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The effect of τ -fluvalinate and λ -cyhalothrin on two parasitic species of *Phradis* spp. (Hymenoptera, Ichneumonidae, Tersilochinae)

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Abstract: The effect of two pyrethroid ester insecticides, as well as their concentration, on the insect survival were studied along with the dynamics of the insect intoxication, as dependent on the application rate of the chemicals. The laboratory tarsal plate test, carried out in May 2006, was based on commercial formulations of τ -fluvalinate and λ -cyhalothrin instead of pure a.i. The study aimed at producing practical recommendations about the reduction of the application rate in the chemical protection of the oil seed rape (OSR) against the pod and the stem weevils and against the pollen beetle. The results suggest that the application rate reduction of τ -fluvalinate does not alter considerably the survival of the tested insect parasitoids, whereas in case of λ -cyhalothrin it is accompanied by an increase in the insects' survival.

Keywords: τ -fluvalinate, MAVRIK, λ -cyhalothrin, KARATE, pyrethroid ester insecticides, oil seed rape, OSR, parasitoids, Tersilochinae, *Phradis interstitialis*, *Phradis morionellus*, *Tersilochus heterocerus*

INTRODUCTION

The insecticidal properties of different stereoisomers of fluvalinate were reported by Henrick *et al.* already in 1980 [1]. During the early stages of the products' development DL-valinate has been used, but later D-valinate was

introduced, the isomer that is not only more effective in terms of insecticidal activity but also works well as an acaricide [2]. Compendium of Pesticide Common Names [3] refers to the same chemical structure, D-valinate, but it specifies its common name as τ -fluvalinate (Figure 1).

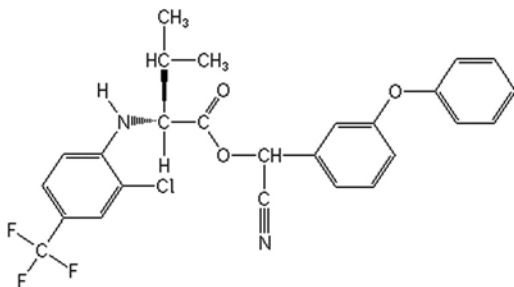


Figure 1. τ -Fluvalinate according to Compendium of Pesticide Common Names [3].

τ -Fluvalinate has been used for a long time in the control of varroa mite, *Varroa jacobsoni* Oudemans, in the form of ready to use, chemical-impregnated plastic strips named Apistan[®] [4, 5] or as plywood hive inserts impregnated by the user with a suspension of a commercial insecticide, Mavrik 24 SC [5]. In both forms it has been merely claimed as “tolerated” by honeybees, but never as entirely harmless to them. In October 2006 a formulation of τ -fluvalinate has been introduced to the insecticide market in Poland under the commercial name MAVRIK 240 EW. It was registered for the use in oil seed rape (OSR) crops and in apple orchards.

The other compound used in the study is cyhalothrin. Its insecticidal and acaricidal properties were described in 1980 by Bentley *et al.* [2 after 6] and in 1982 by Stubbs [2 after 7]. According to Compendium [3] λ -cyhalothrin is a mixture containing equal quantities of two isomers (Figure 2). It has been in use in Poland for many years, including the control of OSR pests, in a number of commercial formulations of different a.i. concentration. It was used in the presented study as a standard, to which τ -fluvalinate was compared.

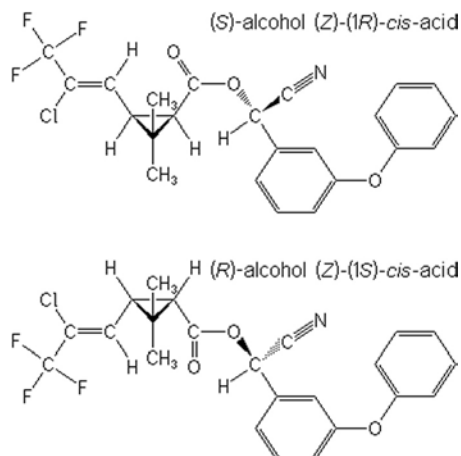


Figure 2. λ -Cyhalothrin according to Compendium of Pesticide Common Names [3].

