The Occurence of Lower Sensitivity Pollen Beetle (*Meligethes* spp.) Populations to Chlorpyriphos in Winter Oilseed Rape (*Brassica napus* L.)

Tanja GOTLIN ČULJAK [1]
Renata PERNAR [2]
Ivan JURAN [1](✉)
Mario ANČIĆ [2]

Summary

Pollen beetle (*Meligethes* spp.) is the most important oilseed rape pest. If no control measures are conducted, this pest could cause even more than 50% reduction of the yield which is the reason of high insecticides input every year. Due to frequent use of insecticides for its control in the last decade, the resistance of pollen beetle to pyrethroids was confirmed. In order to test sensitivity of adult forms to chlorpyriphos, 52 populations were collected from different oilseed rape fields in 2013 and 2014. For each sample were determined coordinates using GPS (Global positioning system). Based on the collected data the database in GIS (Geographic information system) was created. In a laboratory experiment they were tested according to IRAC Susceptibility Test Method No: 025. Results show lower chlorpyriphos efficiency, with potential to be tolerant, on eight pollen beetle populations located in three eastern counties where the highest amount of arable crops is located. This is the first record of low sensitivity pollen beetles to chlorpyriphos in Europe.

Key words

resistance, chlorpyriphos, pollen beetle, oilseed rape

[1] University of Zagreb, Faculty of Agriculture, Department of Agricultural Zoology, Svetošimunska 25, 10000 Zagreb, Croatia
✉ e-mail: ijuran@agr.hr
[2] University of Zagreb, Faculty of Forestry, Department for Forest Inventory and Management, Svetošimunska 25, 10000 Zagreb, Croatia

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Introduction

Pollen beetle (*Meligethes* spp.) is one of the most important oilseed rape pests and it occurs regularly in the whole breeding area of this crop. If no control measures are conducted, this pest could cause even more than 50 % reduction of the yield which is the reason of high insecticides input every year. Chemical control is still common in Europe with at least one application (Richardson, 2008) and insecticides are often applied prophyllacticaly for more than two decades without monitoring of pollen beetle populations and utilization of active ingredients with different mode of action (Žlof, 2008). Consequently lower sensitivity of pollen beetles was occurred and led to insecticides resistance. Pyrethroids are the group of insecticides that are used to the greatest extent and resistance was confirmed for the first time in 2000 in Sweden and Switzerland (Derron et al., 2004; Kazachkova et al., 2007) and later in other EU countries (Ballanger et al., 2003; Hansen, 2003; Wegorek, 2005; Heimbach et al., 2005; Slater et al., 2011). According to Richardson (2008) alternative active ingredients to be used instead of pyrethroids included thiacloprid, bifenthrin, etofenprox, tau fluvalinate, chlorpyriphos and malathion, although pollen beetle resistance to tau fluvalinate was already recorded (Hansen, 2008). The trend of insecticides utilization in Croatia is similar. The use of organophosphorus insecticides, whose efficiency for pollen beetle control at low temperatures was not satisfactory, were followed by the use of pyrethroids that were of greater efficiency at low temperatures (Maceljski, 2002; Jelovčan and Gotlin Čuljak, 2007; Jelovčan et al., 2008). As a result pollen beetle resistance to pyrethroids was confirmed in 2007 (Gotlin Čuljak et al., 2013). Chlorpyriphos, another active ingredient widely used for pollen beetle control in Croatia, is utilized in combination with cypermethrin. Between 0.288 to 0.5 kg of active ingredient is applied per hectare (Richardson, 2008a). In countries where combination of organophosphorus and pyrethroids insecticides was no longer applied, good effect on pollen beetle mortality was achieved (Wegorek et al., 2009; Šmatas et al., 2012; Milovanović et al., 2013). Lower sensitivity of pollen beetles and their potential resistance to chlorpyriphos would have severe implications in successful control of this pest.

The objective of this study was to determine the level and the spatial distribution of the sensitivity of pollen beetles towards the chlorphyriphos.

