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ASSESSMENT OF THE HARLEQUIN LADYBIRD'S (*HARMONIA AXYRIDIS*) RESISTANCE TO THE MOST COMMONLY USED ACTIVE SUBSTANCES IN INSECTICIDES

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Abstract

The objective of this study was to find out if useful insects – in this case, harlequin ladybirds (*Harmonia axyridis*) – are affected by plant protection products (PPP) to the same extent as pests and if this species shows any levels of resistance to chosen active substances in insecticides. Harlequin ladybirds were collected in selected localities during the 2019 vegetation season. Their resistance to active substances (lambda-cyhalothrin and thiacloprid in various concentrations) was then tested. In the thiacloprid test, the commercial formulation of Biscaya 240 OD was used. Testing was performed pursuant to the Insecticide Resistance Action Committee (IRAC) methods using the adult-vial-test.
The efficiency of the active substance used was assessed, and tested individuals were divided into groups (Alive, Dead, or Tremor) according to their physical condition. From the values obtained, mortality was calculated at 100% of the dose of each active substance. Subsequently, the results were elaborated using the specialised program of POLO PLUS 2.0, which calculates the values of a lethal dose – LD50, LD90 and LD95. Mortality was assessed depending on the dose increase of tested active substances. According to the mortality assessed and calculated lethal doses, it was observed that populations of the harlequin ladybird are sensitive to the affect of tested active substances. Since these are one-year results, it is difficult to determine if a selection of resistant populations will occur.

Keywords: *Harmonia axyridis*, resistance, natural enemy, active substance, insecticide

INTRODUCTION

Predatory beetle belonging to the Coccinellidae family, that is Harlequin ladybird (*Harmonia axyridis* Pallas, 1773) (Niedobová *et al.*, 2019). The harlequin ladybird originated from Asia and it varies greatly in terms of elytron colouring (Brown, 2011). The family is considered significant for the economic reason (Ali *et al.*, 2018) – despite the fact that this is an invasive species, it is often used in biological protection (Viglášová, 2017). The next reason is especially its ability to adapt to different habitats (Ali *et al.*, 2018). A major factor in intentional transmission between countries around the world is that most species in this family are natural predators and therefore play an important role in developing strategies for biological plant protection against pests (Roy and Migeon, 2010). The first population of this species was introduced in North America in 1916 in order to eliminate aphids in orchards (Brown, 2008). According to Nedvěd (2014), the first population introduced in the Czech Republic was mainly used in hop gardens in 2003. Its extension was recorded throughout the whole country from 2006 to 2009 (Panigaj *et al.*, 2014). Currently, the harlequin ladybird (with respect to its preference for woody plants) is used especially in orchards as a beneficial predator for aphids (Brown, 2011). Due to its natural link to woody plants and human houses, some of the most common biotopes are ornamental shrubs and trees (Nedvěd, 2014). Osawa (2011) states that some of the environment's heterogeneity plays a significant role in the coexistence of this species with other predators.

Due to the intensive production of crops in monocultures, there has been a higher risk of pest occurrence in recent years, which leads to an increasing need for chemical treatment of vegetation by plant protection products (PPP). Synthetic agents recently became more popular for use in insecticides, the most commonly used are synthetic pyrethroids (Zimmer *et al.,* 2014). The rising need for treatment with these products also causes an increase in the cost of plant protection. The reason is that plant protection products, especially the ones with the same active substance (AS), are applied repeatedly and occasionally inaccurately (Stará *et al.*, 2009). This negatively affects not only the quality of plant products and the environment (Tilman *et al.*, 2002), but also the natural enemies of agricultural crop pests. The selection of pest resistance is also a negative aspect.

Genetical variability, which is the presence of resistance genes between individuals of the certain population, is one of the conditions for the development of resistance (Abrol, 2014). Resistance is the ability to resist adverse influences or the ability that allows to persist against unfavorable environmental conditions (Petráčková and Kraus, 2001). The resistance selection in other generations takes place by an action of the selection factor, e.g. an insecticide active substances. Resistance can occur 2–20 years after the introduction or first use of an insecticide (IRAC, 2020). The result of the resistance selection is reduced efficiency (or complete inefficacy) of an insecticide (Stará *et al.*, 2009). Resistance level monitoring is one of the tools used to assess the level of negative affect of plant protection products on useful insects.

