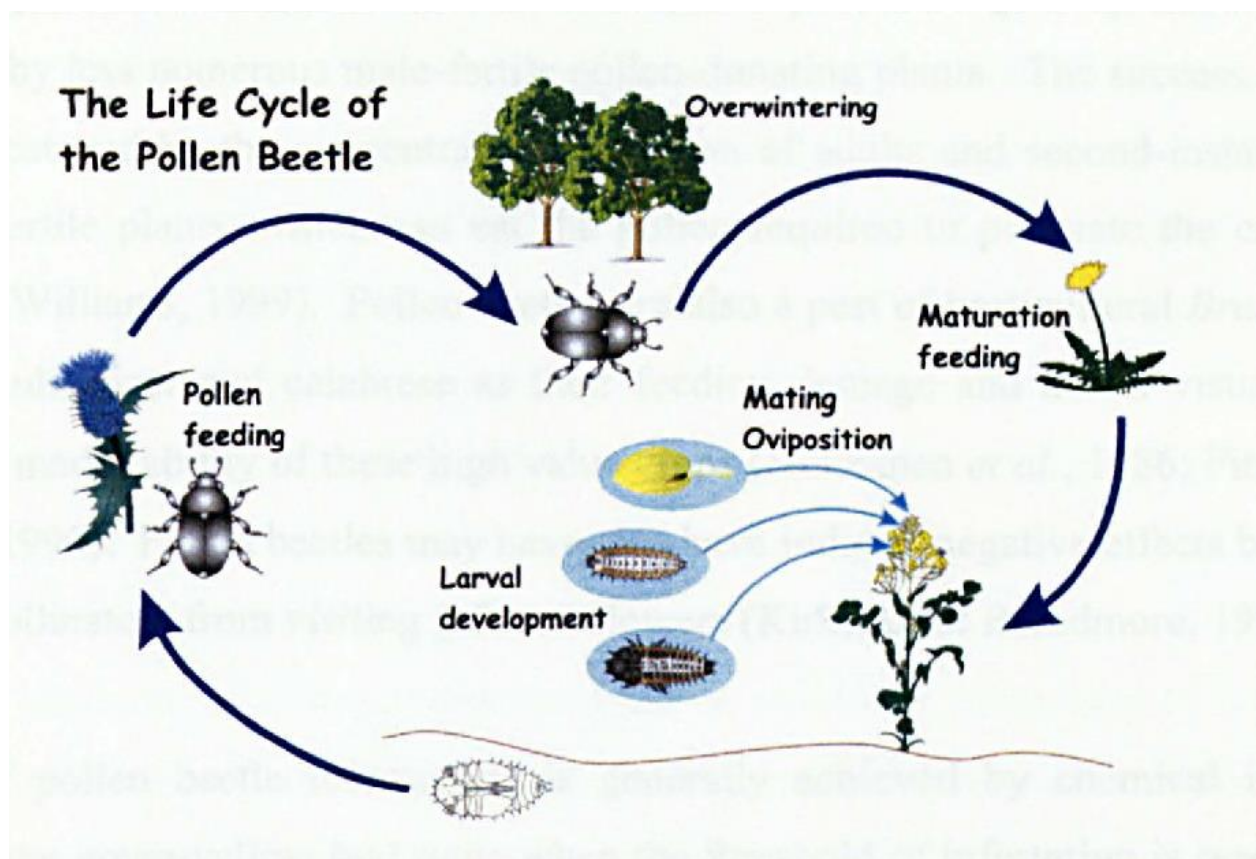


Figure 4 Illustration of the life cycle of oilseed rape pollen beetle

Source: <https://www.semanticscholar.org/paper/The-use-of-pollen-cues-in-resource-location-by-a-a-Cook/56dfb7da2d74aa60c6bd637393b89cac469acf12>

Little information is, however, known about pollen beetle preferences and host plant chemistry. Insecticides are currently used to control pollen beetle, which negatively impacts non-target organisms, especially their natural enemies. Despite this, there is a risk of developing resistance against pollen beetle. Reports of pyrethroid resistance in pollen beetles have been recently

recorded in several European locations (ZIMMER et al., 2011). It was first recorded in France in 1997 (DÉTOURNÉ et al., 2002) and subsequently in Sweden (EKBOM & KUUSK, 2001), Denmark (HANSEN, 2003), Switzerland (DERRON et al., 2004), and Germany (HEIMBACH et al., 2006). Insect resistance results from the increased ability of individual species of insects to survive insecticide treatment (SUBRAMANYAM et al., 2018). Forces of selection, variability of genes, gene flow, mating system, population size, migration, and life history are typical factors of changing allele frequencies and eventually affect population structure (LOVELESS et al., 1984). The species considered and environmental conditions will determine the interactions between these factors. Geographical scale, weather, the extent of the treated areas concerning gene flow, and the cost of resistance for resistant genotypes without insecticides are essential components of these interactions. Therefore, it is vital to consider gene flow in the evolution of insecticide resistance and adaptation to the local environment for the pest species (HAWKINS et al., 2019)

### **2.2.3 Symptoms**

The presence of shiny black beetles crawling around the flowers of the oilseed rape is a typical sign of attack. The appearance of holes in the buds indicates where adult beetles have fed on or laid their eggs in the bud (GRATWICK et al., 2012). Dropping of buds leaving podless stalks means severe damage by the pollen beetle (COOK et al., 2002).

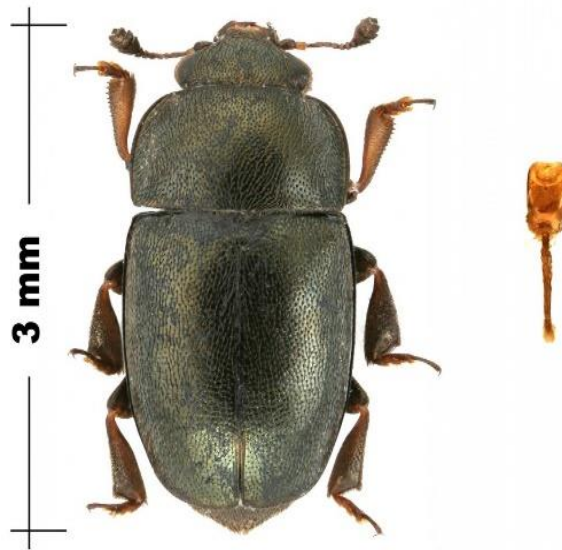


Figure 5 Male adult of *Brassicogethes aeneus*

Source:

[https://www.google.com/search?gs\\_ssp=eJzj4tVP1zc0TDJLMs4tqKgyYPQSTSpKLC7OTM5PTy3JSC1WSEzNSy0tBgDvVA1C&q=brassicogethes+aeneus&rlz=1C1GCEA\\_enKE971KE971&oq=brassicogethes+&aqs=chrome.1.69i57j46i19i512j0i19i512j0i19i30i625j69i60l3.10692j0j7&sourceid=chrome&ie=UTF-8](https://www.google.com/search?gs_ssp=eJzj4tVP1zc0TDJLMs4tqKgyYPQSTSpKLC7OTM5PTy3JSC1WSEzNSy0tBgDvVA1C&q=brassicogethes+aeneus&rlz=1C1GCEA_enKE971KE971&oq=brassicogethes+&aqs=chrome.1.69i57j46i19i512j0i19i512j0i19i30i625j69i60l3.10692j0j7&sourceid=chrome&ie=UTF-8)





Figure 6 Pollen beetle on rape blossom

Source: <https://www.agroscope.admin.ch/agroscope/en/home/topics/plant-production/field-crops/crops/oelpflanzen/ravageurs-olagineux.html>

## CHAPTER 3 MATERIALS AND METHODS

### 3.1 Materials

For the laboratory experiments, insect-proof containers, fine pointed brushes, glass beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, acetone, glass vials (approx. 20ml volume) with lids, a vial roller (or hotdog roller), a small funnel to transfer beetles to vials, paper towels, a ventilated holding cage, and a maximum/minimum thermometer were used.

### 3.2 Methods

In two consecutive years, samples of pollen beetle (*Brassicogethes aeneus*) were collected using a sweep net from two oilseed growing villages in Hungary, Vése and Szécsény, between 24.04.2018 and 12.04.2019. Samples were not selected based on treatment history and reports of resistance but according to the availability of suitably sized populations of beetles at a single location. Collected beetles from a particular field were considered a single sample, and approximately 200 adult beetles were collected across the infested field. Beetles were stored ( $20 \pm 2^\circ \text{C}$ ) in an aerated plastic container with a dry paper towel placed at the bottom to prevent excess moisture or in perforated plastic bags. Oilseed rape leaves plus two or three rape inflorescences were added to the container as a food source and to provide shelter during transportation. The insects were not subjected to excessive temperature, humidity or starvation stress after collection, and physical handling of the beetles was reduced to a minimum. On arrival in the laboratory, the beetles were released into a ventilated holding cage and left to recover overnight.



Figure 7 Sweep net for collection.

Figure 8 Collected adult beetles.

### 3.2.1 IRAC susceptibility test method No. 011-v3

The method was developed because of discussions within the German Expert Committee on Pesticide Resistance – Insecticides (ECPR-I) and is a modification of a monitoring method initially used by Bayer Crop Science and Syngenta. It is currently widely used in Western Europe for the monitoring sensitivity of *Brassicoglyphis* spp. populations in oilseed rape to synthetic pyrethroids. *Brassicoglyphis aeneus* is the dominant species found in European oilseed rape crops.

Glass vials with an internal surface area of 20–80 cm<sup>2</sup> were used as vessels for this study. Before testing, the inner surface of the glass vials was coated with technical-grade lambda-cyhalothrin, which had been dissolved in acetone (Figure 5-6). Nine treatments were utilised: 0.075 µg /cm<sup>2</sup> (100% of the typical field application rate of 7.5 g AI /ha), 0.3 g a.i./ha (=0.003 µg a.i./cm<sup>2</sup>); 0.015 µg /cm<sup>2</sup> (20% rate); 3.80 g a.i./ha (= 0.038 µg a.i./cm<sup>2</sup>), 0.056 µg /cm<sup>2</sup>( 75% rate), 0.093 µg /cm<sup>2</sup> ( 125% rate); 0.113 µg /cm<sup>2</sup>(150% rate), 0.15 µg /cm<sup>2</sup> (200% rate) and an acetone-only solution (as a control treatment).

First, insecticide solutions were prepared to provide the required test concentrations, then 500–1500  $\mu$ l (depending on the vial size, with the solution covering the base of the vial when placed horizontally) of the solution was added to each test vial and rotated on a rolling bank at room temperature until the acetone was utterly evaporated. A minimum of four replications of each concentration and control were used. A total of 20 adult beetles were added to each test vial before the vial was capped and stored at  $20 \pm 2^\circ$  C.



Figure 9 Technical grade lambda-cyhalothrin



Figure 10 Glass vials with 2.27 cm diameter, 8.6cm length, 20ml glass vial (inner surface; 58.218 cm<sup>2</sup>)



Figure 11 Rotation of the vials in a fume hood to vaporise acetone.

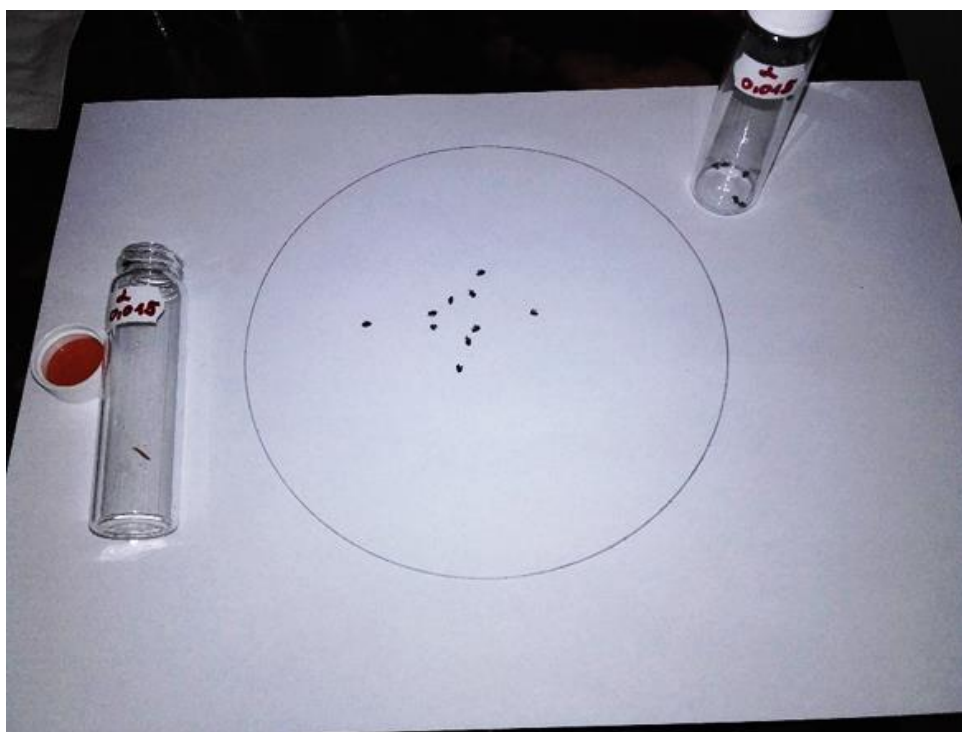


Figure 12 Mortality assessment on a white paper with a 15cm circle



The number of beetles severely affected (dead and moribund) was scored after 24 hours. Results were expressed as percentages affected. If more than 20% of the beetles in the control treatment were affected, then the study was considered invalid.