The study has not aimed at determining LC_{50} or LD_{50} values, which, though perhaps not known specifically for Tersilochinae, must have been determined for these compounds already. Instead, the authors were seeking the insect response to the residual concentrations of a chemical after treating a substrate with the commercial products at the recommended or the reduced application rate. Such results translate more readily into practical recommendations and can be more easily adopted in the farming practice than the highly theoretical indices mentioned above.

MATERIALS AND METHODS

Insecticides

In May 2006, when the study was accomplished, Mavrik 240 EW was not registered in Poland yet, therefore Mavrik 2F, by Makhteshim – Agan Holland B.V., was used for the tests. It contained 240 g of τ -fluvalinate in 1 L of the product, as declared by the producer; a concentration that is equal to the one found in Mavrik 240 EW, already in use in Poland in 2008. The source of λ -cyhalothrin was KARATE ZEON 050 CS, by Syngenta Limited, Great Britain, imported to Poland by Syngenta Crop Protection sp. z o.o., Poland, containing 50 g a.i. per 1L of the product, according to the product label.

Insects

The parasitoids were collected from the winter OSR crop, on the 6th, 11th, 13th and 17th of May 2006, before noon. The crop has not been previously protected with insecticides. The insects were collected by the sweeping net and transferred to glass jars from which they were gently sucked using small aspirator and exhausted immediately into polyethylene containers, in batches of 10 insects, as needed for a single test replicate.

The taxonomic identification of the insects after the experiment revealed that the tested species are *Phradis interstitialis* (Thomson.) and *Phradis morionellus* (Holmgren.), which occurred in samples at an approximately even proportion, making up together more than 95% of the sample population. The third species, *Tersilochus heterocerus* (Thomson.), occurred sporadically. All the species are the larval parasitoids of the pollen beetle *Meligethes aeneus* L.

Tests

The selected test method was the tarsal plate test – the insects were exposed to the surface of a blot paper or OSR leaf rings, within the glass Petri dishes, and closed. The applied blot paper was of 125 mm diameter, the same as the Petri dishes. As only few OSR leaves were that wide, the rings of 54 mm diameter were cut out of the leaves and laid in sets of three on each plate (Figure 3).



Figure 3. Rings of oilseed rape leaves used in the tarsal plate test.

Altogether, four tests were performed, each one focused on a different aspect of the intoxication. They are summarized in Table 1.

Table 1. Tarsal plate tests on *Phradis* sp. – the outline of the experimental design

Test no. substrate	Date	Experimental factors	Factor levels:	Replicates
1. blot	6.05.	product application rate time delay	Mavrik 2F, Karate 050 CS, reference (water) 1, 0.75, 0.50 recommended application rate* 2 h, 3 h, 4 h	27, 27, 9 9, 9, 9 3, 3, 3**
2. blot	11.05.	product application rate	Mavrik 2F, Karate 050 CS, reference (water) 1, 0.75, 0.50 recommended application rate	10
3. leaf	13.05.	product application rate	Mavrik 2F, Karate 050 CS, reference (water) 1, 0.75, 0.50 recommended application rate	10
4. blot, leaf	17.05.	substrate	blot rings, leaf rings	10

* for details see Table 2;

** in the preliminary test 1, 27 replicates for each one of the 2 products were split into 3 application rates, yielding 9 replicates for every product-rate treatment, and these were split into 3 time delay periods, yielding 3 replicates for each one of the product-rate-delay combination. As there was only one application rate for water reference, the reference was only split into 3 time delay levels.

Test 1 was a preliminary one, run in order to determine the appropriate time delay between the substrate treatment and the exposure of insects to the treated surface. The “appropriate” was considered the time short enough to elicit clearly the insect response to the toxin, but long enough to exclude any possibility of the insects` contact with the still-wet surface of the blot or leaf, resulting in the intake of the chemical from the liquid phase. Therefore the experiment allowed to validate, in reference to the tested species of *Phradis* spp., the label “prevention time” intervals, declared for both products. As commonly known, these intervals are determined for honey bee, *Apis mellifera* L., exclusively, and they do not refer in any direct way to other hymenopteran species. Despite of that, they are sometimes used by farmers as an approximation of the hazard to other beneficial insects, caused by the product application. According to the label, the time interval for λ -cyhalothrin in Karate Zeon 050 CS is 1h, whereas

there is no prevention time declared (0 h interval) for τ -fluvalinate in Mavrik 240 EW. The proposed delays between the substrate treatment and the insect exposure were 2, 3 and 4 hours. After the test 1 it was decided which of them was the most suitable one.