MATERIALS AND METHODS

Sampling of Ladybirds *Harmonia Axyridis*

The collection of individuals from the harlequin ladybird (*Harmonia axyridis*) population took place at chosen areas within the Czech Republic in the summer and autumn months of 2019. They were collected on conventional areas that are routinely treated with PPP. The areas were marked by numbers: 1 – Hrušovany u Brna and 2 – Žabčice (Fig. 1). Collection areas of individual populations should always be at least 5 km apart from each other. The foliage beating method was chosen. The number of individuals was set in order to be sufficient for the testing of chosen active substances (AS) – usually ca. 300 individuals for one AS. According to Nedvěd (2014), beetles were placed into plastic bottles after the collection. A small amount of plants infested by aphids was also placed into these bottles; these were used as aliment in order to avoid cannibalism. An absorbent paper

1: *Geographical location of areas 1 and 2* was also inserted into bottles to absorb redundant moisture. Once prepared, the samples were then transported to a laboratory where the beetles were tested immediately. A thermobox was used during transportation to ensure that the temperature would not exceed 20 °C and to avoid the death of sampled individuals.

Laboratory Testing

IRAC methods no. 011 for pyrethroids and no. 021 for neonicotinoids were used for testing. Both methods use the adult-vial-test.

Active substances of lambda-cyhalothrin (pyrethroid) and thiacloprid (neonicotinoid) were used for testing. Doses were derived from the ones registered into the rapeseed plant (*Brassica napus* var. *napus*). This crop was selected because it has a high percentual representation in the sowing procedure; moreover, it is often treated against pests.

One millilitre of active substance solutions was applied into each glass vial using a dosing pipette in concentrations corresponding to the out-of-field dose, i.e. registered into a specific crop. Acetone was used as a solvent. Active substances were applied in various concentrations, namely 0% (control dose, only acetone), 4%, 20% and 100%, which is the registered out-of-field dose in the Czech Republic – for lambda-cyhalothrin it is 7.5 g/ha and for thiacloprid it is 7.2 g/ha. The commercial formulation of Biscaya 240 OD was used in the thiacloprid test. Three repetitions were performed for each AS. The whole inner surface of vials was covered with an active substance using a laboratory roller. Imagoes were inserted into prepared vials using soft entomological tweezers: five individuals into each vial. These vials were then closed with a lid with openings and placed into a thermostat at 20 °C for 24 hours. Overall, 720 individuals of the harlequin ladybird were tested during the experiment. The process of the laboratory testing is illustrated in Figs. 2, 3.

The efficiency of used active substances was assessed after 24 hours. The number of alive, dead and tremorous individuals was evaluated. In cooperation with AGRITEC Plant Research s. r. o., Šumperk, the results of laboratory tests conducted at Mendel University in Brno were further elaborated by using the specialised software of POLO PLUS 2.0. The values of LD50, LD90 and LD95 lethal doses were calculated.

2: *Use of adult-vial-test during testing by IRAC methods – preparation of vials*

3: *Use of adult-vial-test during testing by IRAC methods – inserting imagoes*

RESULTS AND DISCUSSION

The mortality of individuals was assessed for each active substance and repetition for a 100% dose, which can be seen in Tab. I. From the values we calculated, it is clear that there was less sensitivity to the active substance of thiacloprid (Biscaya 240 OD) for tested individuals. In Tab. II. are shown the values of LD50, LD90 and LD95 lethal doses, the highest values are highlighted in bold.

