The influence of pyrethroid cypermethrin on non-target species *Harmonia axyridis* ladybird

Aneta Necasova[1], Zaneta Prazanova[1], Eva Hrudova[1], Marek Seidenglanz[2]
[1]Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]AGRITEC, Research, Breeding and Services, Ltd.
Zemedelska 16, 787 01, Sumperk
CZECH REPUBLIC

aneta.necasova@mendelu.cz

Abstract: This study deals with the influence of insecticide active ingredients on selected non-target organisms. Aphidophagous harlequin ladybird (*Harmonia axyridis)* was the tested species, a non-native species that occurs in large populations across the Czech Republic, even in agrocenosis. The objective was to find out if this useful insect was affected by plant protection products (PPP), as was the case with pests of agricultural crops, and whether the development of resistance against selected active ingredients (AI) of insecticides occurred here as well. During the 2020 vegetation season, individual harlequin ladybirds were collected at selected sites and subsequently tested in a laboratory for sensitivity to cypermethrin AI in different concentrations. The efficiency of this AI was evaluated, and from the values acquired, the percentage mortality at the registered dose was calculated. Results were further processed using the specialised POLO PLUS 2.0 software, which calculates values of lethal doses of LD50, LD90 and LD95. As the results show, the mortality increase was recorded due to the increasing dose of the tested AI. From the calculated mortality values and lethal dose, it was found that harlequin ladybird populations are sensitive to the affect of cypermethrin, and thus, the negative influence of this PPP on non-target organisms was verified.

Key Words: Harmonia axyridis, resistance, natural enemy, active ingredient, insecticide

INTRODUCTION

Harlequin ladybird (*Harmonia axyridis* [Pallas, 1773]) is a predatory beetle that belongs to the Coccinellidae family (Poutsma 2008). This family is significant mainly because of its diversity and ability to adapt to different habitats (Ali et al. 2018). The harlequin ladybird comes from East Asia and is quite variable in terms of elytron colouring (Nedvěd 2014). Even though the harlequin ladybird is an invasive species, it is being introduced and used in biological plant protection against insect pests throughout the world. The first population of this ladybird was introduced for biological protection in the USA in 1916 (Brown et al. 2008). In the Czech Republic, the first individuals were introduced for use in hop gardens in 2003 (Nedvěd 2014). Its extension was recorded throughout the whole country during 2006–2009 (Panigaj et al. 2014). Currently, the harlequin ladybird, because of its preference for woody plants, is used mainly in orchards as a natural predator for aphids and used less in field crops and greenhouses. Thanks to its natural link to woody plants and people's homes, one of the most common biotopes of this species are ornamental shrubs and trees (Nedvěd 2014). In the case of aphid overgrowth, it can be used on cereals (Honěk et al. 2017). In a heterogeneous environment, the harlequin ladybird can coexist with other predator species (Osawa 2011).

Due to the intensive production of monocultural crops, the risk of pest occurrence increases. This fact leads to an increased need for chemical treatment of vegetation by plant protection products (PPP). In the case of insect pests, this means insecticides. Recently, synthetic agents have been used for insecticide protection of plants. Zimmer et al. (2014) state that the most used group of insecticide agents are synthetic pyrethroids. The increasing need for treatment with these products also causes an increase in plant protection costs. The reason is that PPP, especially ones with the same active ingredient (AI), are applied repeatedly and, in some cases, inaccurately (Stará et al. 2009). The result is a negative effect on the quality of the environment and on natural enemies of agricultural crops pests, which are also limited by these interventions.

