

ORIGINAL ARTICLE

A case study on the occurrence of pyrimethanil, cyprodinil and cyflufenamid residues in soil and on apple leaves, blossoms and pollen, and their transfer by worker bees to the hive

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Abstract

A field trial on the transfer of pyrimethanil, cyprodinil and cyflufenamid residues from apple trees of Idared cultivar to hives by honeybees *Apis mellifera* was carried out. Two days after spraying (Faban 500 SC and Kendo 50 EW), and on the day of spraying (Chorus 50 WG), the quantities of residues on leaves and flowers of apple trees and pollen were as follows: pyrimethanil: 1.45 µg per cm² of leaves, 11.51 µg per single flower and 7.18 µg · g⁻¹ of pollen, cyprodinil: 1.35, 8.64 and 7.94 µg, and cyflufenamid: 0.064, 0.266 and 0.11 µg, respectively. All of them subsequently disappeared exponentially. Two days after, and on the day of spraying, pyrimethanil (1.81 µg · g⁻¹), cyprodinil (up to 0.55 µg · g⁻¹) and cyflufenamid (0.04 µg · g⁻¹) were found in worker bees. Residues of all used chemicals were found in the brood, honey and wax samples. The residues of pyrimethanil, cyprodinil and cyflufenamid in worker bees exceeded the level of 0.2% of the LD₅₀, which indicates that their application rates (doses) are safe for the honey bee.

Keywords: consumer and bee safety, cyprodinil and cyflufenamid residues, pyrimethanil, transfer to hive, worker bee

Introduction

Plant protection products (PPPs) are mainly used to protect crops against fungal pathogens and herbivorous insects (pests). Undesirable effects of these compounds on the consumer are brought about by their residues on and in plants, in plant products (Sadło *et al.* 2018; Jankowska *et al.* 2019), in soil (Silva *et al.* 2019) and in water (Kapsi *et al.* 2019). The use of these preparations also means an exposure of beneficial organisms to their harmful effects (Potts *et al.* 2010).

Although the efficiency of pollination by honey bees is low compared to solitary bees (Eraerts *et al.* 2019), this species dominates among crop pollinators, and its share in the pollination process accounts for 9.5% of global agricultural production (Gallai *et al.* 2009).

Sixty species of agricultural plants and 140 horticultural species in Poland depend on pollination. It gives a profit of PLN 4.1 to 7.4 billion (NIK 2017). One such crop is the apple (Majewski 2011), of which

Poland is the third largest producer in the world (GUS 2020). The annual domestic production of this fruit in 2021 was estimated at 4 million tonnes (GUS 2021b). The need to pollinate fruit crops means that pollinating insects during the flowering period will be exposed to PPPs (Mayer and Lunden 1986; Mc Kerchar *et al.* 2020; Piechowicz *et al.* 2021b).

Although many responsible producers stop using insecticides just before and during flowering, thus protecting pollinators against direct contact with the most dangerous preparations, the use of fungicides in this period cannot be completely eliminated. As shown by more and more studies, fungicides are also not indifferent to *Apis mellifera* (Mussen *et al.* 2004; Everich *et al.* 2009; Johnson *et al.* 2013). This is especially true of juvenile stages (Traynor *et al.* 2021), whose exposure to pesticides causes much more lasting damage than in adults, as demonstrated by, e.g., Batista *et al.* (2020) and Costa Domingues *et al.* (2021) in bees and broods treated with picoxystrobin (strobilurin group).

An important result of honeybee exposure to PPPs is also the contamination of honey and bee pollen. Bee pollen is the primary source of protein for developing broods (Bakour *et al.* 2019). It is carried by bees to the hive on an ongoing basis (its reserves in the hive usually do not exceed 1 kg) and is used up relatively quickly, thanks to which the residues contained in it are relatively quickly eliminated. The exposure of bees to the residues accumulated in the honey lasts much longer. It is, at least in the case of wild *A. mellifera* colonies, the only source of energy for the colony during the overwintering season.

The residues of active ingredients of PPPs present in bee products can expose beekeepers to significant economic losses because they are classified as organic products (Bogdanov 2006) and such products should not contain pesticide residues. Therefore, the Maximum Residue Levels (MRLs) of pesticides are set at levels close to the limit of quantification. In the case of pyrimethanil, cyprodinil, cyflufenamid and dithianon used in the orchard, these values are, respectively, 0.05, 0.05, 0.05 and 0.01 mg · kg⁻¹. In apples, these residues may be 15.00, 2.00, 0.06 and 3.00 mg · kg⁻¹, respectively, i.e., 300, 40, 0.2 and 300 times more (EU Pesticides Database 2022). Exceedances of MRLs of pesticides disqualify them as fit for sale, even when they are completely safe for human consumption (Piechowicz *et al.* 2018).