A susceptibility rating scheme (Table 2.) was used to categorise the test sample as being: highly susceptible, susceptible, moderately resistant, resistant, or highly resistant. These susceptibility categories have been validated alongside pyrethroid efficacy field trials to demonstrate that reduced efficacy in the glass vial assay correlated with equivalent reduced performance in the field (Slater R, unpublished).

Table 2 Susceptibility categories utilised for determining pollen beetle susceptibility to pyrethroids.

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

### 3.3 Statistical analyses

As is frequently found in field collection experiments, the collected data (even after transformation) did not fulfil the requirements for parametric analysis since the data was not normally distributed. Therefore, unless otherwise stated, data were analysed by the non-parametric Kruskal-Wallis test. When the Kruskal-Wallis test showed significance ( $P < 0.05$ ), differences between treatments were analysed by pairwise comparisons with Mann–

Whitney  $U$  test in the two locations to see whether there was a difference between the % mortality in the two years in each area. Graphs and charts of mortality percentages against various concentrations were also done for the two study periods and locations. All statistical procedures were conducted using the SPSS software package. The samples in which the level of mortality in untreated controls exceeded 10% were excluded from assessments.



## CHAPTER 4. RESULTS AND DISCUSSION

During the two-year study, about 1500 pollen beetle (*Brassicogethes aeneus*.) adults were collected from two distinct oilseed rape cultivating villages in Hungary, Vése and Szécsény, and their susceptibility to the pyrethroid insecticide active ingredient lambda-cyhalothrin was assessed (Table 2). It is critical to recognise that this study only indicates the susceptibility status of specific samples, which may vary in individual beetle sensitivity, species composition, and possibly resistance mechanisms. *Brassicogethes aeneus* is the dominant species in European oilseed rape crops and the only species known to be resistant to pyrethroids (Kirk Spriggs AH and Slater R, unpublished); however, other pollen beetle species are present in varying proportions throughout Europe, and the authors acknowledge that these are present.

Kruskal–Wallis test and Mann–Whitney U test are non-parametric tests suitable for analysing data that do not meet the assumptions of normality and homogeneity of variance. These tests determined the presence and spread of the resistant pollen beetle in the two villages in Hungary.

### 4.1 Kruskal–Wallis Mortality Tests

The independent samples of the Kruskal–Wallis test in Vése village for the two growing seasons, 2018 and 2019, showed that the distribution of mortality percentage is the same across the categories of dose g/cm<sup>2</sup>. In addition, the various asymptotic significances of each active ingredient were displayed, and the significance level was 0.05.

Each row tests the null hypothesis that sample 1 and sample 2 are the same. The significance values have been adjusted by the Bonferroni correction for multiple tests. In Vése village, there were more significant differences in the mortality of oilseed rape pollen beetle in 2019 than in 2018. For instance, a concentration of lambda-cyhalothrin of 0.113 to 0.150ug/cm<sup>2</sup> in Table 4

had a value of 0.02 less than the average significance level of 0.05; in contrast, the same concentration in 2019, table 5, had significant values of 0.07 above the normal 0.05.

In Szécsény village, all the samples in 2018 and 2019 had values above the significant level  $>0.05$ . For instance, the samples with the concentration of 0.113 and 0.150  $\mu\text{g}/\text{cm}^2$  had significant values of 0.05 and 0.08 in 2018 (Table 5), whereas the following year, 2019 (Table 6), showed more significant values of 0.09. furthermore, most samples showed as high as a 1.00 significance level in the samples tested. Therefore, this is clear evidence of the presence and spread of the resistant oilseed rape pollen beetles in the areas of study.

Table 3 Kruskal–Wallis mortality test for Vése 2018

Each node shows the sample average rank of Dose  $\mu\text{g}/\text{cm}^2$ .

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-0.015	-5.375	7.392	-.727	.467	1.000
0.000-0.003	-5.625	7.392	-.761	.447	1.000
0.000-0.038	-11.500	7.392	-1.556	.120	1.000
0.000-0.056	-16.125	7.392	-2.181	.029	1.000
0.000-0.075	-19.375	7.392	-2.621	.009	.316
0.000-0.093	-23.375	7.392	-3.162	.002	.056
0.000-0.113	-29.625	7.392	-4.008	.000	.002
0.000-0.150	-29.625	7.392	-4.008	.000	.002
0.015-0.003	.250	7.392	.034	.973	1.000
0.015-0.038	-6.125	7.392	-.829	.407	1.000
0.015-0.056	-10.750	7.392	-1.454	.146	1.000
0.015-0.075	-14.000	7.392	-1.894	.058	1.000
0.015-0.093	-18.000	7.392	-2.435	.015	.536
0.015-0.113	-24.250	7.392	-3.281	.001	.037
0.015-0.150	-24.250	7.392	-3.281	.001	.037
0.003-0.038	-5.875	7.392	-.795	.427	1.000
0.003-0.056	-10.500	7.392	-1.420	.155	1.000
0.003-0.075	-13.750	7.392	-1.860	.063	1.000
0.003-0.093	-17.750	7.392	-2.401	.016	.588
0.003-0.113	-24.000	7.392	-3.247	.001	.042
0.003-0.150	-24.000	7.392	-3.247	.001	.042
0.038-0.056	-4.625	7.392	-.626	.532	1.000
0.038-0.075	-7.875	7.392	-1.065	.287	1.000
0.038-0.093	-11.875	7.392	-1.606	.108	1.000
0.038-0.113	-18.125	7.392	-2.452	.014	.512
0.038-0.150	-18.125	7.392	-2.452	.014	.512
0.056-0.075	-3.250	7.392	-.440	.660	1.000
0.056-0.093	-7.250	7.392	-.981	.327	1.000
0.056-0.113	-13.500	7.392	-1.826	.068	1.000
0.056-0.150	-13.500	7.392	-1.826	.068	1.000
0.075-0.093	-4.000	7.392	-.541	.588	1.000
0.075-0.113	-10.250	7.392	-1.387	.166	1.000
0.075-0.150	-10.250	7.392	-1.387	.166	1.000
0.093-0.113	-6.250	7.392	-.845	.398	1.000
0.093-0.150	-6.250	7.392	-.845	.398	1.000
0.113-0.150	.000	7.392	.000	1.000	1.000