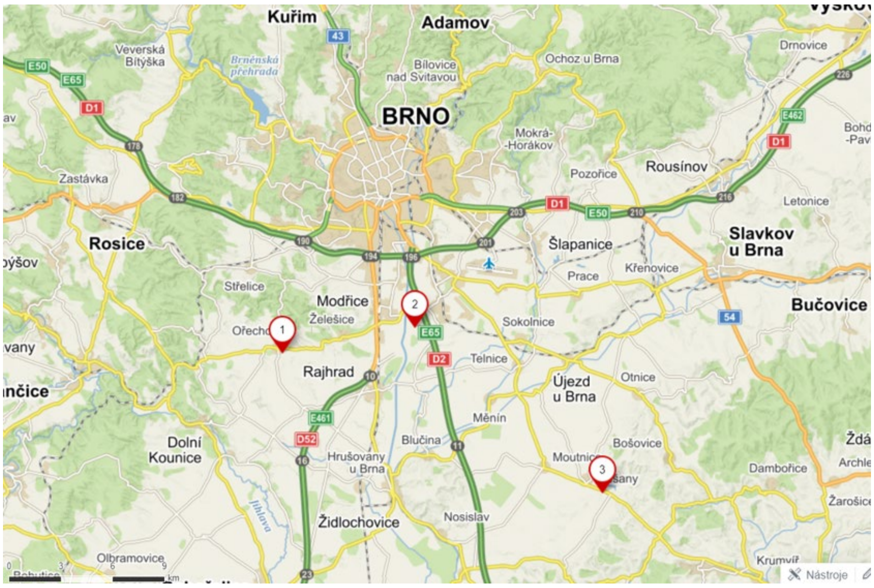
The diversity of population in nature, so-called genetic variability within a population, influences the emergence of resistance. Resistance is defined as an organism's ability to resist adverse influences or a characteristic that allows them to persist against adverse conditions (Petráčková and Kraus 2001). Resistance is an ability inherited or acquired by a process called mutation. According to the Insecticide Resistance Action Committee (IRAC, 2020), resistance can appear within 2–20 years after the introduction or first use of an active ingredients of insecticide. Resistance then results in the inefficiency of an insecticide (Stará et al. 2009). The level of resistance should be monitored, especially in the case of useful organisms. It is the only way to determine the level of the negative influence of PPP on these organisms and effectively support their occurrence at the same time.

MATERIAL AND METHODS

Sampling of *Harmonia axyridis* imagoes

The collection of harlequin ladybirds took place at selected areas during the 2020 vegetation season. The selected areas were numbered: 1 – Ořechov (Brno–venkov), 2 – Brno-Chrlice and 3 – Těšany (Brno–venkov) (Figure 1). Localities of individual populations had to be at least five kilometres apart. The foliage-beating method was chosen for the collection of individuals. The number of individuals was set to be sufficient for testing the chosen AI. According to Nedvěd (2014), beetles should be placed in glass bottles. A small number of plants infested with aphids that served as food to avoid cannibalism within individuals were also placed in these bottles. Absorbent paper was inserted into bottles to absorb surplus moisture. The samples thus prepared were then transported to a laboratory in a thermobox, so they were not subjected to temperatures higher than 20 °C.

Figure 1 Geographical location of areas



Laboratory testing

The IRAC method No. 011 for pyrethroids using the adult-vial-test was used for laboratory testing.

The cypermethrin AI was selected for testing. Dosages were derived from the doses registered for the winter rape plant (*Brassica napus* var. *napus*). The reason for selecting this crop was that rape is very frequently sown, and thus, it is often treated against pests. Additionally, pyrethroids are among the most common AIs.

One millilitre of an AI's solution was applied to each glass vial using a dosing pipette. Acetone was used as a solvent. The AI was applied in various concentrations, namely, 0% (control dose, acetone only), 1.33% dose (= 0.33 g cypermethrin/ha), 4% dose (= 1 g cypermethrin/ha), 20% dose (= 5 g cypermethrin/ha), 100% dose (= 25 g cypermethrin/ha → registered out-of-field dose in the Czech Republic) and 500% dose (= 125 g cypermethrin/ha). Three repetitions were performed for each dose. The whole inner surface of each vial was covered with an AI using a laboratory roller. Imagoes were inserted into prepared vials using soft entomological tweezers: five individuals into each vial. The vials were then closed with a ventilated lid, and subsequently, the vials were placed for 24 hours into a thermostat at 20 °C. Two hundred and seventy specimens of *Harmonia axyridis* were tested during the experiment in total. The progress of the laboratory testing is illustrated in Figure 2.