The aim of this field trial was to estimate the occurrence and disappearance of pyrimethanil, cyprodinil and cyflufenamid in leaves, flowers, pollen and soil, the dynamics of worker bee intoxication by these chemicals and their transport to the hive as well as their residues in broods, honey and wax. An additional goal was to assess the risk to consumer health posed by honey containing the residues of standard fungicides.

Materials and Methods

The apple orchard protection program against pests and diseases

The field trial was carried out in a commercial 70 ha apple orchard located in Józefów nad Wisłą (Poland) protected in accordance with the approved program.

Four bee families of similar numbers were placed in the middle of the orchard. It was assumed that the bees, in order to minimize the energy cost of harvesting, would first obtain food in the immediate vicinity of the hives, thereby significantly reducing the possibility of feeding on neighboring crops. As it was a commercial orchard, the authors of the research had no influence on the treatment program adopted in the orchard.

The field trial was carried out on the Idared variety. During apple blossoming, when infection may occur through the generative organs of plants, the apple trees were sprayed with Faban 500 SC and Kendo 50 EW (both applied April 30), and Chorus 50 WG (applied May 2). The characteristics of the preparations used and their active ingredients are presented in Table 1. Sampling for laboratory analyses began on May 2nd and continued until the end of flowering, i.e., May 20th.

Sampling

Laboratory samples of flowers (only fully grown flowers were collected) and leaves (ripe leaves) were taken from eight randomly selected apple trees, from each of the four rows from which the samples were taken (32 trees in total). Worker bees, broods, honey (from unsealed honeycomb cells), and new wax (deposited by bees in holes specially cut in the hose) were always taken from all the hives on May 2, 6, 9, 13 and 20.

The bees used for analysis were collected from the nest part of the hive, in which there were both forager bees directly exposed to contact with PPPs used in cultivation and younger bees which had not yet left their hives. The pollen samples were taken with catch kits installed at the bottom of the hives. On May 2nd, the pollen collected by bees within the first 10 h was taken. At subsequent dates, pollen deposited by the bees in their traps was collected throughout the entire period from their earlier emptying.

Additionally, soil samples were taken 30–50 cm from each tree from which the samples of leaves and flowers were collected to the depth of 20 cm, using an Egner's stick (eight collections for one sample).

All types of samples were transported to the laboratory in transport refrigerators (leaves, flowers, soil – 4°C; bees, broods, honey, wax, pollen – 18°C).

In the laboratory soil samples were air-dried (22°C) for 3 days prior to analysis and pulverized with

Table 1. Properties of the applied pesticides

Trade name	AI	Chemical classification	Pesticide content	Solubility – in water at 20°C [mg · l ⁻¹]	Field soil degradation DT ₅₀ (days; aerobic)	Aqueous hydrolysis DT ₅₀ (days) at 20°C and pH7	Mechanism of action	LD ₅₀ for bees	Recommended preventive measures for bees
Faban 500 SC	Dithianon*								
	IUPAC name: 5,10-dioxobenzof[1,4]benzodithiine-2,3-dicarbonitrile	Antraquinone	250 g · l ⁻¹ (21.9%)	0.22 (low)	35 (moderately persistent)	non-persistent	by contact and deep-seated	contact: >100 oral: >25.4	application during reduced bees' activity
Kendo 50 EW	Pyrimethanil								
	IUPAC name: 4,6-dimethyl-N-phenylpyrimidin-2-amine	Anilinopyrimidine	250 g · l ⁻¹ (21.9%)	110 (low)	31.4 (moderately persistent)	stable		contact: >100 oral: >100	
Chorus 50 WG	Cyflufenamid								
	IUPAC name: N-[(Z)-N-(cyclopropylmethoxy)-C-[2,3-difluoro-6-(trifluoromethyl)phenyl]carbonimidoyl]-2-phenylacetamide	Phenylacetyl	50 g · l ⁻¹ (5.32%)	0.52 (low)	25.3 (non-persistent)	stable	systemic	contact: >100 oral: >100	application during reduced bees activity
Chorus 50 WG	Cyprodinil								
	IUPAC name: 4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine	Anilinopyrimidine	500 g · kg ⁻¹ (50%)	13 (low)	45 (moderately persistent)	stable	by deep-seated	contact: >75 oral: 112.5	contact with an animal is possible when the liquid dries completely

*the results of the analysis were not presented because they exceeded permissible validation parameters

AI – active ingredient

a Testchem LMG grinder (Testchem Sp. z o.o., Poland), and mixed immediately prior to analysis.

On the day of Chorus 50 WG application, sampling of worker bees commenced approximately 15 min after the sprayer had passed the hives in the immediate vicinity, and then five times at 2-hour intervals.