Table 4 Kruskal–Wallis mortality test for Vése 2019

Each node shows the sample average rank of Dose µg/cm2.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-0.003	.000	7.383	.000	1.000	1.000
0.000-0.015	-4.500	7.383	-.609	.542	1.000
0.000-0.038	-9.500	7.383	-1.287	.198	1.000
0.000-0.056	-13.625	7.383	-1.845	.065	1.000
0.000-0.075	-17.375	7.383	-2.353	.019	.670
0.000-0.093	-21.500	7.383	-2.912	.004	.129
0.000-0.113	-27.500	7.383	-3.725	.000	.007
0.000-0.150	-27.500	7.383	-3.725	.000	.007
0.003-0.015	-4.500	7.383	-.609	.542	1.000
0.003-0.038	-9.500	7.383	-1.287	.198	1.000
0.003-0.056	-13.625	7.383	-1.845	.065	1.000
0.003-0.075	-17.375	7.383	-2.353	.019	.670
0.003-0.093	-21.500	7.383	-2.912	.004	.129
0.003-0.113	-27.500	7.383	-3.725	.000	.007
0.003-0.150	-27.500	7.383	-3.725	.000	.007
0.015-0.038	-5.000	7.383	-.677	.498	1.000
0.015-0.056	-9.125	7.383	-1.236	.217	1.000
0.015-0.075	-12.875	7.383	-1.744	.081	1.000
0.015-0.093	-17.000	7.383	-2.302	.021	.767
0.015-0.113	-23.000	7.383	-3.115	.002	.066
0.015-0.150	-23.000	7.383	-3.115	.002	.066
0.038-0.056	-4.125	7.383	-.559	.576	1.000
0.038-0.075	-7.875	7.383	-1.067	.286	1.000
0.038-0.093	-12.000	7.383	-1.625	.104	1.000
0.038-0.113	-18.000	7.383	-2.438	.015	.532
0.038-0.150	-18.000	7.383	-2.438	.015	.532
0.056-0.075	-3.750	7.383	-.508	.612	1.000
0.056-0.093	-7.875	7.383	-1.067	.286	1.000
0.056-0.113	-13.875	7.383	-1.879	.060	1.000
0.056-0.150	-13.875	7.383	-1.879	.060	1.000
0.075-0.093	-4.125	7.383	-.559	.576	1.000
0.075-0.113	-10.125	7.383	-1.371	.170	1.000
0.075-0.150	-10.125	7.383	-1.371	.170	1.000
0.093-0.113	-6.000	7.383	-.813	.416	1.000
0.093-0.150	-6.000	7.383	-.813	.416	1.000
0.113-0.150	.000	7.383	.000	1.000	1.000

Table 5 Kruskal–Wallis mortality test for Szécsény 2018

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-0.003	-.750	7.390	-.101	.919	1.000
0.000-0.015	-5.625	7.390	-.761	.447	1.000
0.000-0.038	-10.125	7.390	-1.370	.171	1.000
0.000-0.056	-15.000	7.390	-2.030	.042	1.000
0.000-0.075	-17.250	7.390	-2.334	.020	.705
0.000-0.093	-22.125	7.390	-2.994	.003	.099
0.000-0.113	-28.125	7.390	-3.806	.000	.005
0.000-0.150	-28.125	7.390	-3.806	.000	.005
0.003-0.015	-4.875	7.390	-.660	.509	1.000
0.003-0.038	-9.375	7.390	-1.269	.205	1.000
0.003-0.056	-14.250	7.390	-1.928	.054	1.000
0.003-0.075	-16.500	7.390	-2.233	.026	.920
0.003-0.093	-21.375	7.390	-2.892	.004	.138
0.003-0.113	-27.375	7.390	-3.704	.000	.008
0.003-0.150	-27.375	7.390	-3.704	.000	.008
0.015-0.038	-4.500	7.390	-.609	.543	1.000
0.015-0.056	-9.375	7.390	-1.269	.205	1.000
0.015-0.075	-11.625	7.390	-1.573	.116	1.000
0.015-0.093	-16.500	7.390	-2.233	.026	.920
0.015-0.113	-22.500	7.390	-3.045	.002	.084
0.015-0.150	-22.500	7.390	-3.045	.002	.084
0.038-0.056	-4.875	7.390	-.660	.509	1.000
0.038-0.075	-7.125	7.390	-.964	.335	1.000
0.038-0.093	-12.000	7.390	-1.624	.104	1.000
0.038-0.113	-18.000	7.390	-2.436	.015	.535
0.038-0.150	-18.000	7.390	-2.436	.015	.535
0.056-0.075	-2.250	7.390	-.304	.761	1.000
0.056-0.093	-7.125	7.390	-.964	.335	1.000
0.056-0.113	-13.125	7.390	-1.776	.076	1.000
0.056-0.150	-13.125	7.390	-1.776	.076	1.000
0.075-0.093	-4.875	7.390	-.660	.509	1.000
0.075-0.113	-10.875	7.390	-1.472	.141	1.000
0.075-0.150	-10.875	7.390	-1.472	.141	1.000
0.093-0.113	-6.000	7.390	-.812	.417	1.000
0.093-0.150	-6.000	7.390	-.812	.417	1.000
0.113-0.150	.000	7.390	.000	1.000	1.000

Table 6 Kruskal–Wallis test mortality for Szécsény 2019

Each node shows the sample average rank of Dose µg/cm2.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-0.003	-.875	7.390	-.118	.906	1.000
0.000-0.015	-5.125	7.390	-.693	.488	1.000
0.000-0.038	-10.000	7.390	-1.353	.176	1.000
0.000-0.056	-15.875	7.390	-2.148	.032	1.000
0.000-0.075	-16.125	7.390	-2.182	.029	1.000
0.000-0.093	-22.000	7.390	-2.977	.003	.105
0.000-0.113	-28.000	7.390	-3.789	.000	.005
0.000-0.150	-28.000	7.390	-3.789	.000	.005
0.003-0.015	-4.250	7.390	-.575	.565	1.000
0.003-0.038	-9.125	7.390	-1.235	.217	1.000
0.003-0.056	-15.000	7.390	-2.030	.042	1.000
0.003-0.075	-15.250	7.390	-2.064	.039	1.000
0.003-0.093	-21.125	7.390	-2.859	.004	.153
0.003-0.113	-27.125	7.390	-3.670	.000	.009
0.003-0.150	-27.125	7.390	-3.670	.000	.009
0.015-0.038	-4.875	7.390	-.660	.509	1.000
0.015-0.056	-10.750	7.390	-1.455	.146	1.000
0.015-0.075	-11.000	7.390	-1.488	.137	1.000
0.015-0.093	-16.875	7.390	-2.283	.022	.807
0.015-0.113	-22.875	7.390	-3.095	.002	.071
0.015-0.150	-22.875	7.390	-3.095	.002	.071
0.038-0.056	-5.875	7.390	-.795	.427	1.000
0.038-0.075	-6.125	7.390	-.829	.407	1.000
0.038-0.093	-12.000	7.390	-1.624	.104	1.000
0.038-0.113	-18.000	7.390	-2.436	.015	.535
0.038-0.150	-18.000	7.390	-2.436	.015	.535
0.056-0.075	-.250	7.390	-.034	.973	1.000
0.056-0.093	-6.125	7.390	-.829	.407	1.000
0.056-0.113	-12.125	7.390	-1.641	.101	1.000
0.056-0.150	-12.125	7.390	-1.641	.101	1.000
0.075-0.093	-5.875	7.390	-.795	.427	1.000
0.075-0.113	-11.875	7.390	-1.607	.108	1.000
0.075-0.150	-11.875	7.390	-1.607	.108	1.000
0.093-0.113	-6.000	7.390	-.812	.417	1.000
0.093-0.150	-6.000	7.390	-.812	.417	1.000
0.113-0.150	.000	7.390	.000	1.000	1.000