Test 2, performed on the blot paper, investigated the insects' response to 2 chemicals at 3 application rates, 4 h after treatment, in the artificial conditions, whereas test 3 was carried out to determine response to the same factors at the same levels but on semi-natural substrate, *i.e.* on the leaf rings. Test 4 enabled comparison of the insect performance on blot or leaf substrate in the absence of the chemical treatment. Both substrates were treated with water and dried for 4 hours and the insects were managed and observed the same way as in the tests 1-3.

The recommended and experimental application rates and volumes are shown in Table 2. The basic application rate recommended by the producer is 200 mL per ha in 400 L of water for either of the products. The rates applied in the experiment were 1.0, 0.75 and 0.50 of the recommended rate. They convert into concentrations of 0.500 mL, 0.375 mL and 0.250 mL of formulation in 1 L of spray volume (Table 2).

Table 2. The recommended spraying rates for τ -fluvalinate and λ -cyhalothrin preparations and the product volumes used in the experiment

Active ingredient	Recommended product application rate [mL per 1 ha]	a.i. content [g per 1L]	Experimental product application rate [% recomm. rate]	product in 1 L solution [mL]	solution applied per 1 Petri dish [mL]	a.i. per 1 Petri dish* [mg]
τ -Fluvalinate	200	240	100	0.500	2.5	600.00*
			75	0.375	2.5	450.00
			50	0.250	2.5	300.00
λ -Cyhalothrin	200	50	100	0.500	2.5	125.00*
			75	0.375	2.5	93.75
			50	0.250	2.5	62.50

* The value known in test 2, on blot paper, but unknown precisely for the test 3 – see text below

The solutions were prepared and applied to blot rings using 2.5 mL PVC syringe (in test 1) or a glass pipette (in test 2). In the leaf test (3) the leaves were

dipped in the spraying liquid for 3 seconds and then laid on paper sheets to dry completely before the rings were cut out of them. After the chemical treatment was applied and the substrate – dried – the insects were placed on Petri dishes which were then closed with lid. Flowers of the OSR were placed on dishes in all tests in order to “comfort” the insects and to minimize the starvation-induced mortality. The living and dead insects were observed and recorded every 60 minutes in the test 2 and every 30 minutes in the test 3. Observations longer than 7 hours (test 2) or 12 hours (test 3) were considered pointless, as with the elapsing time the action of the tested toxins may have become less critical for the survival than the experimental conditions themselves.

The tests were carried out in ventilated rooms, without a possibility to control temperature or RH of the incoming air. Therefore the temperatures ranged between 25.0-26.0 °C during the tests 1 and 2 and they oscillated between 26.0 and 27.5 °C during the tests 3 and 4.

Survival analysis, using Kaplan-Meier (K-M) procedure, as well as Mantel-Haenszel log-rank test of significance were performed for all the time intervals in which the number of alive and dead insects was recorded during the tests, but only until the percentage of alive insects was higher or equal 3.0, the value close to the most frequently estimated SE value. The K-M test converts the individual survival times into the estimates of survival probability for the investigated organisms. *Chi-square* values were also computed based on the sums of the scores assigned to each survival time by the Mantel’s procedure mentioned above [8, after Mantel 1967].