Figure 2 Use of adult-vial-test during testing by IRAC methods

RESULTS AND DISCUSSION

The efficiency of the AI used on individuals was assessed after 24 hours. The number of live, dead and tremor individuals was evaluated. The mortality percentage was assessed at 100% dose of the AI cypermethrin, which is shown in Table 1. From these values, it is clear that tested individuals were sensitive to the selected AI. In cooperation with the Agritec Plant Research s.r.o. company, Šumperk, the results of laboratory tests executed at Mendel University in Brno were further processed using specialised software, POLO PLUS 2.0. This program calculated the values of lethal doses – LD50, LD90 and LD95. All data are stated in Table 2.

Table 1 Mortality assessment for the active ingredient cypermethrin

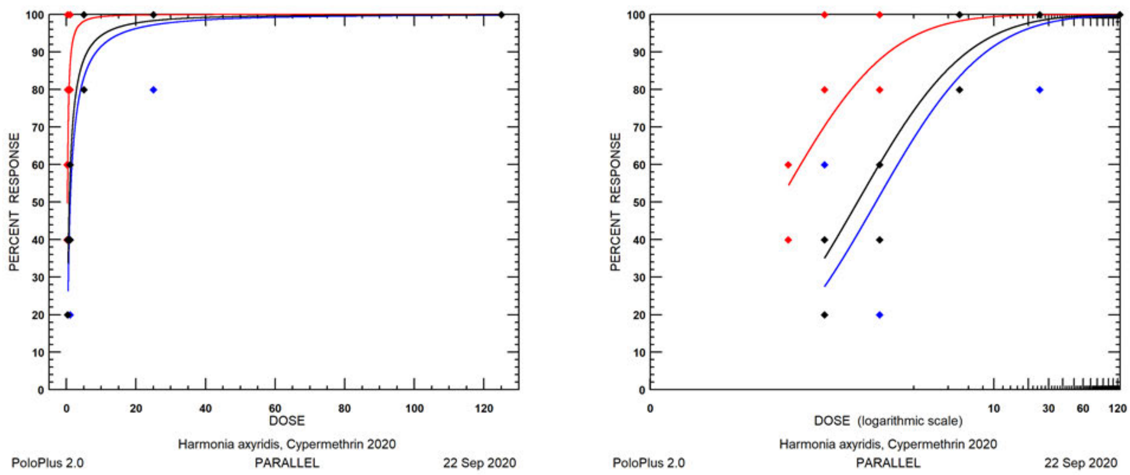
Active substance	**Locality**	**Replicate 1**				**Replicate 2**				**Replicate 3**				**Mortality (%)**
		Total amount	Dead	Alive	% mortality	Total amount	Dead	Alive	% mortality	Total amount	Dead	Alive	% mortality	
Cypermethrin	1	5	4	1	80	5	5	0	100	5	5	0	100	93.33
	2	5	5	0	100	5	5	0	100	5	5	0	100	100
	3	5	5	0	100	5	5	0	100	5	5	0	100	100

Table 2 Evaluation of the adult-vial-test for cypermethrin

Locality	Active substance (preparation)	Registered dose (g/ha)	Mortality (%)	LD 50 (g/ha)	LD 90 (g/ha)	LD 95 (g/ha)
1	Cypermethrin	25	93.33	0.908	9.028	17.314
2	Cypermethrin	25	100	0.099	1.500	3.238
3	Cypermethrin	25	100	0.706	4.106	6.763

In Figure 3 the mortality growth (y) against the increase in dose (x) of an active ingredient is given.