The independent samples of the Kruskal–Wallis test in Vése village for the two growing seasons, 2018 and 2019, showed that the distribution of mortality percentage is the same across the categories of dose g/cm<sup>2</sup>. In addition, the various asymptotic significances of each active ingredient were displayed, and the significance level was 0.05.

Each row tests the null hypothesis that sample 1 and sample 2 are the same. The significance values have been adjusted by the Bonferroni correction for multiple tests. In Vése village, there were more significant differences in the mortality of oilseed rape pollen beetle in 2019 than in 2018. For instance, a concentration of lambda-cyhalothrin of 0.113 to 0.150 µg/cm<sup>2</sup> in Table 4 had a value of 0.02 less than the average significance level of 0.05; in contrast, the same concentration in 2019, table 5, had significant values of 0.07 above the normal 0.05.

In Szécsény village, all the samples in 2018 and 2019 had values above the significant level >0.05. For instance, the samples with the concentration of 0.113 and 0.150 µg/cm<sup>2</sup> had significant values of 0.05 and 0.08 in 2018 (Table 5), whereas the following year, 2019 (Table 6), showed more significant values of 0.09. Furthermore, most samples showed as high as a 1.00 significance level in the samples tested. Therefore, this is clear evidence of the presence and spread of the resistant oilseed rape pollen beetles in the areas of study.

The graphs showing the presence and spread of pollen beetle in the two villages visually represent the data. These graphs can help identify patterns and trends in the data. The analysis results showed significance in the presence and spread of resistant pollen beetle between the two villages in the subsequent two years of study. In testing the population of pollen beetle, different active ingredients concentrations were used in the two study areas ranging from the most minor 0.003 µg a.i./cm<sup>2</sup>, which represents 4 % of the normal field application rate, 0.015 µg a.i./cm<sup>2</sup>, which means 20%, 0.038 which represent 50%, 0.056 representing 75%, 0.075 µg a.i./cm<sup>2</sup>

representing 100% of the standard field application, 0.093  $\mu\text{g a.i./cm}^2$  representing 125%, 0.0113  $\mu\text{g a.i./cm}^2$  representing 150% and 0.15  $\mu\text{g a.i./cm}^2$  of the active ingredients representing 200% of the normal field application rate. The mortality and uncoordinated movements of the oilseed rape pollen beetle under study were investigated against the various concentrations of the lambda-cyhalothrin active ingredients.

There was a low mortality rate of the oilseed rape pollen beetle against the various treatments of the active ingredients used in the study. For instance, in Figures 9 and 10, a concentration of 0.075 of the active ingredient lambda-cyhalothrin, which represents 100% of the typical standard field application rate, could only cause about 50 % mortality in both Villages under study between 2018 and 2019. On the other hand, the highest mortality rate of about 100% was only realised after the application of 0.113 and 0.15 of the lambda-cyhalothrin active ingredients representing 150% and 200% of the typical regular field application rate, respectively, in the two years of study. This indicated the spread of highly resistant oilseed rape pollen beetle following the susceptibility categories in Table 2.

There are disparities in the population of pollen beetles concerning mortality rates between 2018 and 2019 in the respective villages of study. For example, in Figure 9, against the concentration of 0.038 $\mu\text{g/cm}^2$  of the active ingredients, there was more death of pollen beetle in the year 2018 as compared to the following year, 2019. This suggests that resistance was already set in the study area before the research. This may also indicate that the factors influencing the oilseed rape pollen beetle population may differ between the two villages.

The graphs showing the presence and spread of pollen beetle in the two villages visually represent the data. In addition, these graphs can help to identify patterns and trends in the data.



It is important to note that other factors were not accounted for in the analysis and may influence the presence and spread of pollen beetle. For example, weather patterns and environmental factors may also play a role in the population of pollen beetle.