Materials and methods

Adult forms of pollen beetle (*Meligethes* spp.) were collected during 2013 and 2014 from 52 diverse sites in continental counties of Croatia where oilseed rape is grown regularly. Sites were chosen randomly and were not selected on the basis of any criteria such as treatments history or reports of resistance. For each sample coordinates using GPS (Global positioning system) were determined. Based on the collected data it database in GIS (Geographic information system) was created. This enabled to make thematic layers, by which was shown the spatial distribution of the test sites during years of monitoring and level of chlorpyriphos efficiency. This allowed implementation of the spatial analysis by layers within the established GIS - models (e.g. level of chlorpyriphos efficiency during the years of monitoring and counties, etc.). Beetles collected from individual field were considered as one sample. Approximately 150 individuals of pollen beetle adults were collected from each site and were stored in aerated plastic insect cages with the filter paper at the bottom., Few oilseed rape leaves and inflorescences were put inside each cage as a food source and shelter. After collection and during transportation beetles were not exposed to any stress in terms of large temperature amplitudes, humidity or starvation. On arrival in the laboratory beetles were left to recover overnight.

Table 1. Susceptibility rating scheme for chlorpyriphos (according to IRAC method No. 025)

Rate	% affected	Classification	Code
0.3 µg/cm^2	≤ 100 to 90	susceptible	1
	< 90	potential to be tolerant	2

The test was set up according to IRAC susceptibility method No. 025 (2014). Glass vials with an internal surface area of 61 cm^2 were used for this study and prior to start testing each vial was coated in acetone and left to dry out. The technical grade of chlorpyriphos was diluted in acetone. One treatment of 0.3 µg/cm^2 (16% of the typical field application rate of 187.5 g of active ingredient/ha) and acetone-only solution (control treatment) were utilized. Two replicates of one concentration and one control were used for each site. Glass vials were filled with 0.5 ml of chlorpyriphos or acetone-only solution and rotated on roller mixer at room temperature until the acetone was completely evaporated. Adult forms of pollen beetle were aspirated from insect cage and total of 10 individuals were added to each test vial before the vials were loosely capped and stored at 20°C.

The experiment was scored after 24 hours period. Total number of pollen beetles from each test vial was placed on the paper in the centre of 8 cm drawn circle. The assessment was done in bright light in order to stimulate beetle movement. All beetles which were not able to exit the circle within one minute period were considered as severely affected. Results were expressed as percentage of mortality which was calculated using Schneider – Orelli's formula (Püntener, 1981). To categorise the test samples a susceptibility rating scheme (Table 1) was used.

Results and discussion

Lower efficiency of chlorpyriphos was recorded on eight out of 52 tested pollen beetle populations from 11 counties (Table 2).

Among lower sensitivity populations the highest mortality (89.5% of affected pollen beetles) was recorded at Drenje site. At four sites pollen beetle mortality was around 85% (V. Kopanica, Slakovci, Tovarnik, Š. Polje and Tenja). The lowest mortality was recorded at Đurđanci (75%) and at Magadenovac site (70%). Spatial distribution of tested sites and susceptibility level of pollen beetle to chlorpyriphos are presented in Figure 1.

Table 2. Tested sites, percent of affected pollen beetle adults and classification during 2013 and 2014