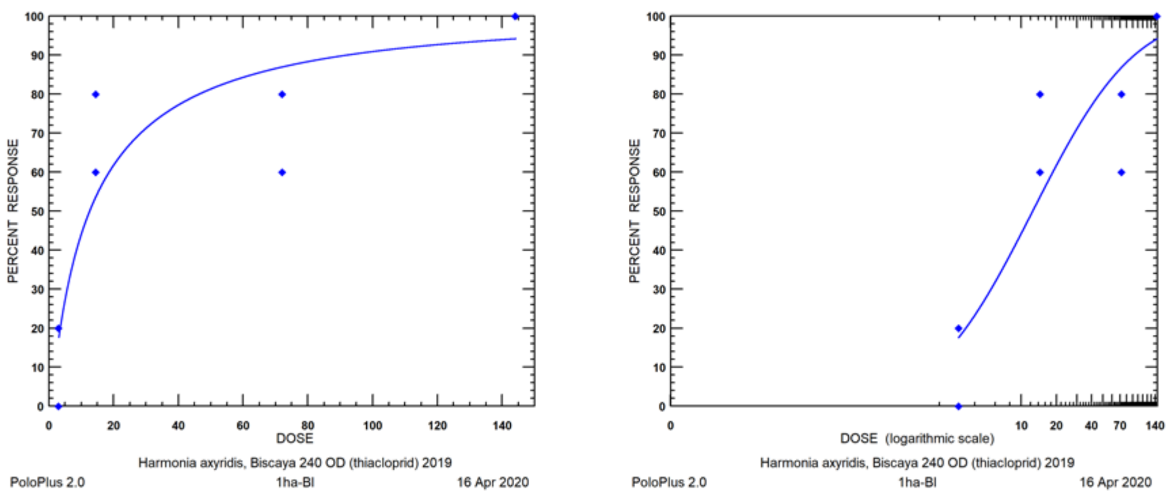
Figs. 4–11 show the rise of mortality rates (y) depending on the dose increase for individual active substances (x).

I: *Mortality assessment for the active ingredients lambda-cyhalothrin and thiacloprid (Biscaya 240 OD)*

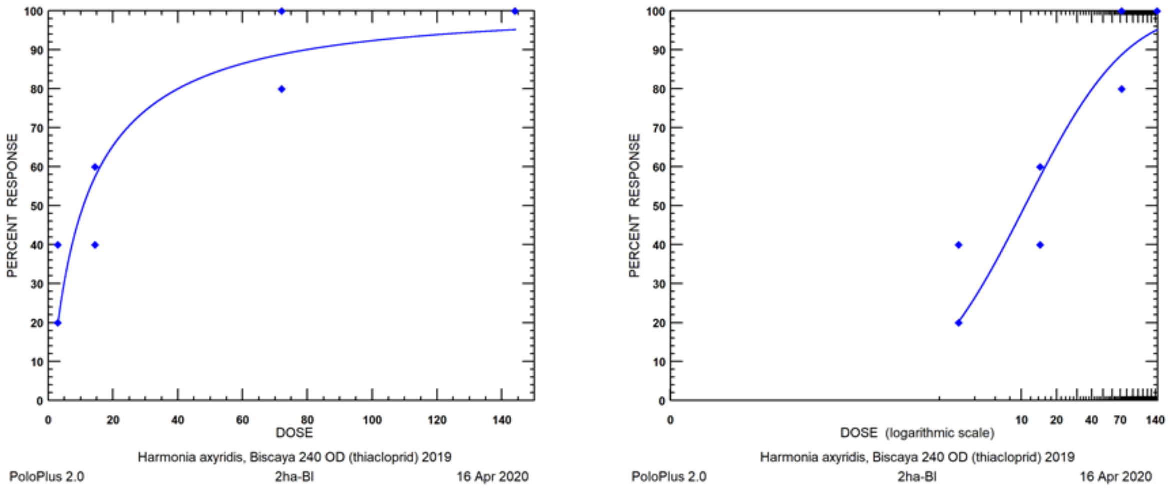
Active ingredient	Locality	Replicate 1				Replicate 2				Replicate 3				Mortality (%)
		Total amount	Dead	Alive	% mortality	Total amount	Dead	Alive	% mortality	Total amount	Dead	Alive	% mortality	
Lambda-cyhalothrin	1	5	5	0	100	5	5	0	100	5	5	0	100	100
	2	5	5	0	100	5	5	0	100	5	5	0	100	100
	2	5	5	0	100	5	4	1	80	5	5	0	100	93.33
	1	5	5	0	100	5	5	0	100	5	5	0	100	100
Thiacloprid	1	5	4	1	80	5	3	2	60	5	4	1	80	73.33
	2	5	5	0	100	5	5	0	100	5	5	0	100	100
	2	5	4	1	80	5	4	1	80	5	5	1	100	86.67
	1	5	5	0	100	5	4	1	80	5	5	0	100	93.33