Figure 3 Mortality growth (y) against the increase in dose (x) for cypermethrin in real quantities and transformed (log = decimal logarithm) values



Based on the monitored mortality and calculation of lethal doses, it can be stated that tested populations of the harlequin ladybird (*Harmonia axyridis*) were sensitive to the influence of the cypermethrin pyrethroid. Rodrigues et al. (2013) monitored the reaction of *Hippodamia convergens* populations of lambda-cyhalothrin pyrethroid, and their results confirmed that resistance is a genetically conditioned feature. Costa et al. (2018) also tested field populations of *Eriopis connexa* for lambda-cyhalothrin pyrethroid, and 50% of their tested samples showed resistance against this AI. All these authors confirmed that the resistance of the above stated AI was ten times higher after 54.5 generations of selection.

The problem of pest resistance against insecticides, namely to pyrethroid AIs, is commonly monitored within the Czech Republic (e.g. Seidenglanz et al. 2017), as well as abroad (e.g. Joseph et al. 2017). However, in terms of natural predators, it is still monitored only rarely, and there is a lack of data about it.

CONCLUSION

The submitted study clearly shows that monitored populations of the harlequin ladybird are negatively affected by the AIs from the pyrethroids group. The repeated application of plant protection products can subsequently cause resistance to used active ingredient. In the case of agricultural crops pests, this phenomenon is a significant problem in practice. In the case of natural predators, however, the opposite is true because the selection of resistant populations guarantees that there always will be individuals in vegetation that will be able to suppress survivor pests so that they can slow down their subsequent overgrowth.

ACKNOWLEDGEMENTS

This research was financially supported by grant No. AF-IGA2020-IP055.

REFERENCES

Ali, M. et al. 2018. An annotated checklist of Coccinellidae with four new records from Pakistan (Coleoptera, Coccinellidae). ZooKeys [Online], 803: 93–120. Available at: https://zookeys.pensoft.net/article/22543/. [2020-08-27].

Brown, P.M.J. et al. 2008. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. Biological Control, 53(1): 5–21.

Costa, P. et al. 2018. Field-evolved resistance to λ-cyhalothrin in the lady beetle *Eriopis connexa*. Bulletin of Entomological Research, 108(3): 380–387.

Honěk, A. et al. 2017. Mšice na obilninách: biologie, prognóza a regulace. Certified methodology. Praha: Výzkumný ústav rostlinné výroby, v.v.i.

IRAC (Insecticide Resistance Action Committee) Test Methods. 2020. [Online]. Available at: https://www.irac-online.org/. [2020-08-27].

Joseph, S.H. et al. 2017. Outlook of pyrethroid insecticides for pest management in the Salinas Valley of California. Journal of Integrated Pest Management, 8(1): 1–11.

Nedvěd, O. 2014. Slunéčko východní (*Harmonia axyridis*) – pomocník v biologické ochraně nebo ohrožení biodiverzity? Certifikovaná metodika pro praxi. 2nd ed., České Budějovice: Jihočeská univerzita v Českých Budějovicích.

Osawa, N. 2011. Ecology of *Harmonia axyridis* in natural habitats within its native range. Biological Control, 56(4): 613–621.

Panigaj, L. et al. 2014. The invasion history, distribution and colour pattern forms of the harlequin ladybird beetle *Harmonia axyridis* (Pall.) (Coleoptera, Coccinellidae) in Slovakia, Central Europe. ZooKeys [Online], 412: 89–102. Available at: https://zookeys.pensoft.net/articles.php?id=3822. [2020-08-27].

Petráčková, V., Kraus, J. 2001. Akademický slovník cizích slov A–Ž. 1st ed., Praha: Nakladatelství Akademie Věd ČR.

Poutsma, J. et al. 2008. Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model. Biological Control, 53: 103–125.

Rodrigues, A.R.S. et al. 2013. Pyrethroid resistance and its inheritance in a field population of *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae). Pesticide Biochemistry and Physiology, 105(2): 135–143.

Seidenglanz, M. et al. 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(2): 108–117.

Stará, J. et al. 2009. Metodika hodnocení rezistence blýskáčka řepkového k insekticidům. Methodology for practice. Praha: Výzkumný ústav rostlinné výroby, v.v.i.

Zimmer, C. et al. 2014. 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(3): 279–288.