RESULTS AND DISCUSSION

Table 3. Variation between the survival probability of *Phradis* spp. exposed to different spraying rates of τ -fluvalinate (Ma-1, Ma-3/4, Ma-1/2) and λ -cyhalothrin (Ka-1, Ka-3/4, Ka-1/2)

Groups	Mantel-Haenszel log-rank <i>Chi-square</i>	Mantel-Haenszel probability level	Mantel-Haenszel log-rank <i>Chi-square</i>	Mantel-Haenszel probability level
	Blot		Leaf	
Reference / Ka-1	150.020	0.0000	100.715	0.0000
Reference / Ka-1/2	123.019	0.0000	42.513	0.0000
Reference / Ka-3/4	119.394	0.0000	76.746	0.0000
Reference / Ma-1	105.759	0.0000	73.164	0.0000
Reference / Ma-1/2	110.872	0.0000	47.351	0.0000
Reference / Ma-3/4	67.931	0.0000	48.767	0.0000
Ka-1 / Ka-1/2	3.297	0.0694	11.253	0.0008
Ka-1 / Ka-3/4	2.807	0.0939	0.100	0.7513
Ka-1/2 / Ka-3/4	0.095	0.7579	11.543	0.0007
Ka-1/2 / Ma-1	0.802	0.3706	10.152	0.0014
Ka-1/2 / Ma-1/2	0.096	0.7564	1.728	0.1887
Ma-1 / Ka-1	0.200	0.6186	2.122	0.1451
Ma-1 / Ma-3/4	3.400	0.0662	1.101	0.2940
Ma-3/4 / Ma-1/2	1.000	0.4670	0.487	0.4851

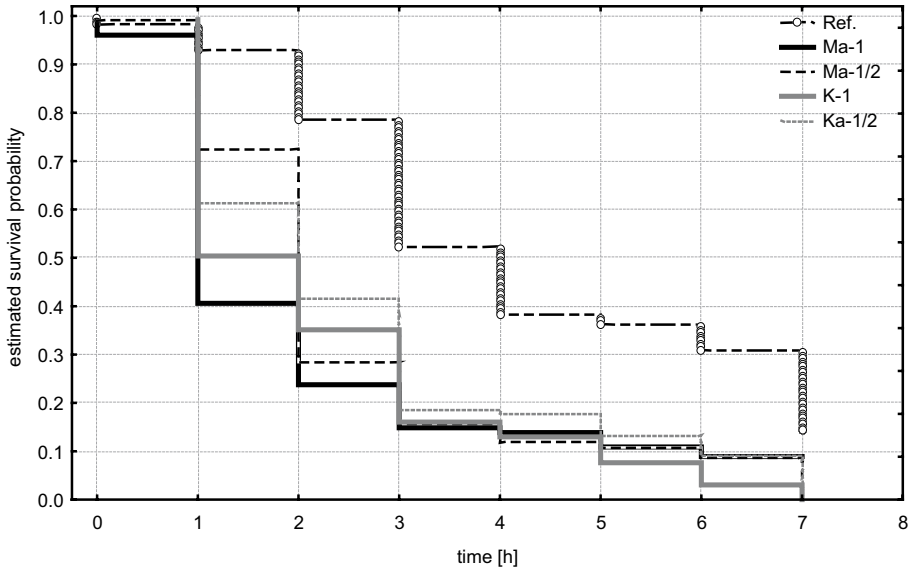


Figure 4. Survival probability of *Phradis* sp. at the spraying rates 1 and 1/2. Substrate: BLOT, test 2.