II: *Evaluation of the adult-vial-test for lambda-cyhalothrin and thiacloprid (Biscaya 240 OD)*

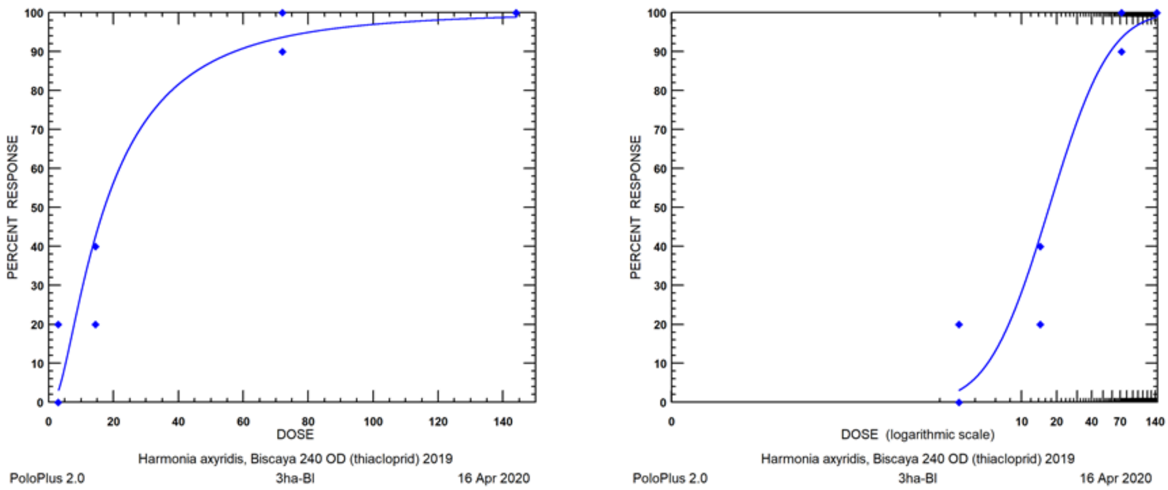
Locality	Active ingredient (preparation)	Registered dose (g/ha)	Mortality (%)	LD50 (g/ha)	LD90 (g/ha)	LD95 (g/ha)
1	Lambda–cyhalothrin	7.5	100	0.027	0.592	1.421
2	Lambda–cyhalothrin	7.5	100	0.334	1.722	2.743
2	Lambda–cyhalothrin	7.5	93.33	0.208	1.667	3.009
1	Lambda–cyhalothrin	7.5	100	0.366	2.678	**4.710**
1	Thiacloprid (Biscaya 240OD)	72	73.33	12.423	92.269	**162.901**
2	Thiacloprid (Biscaya 240OD)	72	100	10.664	79.645	140.835
2	Thiacloprid (Biscaya 240OD)	72	86.66	17.035	57.252	80.730
1	Thiacloprid (Biscaya 240OD)	72	93.33	11.150	41.238	59.748