County	Site	% affected	Classification (code)
Bjelovar - Bilogora	Dežanovac	100	susceptible (1)
	Grubišno Polje	94.7	susceptible (1)
	Hercegovac	100	susceptible (1)
	Miljanovac	95	susceptible (1)
	Trnovitica	100	susceptible (1)
Koprivnica - Križevci	Kalinovac	100	susceptible (1)
	Koprivnički Bregi	100	susceptible (1)
	Redeljevo Selo	100	susceptible (1)
	Subotica Podravska	100	susceptible (1)
Međimurje	Kotoriba	100	susceptible (1)
	Prelog	100	susceptible (1)
	Savska Ves	100	susceptible (1)
Osijek - Baranja	Brestovac	100	susceptible (1)
	Drenje	89.5	potential to be tolerant (2)
	Đurđanci	75	potential to be tolerant (2)
	Ladimirevci	100	susceptible (1)
	Magadenovac	70	potential to be tolerant (2)
	Moslavina Podravska	100	susceptible (1)
	Piškorevci	90	susceptible (1)
	Širine	100	susceptible (1)
	Široko Polje	85	potential to be tolerant (2)
	Tenja	85	potential to be tolerant (2)
Požega - Slavonija	Lipik	100	susceptible (1)
	Lukač	100	susceptible (1)
	Vidovci	94.8	susceptible (1)
Slavonski Brod - Posavina	Batrina	100	susceptible (1)
	Bodovaljci	100	susceptible (1)
	Poljane	100	susceptible (1)
	Šušnjevci	100	susceptible (1)
	Velika Kopanica	84.2	potential to be tolerant (2)
	Zapolje	100	susceptible (1)
Sisak - Moslavina	Dužica	95	susceptible (1)
	Galdovo	100	susceptible (1)
	Jamarice	100	susceptible (1)
	Lipovljani	100	susceptible (1)
	Okoli	100	susceptible (1)
	Voloder	100	susceptible (1)
Varaždin	Križovljan	100	susceptible (1)
	Zelendvor	100	susceptible (1)
Virovitica - Podravina	Antunovac	100	susceptible (1)
	Pitomača	100	susceptible (1)
	Suhopolje	100	susceptible (1)
	Vaška	100	susceptible (1)
	Virovitica	100	susceptible (1)
	Virovitica 1	100	susceptible (1)
	Zdenci	100	susceptible (1)
Vukovar - Srijem	Drenovci	90	susceptible (1)
	Gunja	100	susceptible (1)
	Novi Jankovci	95	susceptible (1)
	Slakovci	85	potential to be tolerant (2)
	Tovarnik	83.3	potential to be tolerant (2)
Zagreb	Šašinovec	100	susceptible (1)

The highest number of sites with potential resistance is located in Osijek - Baranja County where 63% of tested sites show lower chlorpiryphos efficiency. Two sites with lower chlorpiryphos efficiency are located in Vukovar - Srijem County and one site in Slavonski Brod - Posavina County. Potential resistant pollen beetle populations on chlorpyriphos are located in three eastern counties where the highest amount of arable crops is located. The reason for low chlorpyriphos efficiency includes high input of tested chemical into pest control without any restrictions comparing to other European countries where chlorpyriphos application was limited or active ingredient even banned. Due to intensive crop production and pest control, potential resistant pollen beetle populations can expand to the other sites where high chlorpyriphos efficiency was recorded.

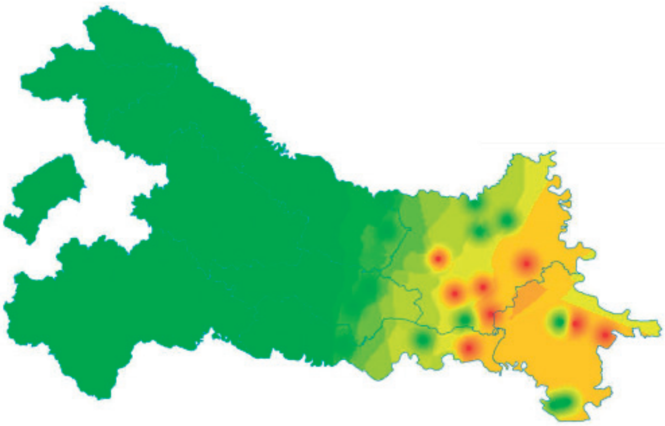

Figure 1. Spatial distribution of tested sites and level of chlorpyriphos efficiency during 2013 and 2014 (green color present sites with high chlorpyriphos efficiency and red color present sites with lower chlorpyriphos efficiency to pollen beetle adults)

Conclusion

Current pollen beetle control is faced with great difficulties. Due to resistance, chemical control is limited with one alternative active ingredient (thiacloprid), which has reduced activity on lower temperatures. Non-chemical control measures that are introduced from organic production include trap crop plants. They have great potential to push down pollen beetle populations below the damage threshold, but further researches are still needed. Lower sensitivity pollen beetle populations to chlorpyriphos with potential to be resistant are recorded for the first time in Europe (APRD, 2014) and together with pyrethroid resistant populations will demand new control strategies.

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