Extraction of pyrimethanil, cyprodinil and cyflufenamid residues

Reagents and materials

In field cultivation, three protective preparations were used: Faban 500 SC (Prod.: BASF SE, Ludwigshafen, Germany), Kendo 50 EW (Prod.: Nisso Chemical Europe GmbH, Düsseldorf, Germany), and Chorus 50 WG (Syngenta Polska Sp. z oo, Warsaw, Poland). The properties of the determined pesticides are given in Table 1.

The analytical standards of pesticides were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) (cyflufenamide) and the Institute of Industrial Organic Chemistry (Poland) (cyprodinil, pyrimethanil).

Petroleum ether, dichloromethane, acetonitrile, acetone, tri-sodium citrate, anhydrous sodium sulphate(VI), di-sodium hydrogen citrate and sodium chloride were obtained from Chempur (Piekary Śląskie, Poland). Florisil (0.15–0.25 mm) was obtained from Macherey-Nagel (Düren, Germany) and C18 EC sorbent from Srobtch (Norcross, USA).

Extraction from the surface of flowers and leaves

Extraction of the residue from the surface of flowers and leaves was carried out in accordance with the methodology presented by Piechowicz *et al.* (2021b).

It was decided to use petroleum ether as a solvent because it has strong hydrophobic properties and does not penetrate into the samples, thereby extracting the residues only from their surface. This made it possible to identify only those residues with which the bee may have come into direct contact.

Extraction from soil and beeswax

Extraction of the residue from soil and wax was carried out in accordance with the methodology presented by Piechowicz *et al.* (2021b).

Extraction from worker bees, broods, pollen, and honey

Extraction of residues from bees, broods, pollen, and honey was performed analogously to the methodology of Piechowicz *et al.* (2021b), while freeze-drying was carried out at -50°C , and 150 mg of PSA was replaced with 300 mg of Florosil.

Chromatographic determination of pyrimethanil, cyprodinil and cyflufenamid residues

Chromatographic analysis

Chromatographic analysis was performed in the same way as in our previous studies (Piechowicz *et al.* 2021a).

Standard solutions were prepared in a clean matrix at the following concentrations: 0.01, 0.05, 0.10, 0.50 and $1.0 \text{ mg} \cdot \text{l}^{-1}$. Linearity was described with determination coefficients ($r^2 > 0.99$). Excellent linearity was achieved for the studied pesticides when using matrix-matched standards.

Method validation

The method was validated for evaluation of the following parameters: linearity (expressed as the coefficient of determination, r^2) and working range, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and repeatability. The acceptance criteria for the method were as follows: average recoveries for the tested pesticides in the range of 70–120% and precision expressed as $RSD \leq 20\%$. Validation was performed according to the European Union guideline SANTE (2017). Validation parameters are presented in Table 2.

Data analysis

Residues (R) were expressed in μg per single flower, μg per cm^2 (leaves) and μg per g of the worker bees, brood, honey, pollen, wax, and soil. The obtained values were corrected for recoveries determined for a given sample type (the matrix concentration: $0.001 \mu\text{g}$ per flower, $0.001 \mu\text{g}$ per cm^2 of leaf, and $0.01 \mu\text{g} \cdot \text{g}^{-1}$ in the case of bees, brood, pollen, honey, wax, and soil). The LOQs of all pesticides were: $0.001 \mu\text{g}$ per flower, $0.001 \mu\text{g}$ per cm^2 of leaf and $0.01 \mu\text{g} \cdot \text{g}^{-1}$ of worker bees, brood, honey, pollen, wax, and soil.

The trends of disappearance, similar to the research of Szpyrka and Sadło (2009), were described by exponential function using Equation 1:

$$R_t = R_0 \times e^{-kt}, \quad (\text{Eq. 1})$$

where: R_t – the concentration (residue) of any pesticide after time (in days), R_0 – the initial concentration of the pesticide ($\text{mg} \cdot \text{kg}^{-1}$), k – the rate constant (day^{-1}). Based on Equation 1 the half-lives ($t_{1/2}$) for tested pesticides were calculated using Equation 2:

$$t_{1/2} = \ln \frac{2}{k}. \quad (\text{Eq. 2})$$

$\%LD_{50}$ in bees was calculated according to Equation 3:

$$\%LD_{50} = R_{i(\text{for single bee})} \frac{100}{LD_{50}}, \quad (\text{Eq. 3})$$

where: (R_i) represents residue level for a single bee, assuming the body weight ($b.w.$) of a single individual of 0.12 g^{-1} . Values of LD_{50} for intoxication by ingestion

Table 2. Average recovery and relative standard deviation (*RSD*)