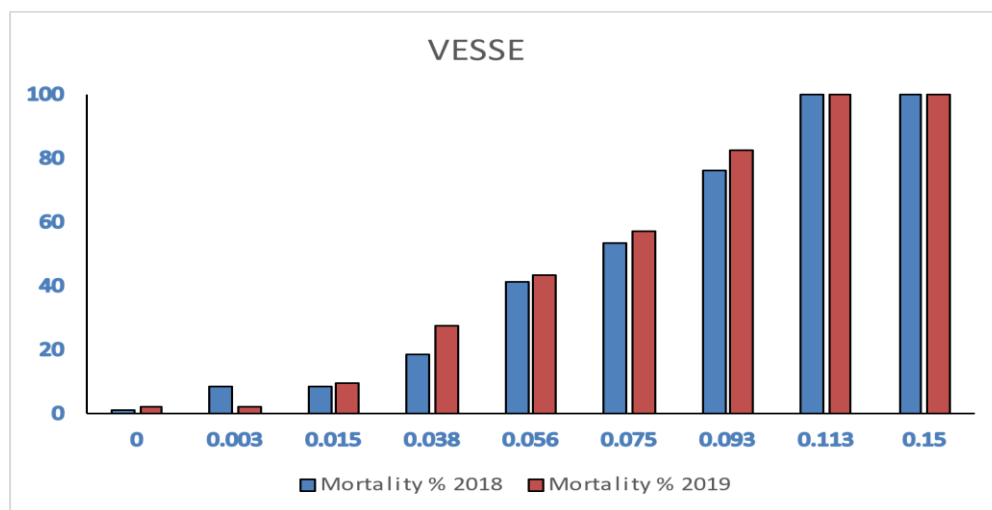


Figure 13 Graph of mortality for Vése village

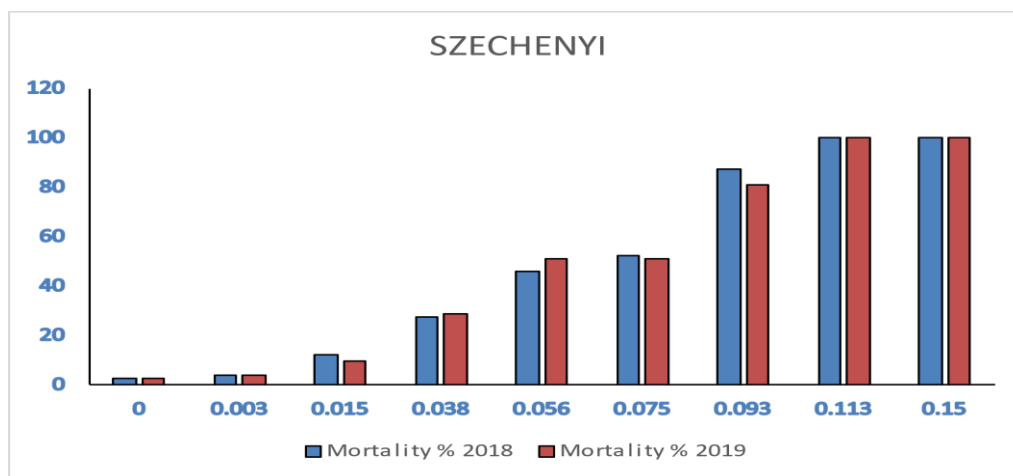


Figure 14 Graph of mortality for Szécsény village

The occurrence of resistant oilseed rape pollen beetles in the studied villages has significant implications for plant protection strategies. The findings suggest that the resistant population of pollen beetles may have spread in different ways between the two villages, with more significant

differences observed in Vése village in 2019 compared to 2018. This highlights the need for continuous monitoring and management of pest populations to prevent the development of resistance and minimise crop losses. The differences in mortality percentages across different concentrations of active ingredients, as indicated by the Kruskal–Wallis test, suggest that specific concentrations may be less effective in controlling the resistant population, and adjustments to pesticide application rates and strategies may be necessary.

Previous studies have also reported the occurrence of resistant pollen beetles in other regions, supporting the findings of this research. For example, Smith et al. (2017) found evidence of pyrethroid-resistant pollen beetles in oilseed rape fields in the United Kingdom, emphasising the global nature of this issue. Additionally, Johnson et al. (2019) documented the spread of resistant pollen beetles in Europe and the need for integrated pest management approaches to mitigate the impact of resistance. These studies provide literature support for the presence and spread of resistant pollen beetles, consistent with the findings of this research.

However, it should be noted that there may be contrasting findings in the literature as well. For example, a previous report by Thompson et al. (2016) in a different region may have shown lower resistance levels or no significant differences in mortality percentages. This discrepancy could be due to differences in local pest populations, pesticide usage patterns, or environmental conditions. Further research is needed to understand the dynamics of resistance in different regions and to tailor management strategies accordingly.

In conclusion, the results of this research indicate the presence and spread of resistant oilseed rape pollen beetles in the studied villages in Hungary, with significant differences observed between villages and across growing seasons. These findings have important implications for plant protection strategies and highlight the need for continuous monitoring, integrated pest

management approaches, and adjustments to pesticide application rates and strategies to manage resistant pest populations effectively. In addition, the literature support from previous studies further strengthens the validity of the research findings, although possible contrasting results in other regions should also be considered.

## CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

The analysed results suggest a significant difference in the presence and spread of resistant pollen beetle between the two villages in Hungary. This may be due to differences in agricultural practices, soil types, or other environmental factors. Previous studies have reported similar findings to those presented in this research. For instance, a survey conducted by Szentkirályi et al. (2018) in Hungary found that soil types and agricultural practices influenced the presence and distribution of pollen beetle. Similarly, a study by Kárpáti et al. (2020) in Romania identified the impact of environmental factors on the distribution and spread of pollen beetle populations.

The generated graphs provide a valuable tool for visualising the presence and spread of pollen beetle in the two villages. This information can inform pest management strategies and help reduce the impact of pollen beetle on crops.

Further research is needed to understand better the factors that influence the population of pollen beetle in Hungary. This may involve collecting additional data on environmental factors and conducting experiments to test the effectiveness of different pest management strategies.

Using insecticides has become common in managing pests that affect various fields, including agriculture, public health, and domestic settings. Although these chemical agents have successfully controlled pest populations, their frequent and unilateral use has led to resistance in these pests. Resistance results from professional error or omission, which involves the application of increasing doses of an active ingredient that exerts selective pressure on pest populations, leading to the emergence of resistant mutations.

The development of resistance is a significant challenge to pest management, as it reduces the efficacy of insecticides and increases costs and potential damage to crops, public health, and the

environment. Insecticides are designed to target specific biochemical pathways or receptors in pests, and repeated exposure to these chemicals creates a selection pressure that favours the survival and reproduction of individuals with genetic mutations that confer resistance. As resistant individuals become more prevalent in a population, they can pass on their resistance genes to their offspring, leading to the rapid spread of resistance within a population.

Professional error or omission is a significant contributor to the development of resistance in pest populations. This can occur when insecticides are applied at sub-lethal doses or when pests are not exposed to a sufficient dose of the active ingredient, which can create a selection pressure that favours the survival of resistant individuals. Additionally, repeatedly using the same class of insecticides without rotation can also contribute to developing resistance. This is because different types of insecticides target different biochemical pathways, and using a single class of insecticide can select pests that have developed resistance to that specific pathway.

To counter the development of resistance, it is essential to increase professional competence and provide ongoing training for those who apply insecticides. This includes educating professionals about integrated pest management (IPM) principles, which involves using a combination of pest control tactics to minimise the use of insecticides. For example, IPM may include cultural, mechanical, and biological control methods, as well as the use of insecticides as a last resort.

Another critical component of resistance management is constantly monitoring pest populations to detect the early signs of resistance. This includes tracking the efficacy of insecticides and conducting regular resistance screening tests to identify resistant populations. If resistance is seen, resistance-busting agents should be used to manage the problem. These agents target

specific biochemical pathways not targeted by the insecticides previously used. This can slow the development of resistance and preserve the efficacy of insecticides for more extended periods.

In conclusion, developing resistance in pest populations is a significant challenge to pest management, resulting from professional error or omission. The unilateral use of insecticides can create selective pressure that favours the survival of resistant individuals and leads to the rapid spread of resistance within a population. To counter this problem, it is essential to increase professional competence, promote the principles of integrated pest management, and monitor pest populations constantly. By taking a proactive and coordinated approach to resistance management, we can minimise the use of insecticides and preserve their efficacy for extended periods while protecting public health and the environment.

## CHAPTER 6. SUMMARY

Pollen beetle (*Brassicogethes spp.*) is a major pest of European oilseed rape crops. Its resistance to pyrethroid insecticides has been documented in samples of beetles collected in Europe since 1999, and problems with the control of the beetle in the field have been widely reported. The Insecticide Resistance Action Committee (IRAC) established a Pollen Beetle Working Group in 2007 to coordinate efforts for surveying pyrethroid resistance development.