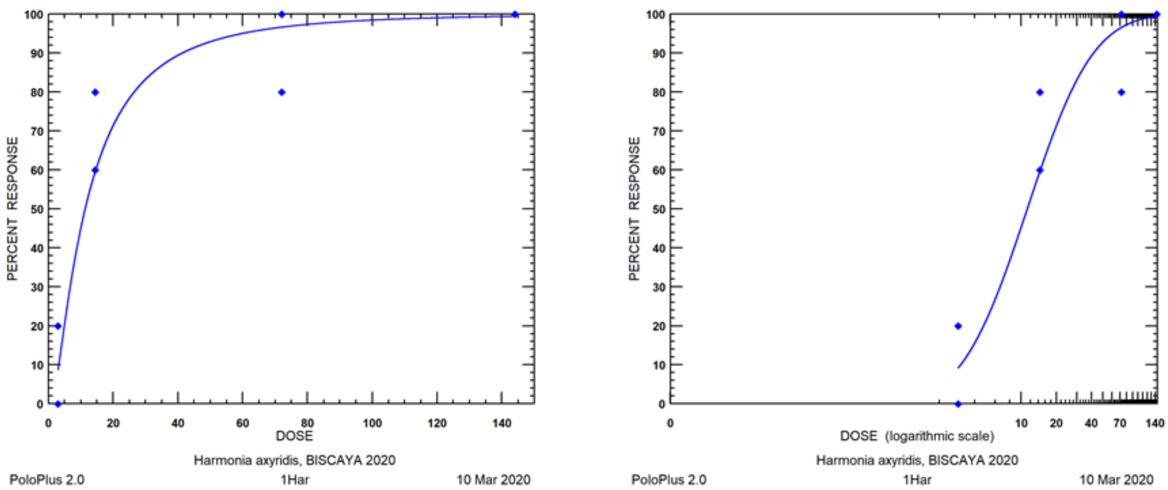
4: *Mortality growth (y) depending on the dose increase (x) for thiacloprid (Biscaya 240 OD) and the first population in real quantities and transformed (log = decimal logarithm) values*



5: *Mortality growth (y) depending on the dose increase (x) for thiacloprid (Biscaya 240 OD) and the second population in real quantities and transformed (log = decimal logarithm) values*



6: *Mortality growth (y) depending on the dose increase (x) for thiacloprid (Biscaya 240 OD) and the third population in real quantities and transformed (log = decimal logarithm) values*

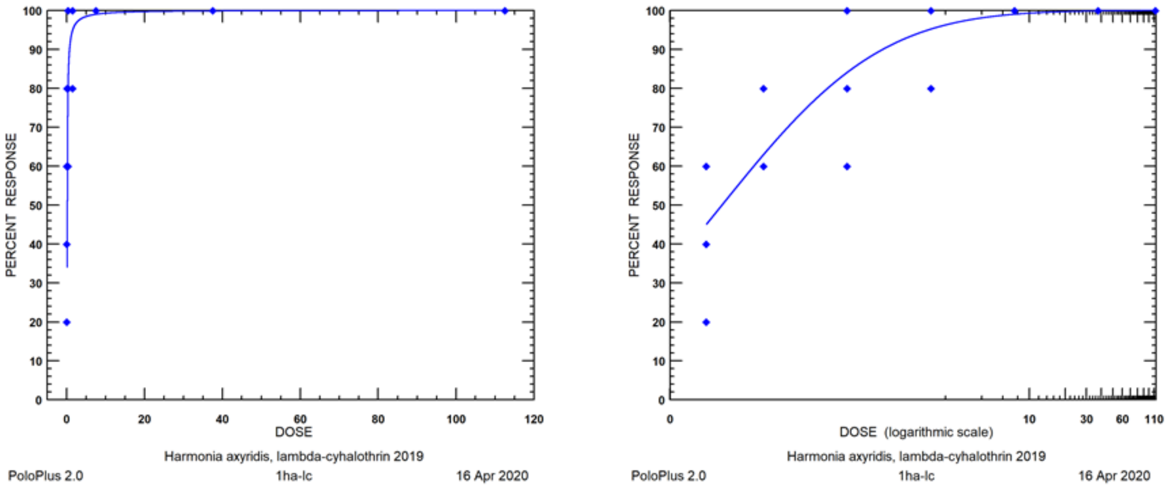


7: *Mortality growth (y) depending on the dose increase (x) for thiacloprid (Biscaya 240 OD) and the fourth population in real quantities and transformed (log = decimal logarithm) values*