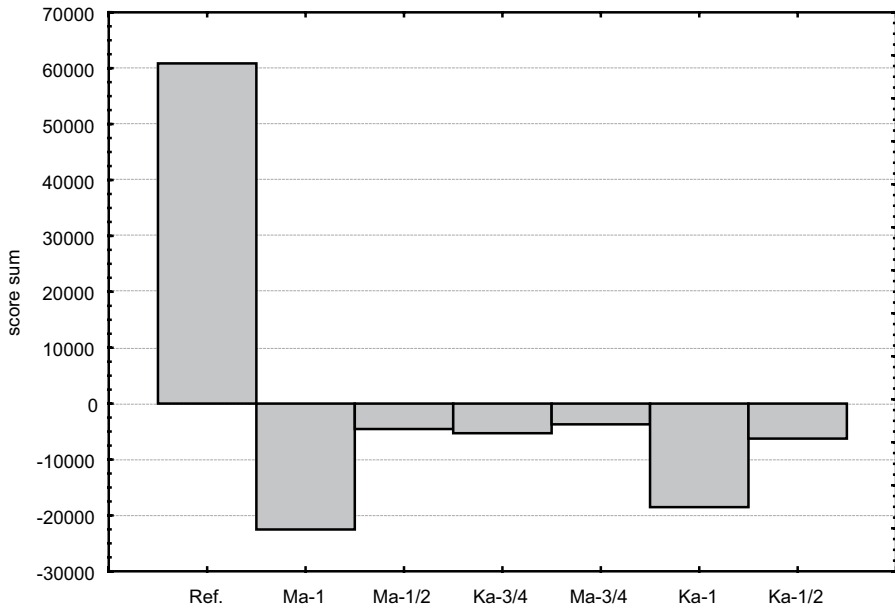


Figure 5. Mantel's score sum for survival at the spraying rates 1, 3/4 and 1/2. Test 2. Substrate: BLOT.

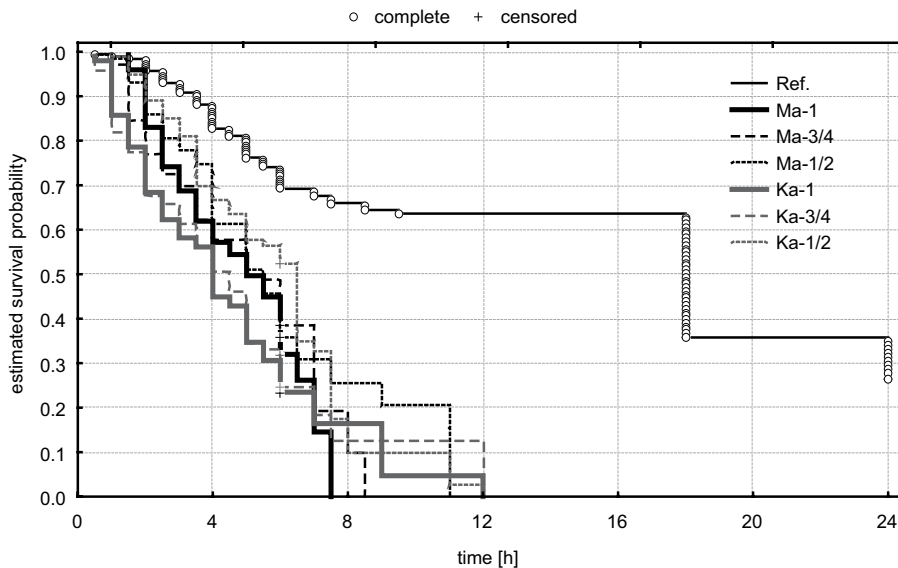


Figure 6. Survival probability of *Phradis* sp. at the spraying rates 1, 3/4 and 1/2. Substrate: LEAF, test 3.

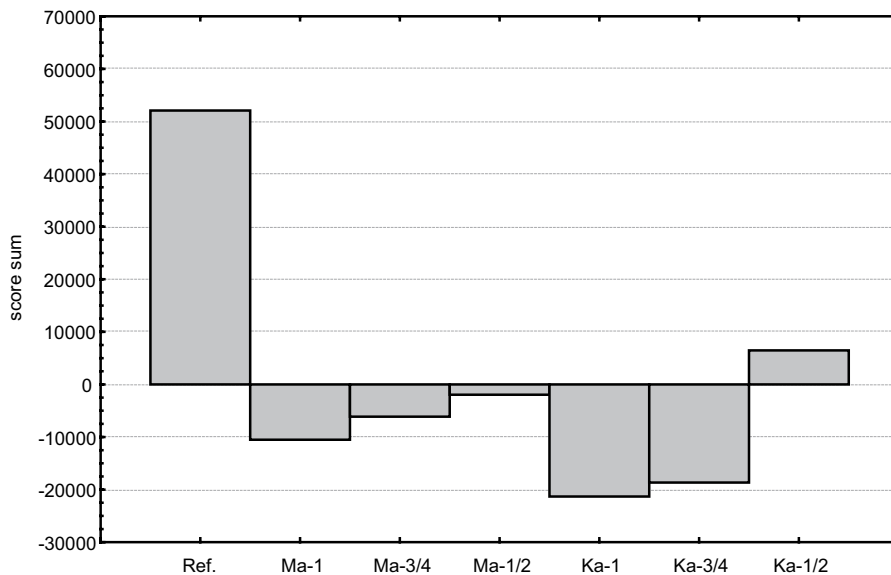


Figure 7. Mantel's score sum for survival at the spraying rates 1, 3/4 and 1/2. Test 3. Substrate: LEAF.