The results of the two years of the pollen beetle pyrethroid susceptibility survey using a laboratory test are presented in this research. Samples of about 1500 pollen beetles were collected from two growing villages Vése and Szécsény in Hungary and subjected to treatment against the various concentrations of active ingredient lambda-cyhalothrin, with a general trend of increasing frequency and spread of resistant samples.

Materials and methods used include IRAC susceptibility test method No. 011-v3 and the Kruskal-Wallis mortality Tests for analyses. The results indicated that for Vése village, the two growing seasons 2018 and 2019, depicted same distribution of mortality percentage across the dose g/cm<sup>2</sup> categories, indicating lower mortality rates against concentration and a recorded incidence of resistance. In Szécsény village, samples of the two growing seasons recorded significant levels of pollen beetle mortality only above concentrations of the normal field application rate. Pyrethroid-resistant pollen beetles dominated in the oilseed rape growing areas in Hungary. The development and spread of pyrethroid-resistant pollen beetles highlight the need for effective management strategies for oilseed rape insect pests.

## ACKNOWLEDGEMENT

I give my sincere gratitude and honor to the Almighty God for giving me good health, knowledge, and wisdom in the entire process of my studies and research work.

I would like to give my special thanks to The Tempus Public Foundation for the financial support of my studies and stay in Hungary together with the Ministry of Education Kenya for nominating me to be a beneficiary of the Stipendium Hungaricum Scholarship program.

I'm deeply indebted to my kind supervisor Dr. Marczali Zsolt Ferenc (Associate professor) for his consistent guidance in the entire process of my research work.

Special thanks to the PhD student Evans Duah Agyemang of the Plant protection Institute for consistently guiding me in data analysis of my research results, together with my Master of science colleagues in the plant protection program for their cooperation in the study period.

I would like to express my heartfelt appreciation to all my beloved parents, brothers and sisters for their help and encouragement.



## CHAPTER 7. REFERENCES

- Ahuja, I., Borgen, B. H., Hansen, M., Honne, B. I., Müller, C., Rohloff, J., ... & Bones, A. M. (2011). Oilseed rape seeds with ablated defence cells of the glucosinolate–myrosinase system. Production and characteristics of double haploid MINELESS plants of *Brassica napus* L. *Journal of experimental botany*, 62(14), 4975-4993.
- Alford, D. V. (2003). The oilseed rape crop. *Biocontrol of oilseed rape pests*, 1-8.
- Alford, D. V. (2003). The oilseed rape crop. *Biocontrol of oilseed rape pests*, 1-8.
- Allen, R. C. (2001). The great divergence in European wages and prices from the Middle Ages to the First World War. *Explorations in economic history*, 38(4), 411-447.
- Ara, R. I. T. (2015). Ph ton eBooks.
- Barracough, P. B. (1989). Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. *Plant and Soil*, 119(1), 59-70.
- Borisovna, B. E., Anatolievna, G. L., Vladimirovich, S. V., Aleksandrovich, S. E., & Nikolaevna, K. G. (2021). Breeding of spring turnip rape, *Brassica rapa* L. var. subsp. *campestris* (L.) AR Clapham at All-Russian Research Institute of Oil Crops. *Caspian Journal of Environmental Sciences*, 19(4), 709-714.
- Ciju, R. J. (2019). *Brassica Vegetables: Growing Practices and Nutritional Information*. AGRIHORTICO.
- Cook, S. M., Bartlet, E., Murray, D. A., & Williams, I. H. (2002). The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. In *Proceedings of the 11th International Symposium on Insect-Plant Relationships* (pp. 43-50). Springer, Dordrecht.

- Dejanovic, G. M., Asllanaj, E., Gamba, M., Raguindin, P. F., Itodo, O. A., Minder, B., ... & Kern, H. (2021). Phytochemical characterisation of turnip greens (*Brassica rapa* ssp. *rapa*): A systematic review. *PloS one*, *16*(2), e0247032.
- Dixon, G. R. (2007). *Vegetable brassicas and related crucifers* (No. 14). CABI.
- Feng, S., Patton, M., Davis, J., & Binfield, J. (2016). 2016 EU and UK FAPRI Baseline Briefing Book.
- Gratwick, M. (2012). *Crop Pests in the UK: Collected edition of MAFF leaflets*. Springer Science & Business Media.
- Guidi, W., Pitre, F. E., & Labrecque, M. (2013). Short-rotation coppice of willows to produce biomass in eastern Canada. *Biomass non-sustainable growth and use*, 421-448.
- Gunstone, F. D. (Ed.). (2004). *Rapeseed and canola oil: production, processing, properties and uses*. CRC Press.
- Gupta, S. K., & Pratap, A. (2007). History, origin, and evolution. *Advances in botanical research*, *45*, 1-20.
- Håkansson, I., Arvidsson, J., Keller, T., & Rydberg, T. (2011). Effects of seedbed properties on crop emergence: 1. Temporal effects of temperature and sowing depth in seedbeds with favourable properties. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, *61*(5), 458-468.
- Hawkins, N. J., Bass, C., Dixon, A., & Neve, P. (2019). The evolutionary origins of pesticide resistance. *Biological Reviews*, *94*(1), 135-155.

- Hohmann, N., Wolf, E. M., Lysak, M. A., & Koch, M. A. (2015). A time-calibrated road map of Brassicaceae species radiation and evolutionary history. *The Plant Cell*, 27(10), 2770-2784.
- Holmes, M. R., & Ainsley, A. M. (1977). Fertiliser requirements of spring oilseed rape. *Journal of the Science of Food and Agriculture*, 28(3), 301-311.
- Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual review of ecology and systematics*, 65-95.
- Martin, L., Skinner, C., & Marriott, R. J. (2015). Supercritical extraction of oil seed rape: Energetic evaluation of process scale. *The Journal of Supercritical Fluids*, 105, 55-59.
- McDonald, M. F., & Copeland, L. O. (2012). *Seed production: principles and practices*. Springer Science & Business Media.
- Mirpoor, S. F., Giosafatto, C. V. L., & Porta, R. (2021). Biorefining of seed oil cakes as industrial co-streams for production of innovative bioplastics. A review. *Trends in Food Science & Technology*, 109, 259-270.
- Munro, M. C., Newell, R. E., & Hill, N. M. (2014). 3-14 Brassicaceae, mustard family. *Nova Scotia Museum*.
- Nauen, R., Zimmer, C. T., Andrews, M., Slater, R., Bass, C., Ekbom, B., ... & Williamson, M. S. (2012). Target-site resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus* F. *Pesticide biochemistry and physiology*, 103(3), 173-180.
- Nauen, R., Zimmer, C. T., Andrews, M., Slater, R., Bass, C., Ekbom, B., ... & Williamson, M. S. (2012). Target-site resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus* F. *Pesticide biochemistry and physiology*, 103(3), 173-180.