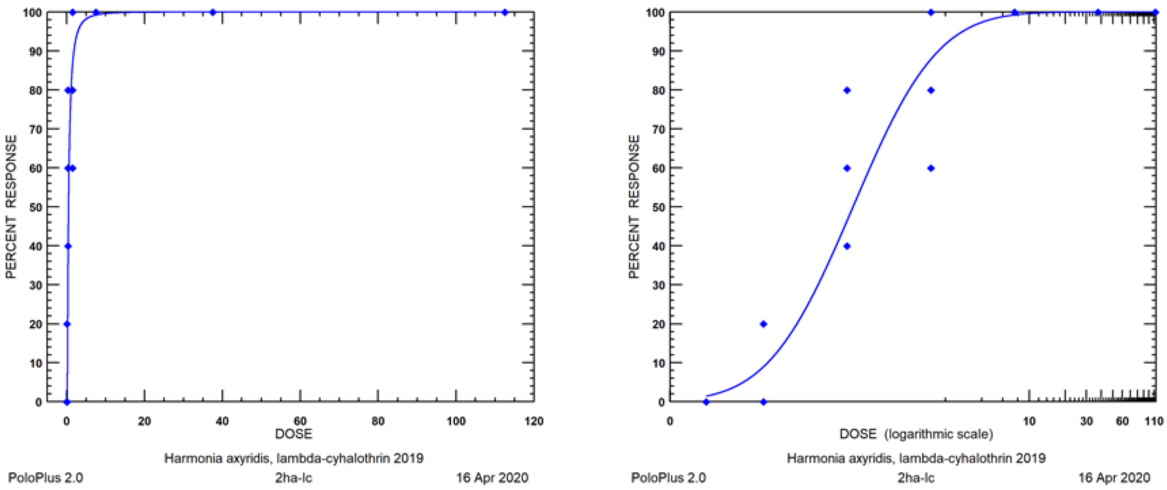
As the assessment of mortality and lethal doses show, the sensitivity to neonicotinoid thiacloprid in the tested agent of Biscaya 240 OD was slightly reduced for all monitored populations of the harlequin ladybird (*Harmonia axyridis*) (Figs. 4–7). Krischik *et al.* (2015) evaluated the affect of the repeated application of the neonicotinoid imidacloprid on four ladybird species. This substance caused significant mortality for three of them: *Harmonia axyridis*, *Coleomegilla maculata* and *Hippodamia convergens*. Thus, the negative affect of this group of active substances on natural predators of pests can be confirmed.

Based on the observed mortality and calculations of lethal doses, it can be said that the tested populations of the harlequin ladybird (*Harmonia axyridis*) were sensitive to the action of the lambda-cyhalothrin pesticide (Figs. 8–11). Costa *et al.* (2018) exposed the field populations of the *Eriopis connexa* ladybird to lambda-cyhalothrin; 50% of the tested samples showed a resistance to this active substance. Rodrigues *et al.* (2013) also monitored the reaction of the *Eriopis connexa* ladybird on lambda-cyhalothrin and their research demonstrated that resistance is a genetically determined feature. The authors stated above confirm that the resistance to this active substance has increased 10 times after 54.5 generations of selections.

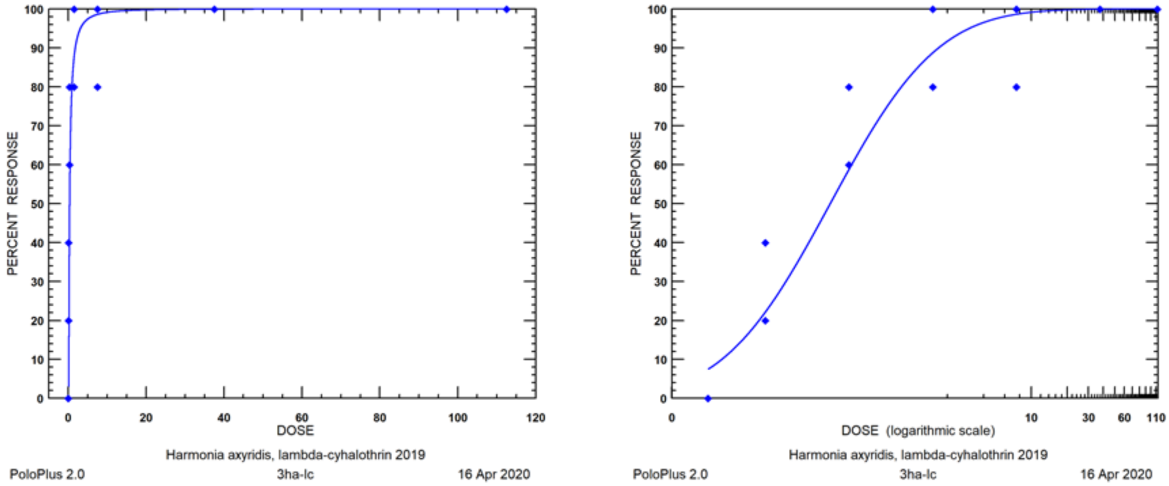
While the resistance state of crop pests to insecticides, including neonicotinoids, is monitored abroad (e.g. Thany, 2010; Nauen and Denholm, 2005 or Elbert and Nauen, 2000), and in the Czech Republic (e.g. Spitzer *et al.*, 2020 or Seidenglanz *et al.*, 2017), the resistance of non-target species is monitored only rarely and there is insufficient data on it.