Table 4. Product-Limit Survival Analysis. *Phradis* spp. survival S(T) 6.5 h after insect exposure to the treated surface. Group names as in Table 3.

Groups	Cumulative survival S(T)	Standard error of S(T)
Blot		
Ref.	0.491	±0.033
Ma-1	0.089	±0.028
Ka-1	0.031	±0.015
Ma-1/2	0.088	±0.028
Ka-1/2	0.089	±0.025
Leaf		
Ref.	0.659	±0.033
Ma-1	0.262	±0.049
Ka-1	0.178	±0.046
Ma-3/4	0.269	±0.059
Ka-3/4	0.208	±0.058
Ma-1/2	0.308	±0.061
Ka-1/2	0.350	±0.064

The 6.5 h interval was chosen as a relevant representation of the situation (Table 4, Figures 5 and 7), for at least two reasons. About that time the lines representing survival dynamics at different spraying rates and different products stop overlapping and split sufficiently to make the picture easier to interpret (leaf test, Figure 6). Furthermore, close to the 6.5 h interval the dynamics of Ma-1 and Ka-1 cross one another, marking the point in time since when the final insect survival dynamics at these two treatments apparently diverge. Bearing in mind the 4 h interval between the substrate treatment and the insect exposure in each test, the 6.5 h analysis mirrors the insect survival 10.5 h after the chemicals application to the blot or to the leaf.

Both in the leaf test and in the blot test all the treatments were statistically different from the reference (Table 3, Mantel Haenszel log-rank test); the observed total mortality of Tersilochinae was significantly higher than in the reference plates. The difference may be reliably assigned to the toxic action of the tested insecticides.

After 6.5 h 49.12% or more than 65% individuals survived in the reference Petri dishes (Table 4, Blot, Leaf, respectively). The insecticidal action of Mavrik

at full spraying rate results in 5% or 8% lower parasitoid mortality, as compared to Karate at the full rate and after the same time elapsed (Table 4, Ma-1, Ka-1, Blot and Leaf, respectively). Taking into account the relatively high, > 50%, mortality at reference, one may generally assume that the difference between the parasitoid mortality caused by the two products is 10%, or even 16% (Blot test, Leaf test, respectively).

When used at 50% spraying rate, Mavrik and Karate allowed survival of more than 8.8% individuals (Table 4, Blot). Nevertheless, in case of Karate already 1 h later 100% mortality occurred, whereas of all the insects exposed to Mavrik residues, 5% survived (Figure 4). Furthermore, Mavrik results in very similar mortality at the time 6.5 h after the exposure, irrespective of the spraying rate being 50% or full and in both, the blot and the leaf tests. The same is not valid for Karate (Table 4, Figures 4 and 6).

Despite the relations described above as concluded from Kaplan-Meier test, Mantel-Haenszel log-rank test demonstrates that it is not the product brand or active ingredient but the spraying dose, that is responsible for the principal difference in insect mortality dynamics between the investigated treatments (Table 3, Ka-1 / Ka-1/2, Ka-1/2 / Ka-3/4, Ka-1/2 / Ma-1: significant in the leaf test, Ma-1/Ka-1: not significant in leaf and in blot test, see also Figures 5 and 7). 50% dose reduction decreases the dynamics slope and results in the lower terminal mortality of the parasitoids (Figures 4 and 6, higher survival probability). Moreover, the dose reduction is more efficient in terms of the parasitoid survival in the case of Karate, than in the case of Mavrik (Table 3). Nevertheless, the graphical analysis of these differences gives rise to the question, whether the elusive increase in parasitoid survival should be traded off for the possible reduction of the treatment efficacy at 50% spraying rate.