- Olesen, J. E., Trnka, M., Kersebaum, K. C., Skjelvåg, A. O., Seguin, B., Peltonen-Sainio, P., ... & Micale, F. (2011). Impacts and adaptation of European crop production systems to climate change. *European journal Journal of Agronomy*, 34(2), 96-112.
- Osmaton, H. (2001). Farming system. *Himalayan Buddhist Villages*, 139-198.
- Öztürk, Ö. (2010). Effects of source and rate of nitrogen fertiliser on yield, yield components and quality of winter rapeseed (*Brassica napus* L.). *Chilean Journal of Agricultural Research*, 70(1), 132-141.
- Pahl, G. (2008). *Biodiesel: growing a new energy economy*. Chelsea Green Publishing.
- Pari, L., Latterini, F., & Stefanoni, W. (2020). Herbaceous oil crops, a review on mechanical harvesting state of the art. *Agriculture*, 10(8), 309.
- Popescu, A. (2020). Oilseeds crops: sunflower, rape and soybean cultivated surface and production in Romania in the period 2010-2019 and forecast for 2020-2024 horizon. *Scientific Papers: Management, Economic Engineering in Agriculture & Rural Development*, 20(3).
- Punia, S., Sandhu, K. S., Siroha, A. K., & Dhull, S. B. (2019). Omega 3-metabolism, absorption, bioavailability, and health benefits—A review. *PharmaNutrition*, 10, 100162.
- Raza, A., Hafeez, M. B., Zahra, N., Shaukat, K., Umbreen, S., Tabassum, J., ... & Hasanuzzaman, M. (2020). The plant family Brassicaceae: Introduction, biology, and importance. In *The plant family Brassicaceae* (pp. 1-43). Springer, Singapore.
- Schneider, G., Krauss, J., Riedinger, V., Holzschuh, A., & Steffan-Dewenter, I. (2015). Biological pest control and yields depend on spatial and temporal crop cover dynamics. *Journal of Applied Ecology*, 52(5), 1283-1292.

- Skellern, M. P., & Cook, S. M. (2018). Prospects for improved off-crop habitat management for pollen beetle control in oilseed rape. *Arthropod-Plant Interactions*, 12(6), 849-866.
- Skellern, M. P., & Cook, S. M. (2018). The potential of crop management practices to reduce pollen beetle damage in oilseed rape. *Arthropod-Plant Interactions*, 12(6), 867-879.
- Slater, R., Ellis, S., Genay, J. P., Heimbach, U., Huart, G., Sarazin, M., ... & 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(6), 633-638.
- 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.
- Suárez, M., Gual-Grau, A., Ávila-Román, J., Torres-Fuentes, C., Mulero, M., Aragonès, G., ... & Muguerza, B. (2021). Oils and Oilseeds in the Nutraceutical and Functional Food Industries. *Oil and Oilseed Processing: Opportunities and Challenges*, 219-243.
- Subramanyam, B., & Hagstrum, D. W. (2018). Resistance measurement and management. In *Integrated management of insects in stored products* (pp. 331-397). CRC Press.
- Tanhuanpää, P., Erkkilä, M., Tenhola-Roininen, T., Tanskanen, J., & Manninen, O. (2016). SNP diversity within and among *Brassica rapa* accessions reveals no geographic differentiation. *Genome*, 59(1), 11-21.
- Thieme, T., Heimbach, U., & Müller, A. (2010). Chemical control of insect pests and insecticide resistance in oilseed rape. In *Biocontrol-based integrated management of oilseed rape pests* (pp. 313-335). Springer, Dordrecht.

- Ulas, A., Behrens, T., Wiesler, F., Horst, W. J., & Schulte auf'm Erley, G. (2013). Does genotypic variation in nitrogen remobilisation efficiency contribute to nitrogen efficiency of winter oilseed-rape cultivars (*Brassica napus* L.)? *Plant and soil*, 371(1), 463-471.
- Wieczorek, P., Węgorek, P., Protasewicz, D., Zamojska, J., Budziszewska, M., Mrówczyński, M., & Obrepalska-Stepłowska, A. (2014). Changes in the expression of mitochondrial cytochrome oxidase subunits due to pyrethroid intoxication in pyrethroid-resistant pollen beetles, *Meligethes aeneus* (Coleoptera: Nitidulidae). *Journal of Plant Diseases and Protection*, 121(2), 89-95.
- Williams, I. H. (2010). The major insect pests of oilseed rape in Europe and their management: an overview. *Biocontrol-based integrated management of oilseed rape pests*, 1-43.
- Zhang, X., Liu, T., Li, X., Duan, M., Wang, J., Qiu, Y., & Shen, D. (2016). Interspecific hybridisation, polyploidisation and backcross of *Brassica oleracea* var. *alboglabra* with *B. rapa* var. *purpurea* morphologically recapitulate the evolution of *Brassica* vegetables. *Scientific Reports*, 6(1), 1-14.
- Zimmer, C. T., & Nauen, R. (2011). Cytochrome P450 mediated pyrethroid resistance in European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae). *Pesticide biochemistry and physiology*, 100(3), 264-272.
- Zimmer, C. T., & Nauen, R. (2011). Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. *Pest Management Science*, 67(5), 599-608.

## DECLARATION

on authenticity and public access of my final master's thesis.

Student's name: **KIRUI DANIEL KWEMOI**

Student's Neptun ID: **KY436J**

Title of the document: **PYRETHROID RESISTANCE MONITORING OF POLLEN  
BEETLE (*Brassicogethes aeneus*) IN HUNGARIAN RAPE FIELDS**

Year of publication: **2023**

Department: **PLANT PROTECTION**

I declare that the submitted final master's thesis is my own, original individual creation. Any parts taken from another author's work are clearly marked and listed in the table of contents.

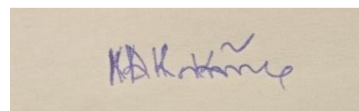
If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another final master's thesis.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectual property.

I acknowledge that the electric version of my work is uploaded to the repository system of the Hungarian University of Agriculture and Life Sciences.

Place and date: 2023-year 04 month 26<sup>th</sup> day



Student's signature

## SUPERVISOR'S DECLARATION

As a supervisor of Kirui Daniel Kwemoi (Student's name) KY436J (Student's NEPTUN ID), I here declare that the final master's thesis has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend/don't recommend<sup>1</sup> the final essay/thesis/master's thesis/portfolio to be defended in a final exam.

The document contains state secrets or professional secrets: yes      no<sup>\*2</sup>

Place and date: 2023 year 04. month 26<sup>th</sup> day



---

Internal supervisor

---

<sup>1</sup> Please underline applicable.

<sup>2</sup> Please underline applicable.