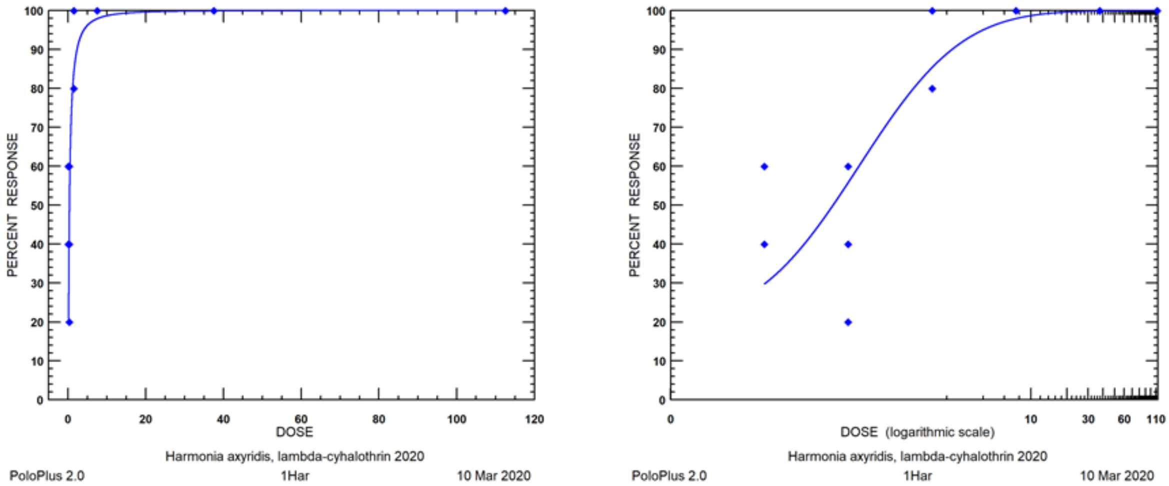
8: *Mortality growth (y) depending on the dose increase (x) for lambda-cyhalothrin and the first population in real quantities and transformed (log = decimal logarithm) values*



9: *Mortality growth (y) depending on the dose increase (x) for lambda-cyhalothrin and the second population in real quantities and transformed (log = decimal logarithm) values*



10: *Mortality growth (y) depending on the dose increase (x) for lambda-cyhalothrin and the third population in real quantities and transformed (log = decimal logarithm) values*



11: *Mortality growth (y) depending on the dose increase (x) for lambda-cyhalothrin and the fourth population in real quantities and transformed (log = decimal logarithm) values*

CONCLUSION

The experiment has shown that populations of the harlequin ladybird are negatively affected by the application of neonicotinoids and pyrethroids. Repeated application may result in the selection of insecticide resistance over time. In the case of plant pests, active substance resistance is undoubtedly a negative phenomenon causing problems in practice; however, in terms of predators, the opposite is true. The selection of resistant populations of natural predators should be a guarantee that there will always be individuals that are able to suppress pests and slow their rapid multiplication.

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