The knock-down effect, typical for the oldest pyrethroids and visible in the first hours of the action of Karate Zeon, ceases gradually around the hour 6 of the leaf test. The plotted survival probability line for Ka-1 (full spraying rate) flattens and remains close to 0.1783 value until the hour 9 (Figure 6, Table 4, Ka-1). The opposite can be seen for Mavrik, where at the same time the probability line for Ma-1 plunges down to reach 0 before the hour 8 (Figure 6, Table 4, Ma-1), partially overlapping with Ma-1/2 line, which finally reaches 0 at the hour 8.5 (Figure 6). The survival at the spraying rate 50% for Karate Zeon remains twice as high as for the full spraying rate until the hour 9 (Figure 6, Table 4, Ka-1/2). Such results give a rather clear picture of the fast acting and fast degraded λ -cyhalothrin and the “gently” acting but presumably more persistent τ -fluvalinate. According to Stenersen [9], giving brief review of the pyrethroid research, replacing side chain of the alcoholic part of molecules with aromatic rings increases the stability

of synthetic compounds compared to natural pyrethroids. Both the investigated substances are examples of such replacement (Figures 1 and 2), but contrary to λ -cyhalothrin, τ -fluvalinate is also lacking the cyclo-propane ring on the opposite side of the ester bond, having another aromatic ring instead. This may contribute to its greater persistence and may also explain the observed very consistent survival probability for Mavrik, irrespective of the used spraying rates.

The risk of bee and parasitoid poisoning are the two entirely distinct issues and they should not be treated as one problem. The body mass of any single parasitoid species of oilseed rape pests is a level of magnitude lower than that of a honey bee. Therefore the overall, individual dose required to kill the former is many times lower than the one needed to poison the latter. In the light of the presented results, the post treatment interval preventing bees poisoning, as given on the product label (1 h for Karate Zeon) is irrelevant for the tested parasitoid species. Furthermore, the other investigated product, Mavrik, at least as registered in Poland, has no declared post-treatment interval for bees safety, suggesting its being extremely friendly for these pollinators. Apart from the presented results of this study, showing considerable risk to Tersilochinae parasitoids, such statement is contrary to the previously known facts about fluvalinate action, coming from its use in the hives against honey bee parasites [4, 5]. Both the chemicals, irrespectively of the variable dynamics of their toxicity to the tested *Phradis* spp., represent a threat to these parasitoid species.

According to the common sense of the insecticide research and their use in practice, the temperatures prevalent during the tests were far from the optimum for the effective action of pyrethroids. Nevertheless, it was possible to measure and quantify at least some of the variation between the products themselves and between their applied doses. One may only speculate, that should the temperatures be lower, the results might be even easier to interpret. On the other hand, the presented data convey another message: that we may sometimes, in the farming practice, underestimate the potential of the pyrethroids working in supraoptimal temperatures.

CONCLUSIONS

1. 10.5 h after treating the substrate with insecticides and 6.5 h after the insects exposure to the treated surface all the treatments have shown mortality of Tersilochinae significantly higher than the one observed in the reference.
2. The crucial factor of the observed differences in parasitoid response is not the active ingredient or a product brand, but their spraying rates used in the

- treatments. Nevertheless, although the spraying rate reduction by 50% may increase parasitoid survival in the case of Karate Zeon, it makes no important difference in relation to the case of Mavrik.
3. Of the two investigated products, Mavrik appears to act less rapidly than Karate Zeon, giving an appearance of the product that is milder and more friendly to the tested beneficial insects. Still, as it seems to be degraded at a slower pace, its negative effect on the parasitoids lasts longer than that of Karate Zeon and this negatively compensates for the expected effect of the dose reduction, resulting the similar mortality at full and at the half dose.
 4. Considerable mortality of the investigated parasitoids, observed after delayed exposure and long time after treatment demonstrates that the post-treatment interval declared on the products' labels may be indicative only for *Apis mellifera* L., but shall not be, in any case, used as a proxy for parasitoid safety. Despite of the variable dynamics of their toxicity to *Phradis* spp., both the tested products represent a threat to these parasitoid species.

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