Sample	Pyrimethanil				Cyprodinil				Cyflufenamid			
	recovery [%] 0.01 mg · kg ⁻¹	<i>RSD</i>	recovery [%] 1.0 mg · kg ⁻¹	<i>RSD</i>	recovery [%] 0.01 mg · kg ⁻¹	<i>RSD</i>	recovery [%] 1.0 mg · kg ⁻¹	<i>RSD</i>	recovery [%] 0.0 mg · kg ⁻¹	<i>RSD</i>	recovery [%] 1.0 mg · kg ⁻¹	<i>RSD</i>
Worker bees	101.8	5.5	106.8	4.9	110.2	11.6	106.9	3.6	112.3	15.6	107.5	6.3
Brood	107.3	6.3	110.6	5.3	88.3	9.1	90.1	8.1	114.9	10.4	111.7	6.4
Pollen	102.5	4.8	99.0	4.9	103.8	4.5	106.0	7.9	108.4	4.6	96.8	8.6
Beeswax	89.4	6.1	80.7	7.3	77.5	3.1	85.2	12.5	79.5	8.3	83.6	10.2
Honey	93.2	8.9	85.4	10.6	86.4	8.4	109.5	9.4	87.3	7.3	82.5	10.1
Flowers	99.2	4.7	97.6	6.7	95.7	5.9	104.9	5.3	106.2	3.9	102.7	5.2
Leaves	94.3	8.6	96.2	11.7	95.4	10.6	98.6	7.7	102.3	4.6	97.5	3.1
Soil	87.4	5.7	80.9	8.3	77.3	5.1	71.8	13.4	113.9	10.8	110.6	4.5

and by contact were taken from the Pesticides Properties Database (PPDB 2022).

The residue level of a given pesticide (R_i) was divided by its *MRL* and expressed as %*MRL*. The %*MRLs* of all pesticides found in a given sample were summed, and then the total %*MRL* was calculated using Equation 4:

$$\%MRL = 100 \sum_{i=1}^n \frac{R_i}{MRL}, \quad (\text{Eq. 4})$$

which appears to be a useful tool for evaluation of the total level of the so-called multiple residues.

Using the same residue level (R_i) and assuming a body weight (*b.w.*) of 76 kg and a daily consumption (*C*) of 0.00129 kg of honey by an adult Polish consumer, the long-term daily intake of pesticides along with honey was calculated and expressed as %*ADI* (Acceptable Daily Intake). When calculating the daily honey consumption, the annual honey production in Poland was taken into account, amounting to 181,185 tonnes in 2019 (Kobylińska 2021), and the total number of Poles in 2019 amounted to 38,382,600 people (GUS 2021a).

Similarly, assuming an additive impact of different chemicals on the human body, the total long-term daily intake (expressed as %*ADI*) of all pesticides was calculated according to Equation 5:

$$\%ADI = 100 \frac{C}{b.w.} \sum_{i=1}^n \frac{R_i}{ADI}, \quad (\text{Eq. 5})$$

where: *C* – daily consumption, *b.w.* – body weight, R_i – residue level.

MRL and *ADI* values were taken from the EU Pesticides Database (2021) website. According to European Union law, the obtained percentage value of *MRL* should not exceed 100%*MRL*.

Finally, using the long-term daily intake with honey expressed as %*ADI* (Eq. 5) and a daily honey consumption by an adult Polish consumer $C = 0.00129$ kg, the safe consumption level (C_{safe}) in kg of honey per day was calculated using Equation 6:

$$C_{\text{safe}} = 100 \frac{C}{\%ADI}. \quad (\text{Eq. 6})$$

Statistics

The Spearman's rank Correlation Coefficient (r_s) was used to assess the strength, direction and statistical significance of dependencies between the residue concentrations of applied fungicides found in the various sample types. The strength of their correlation (positive or negative) is estimated using the following guide:

- 1 – perfect correlation: $r_s = 1.0$;
- 2 – very strong correlation: $0.8 \leq r_s < 1.0$;
- 3 – strong correlation: $0.6 \leq r_s < 0.8$;
- 4 – moderately strong correlation: $0.4 \leq r_s < 0.6$;
- 5 – weak correlation $r_s < 0.4$.

The Kruskal-Wallis test was carried out to estimate the statistical significance of differences between the concentrations of fungicide residues found in different types of samples. The strength of these differences is expressed by *p*-value.

Results and Discussion

Occurrence of pyrimethanil, cyprodinil and cyflufenamid on leaves, apple flowers and pollen as a result of direct application and on the soil as a result of drift of the preparation during the treatment

Disappearance of pyrimethanil, cyprodinil and cyflufenamid on leaves, apple flowers, pollen and soil

When spraying a flowering apple orchard, the PPP reaches the leaves and flowers (including pollen) of the apple trees, as well as the soil in their vicinity.

Chemical analyses of their representative samples collected on May 2nd showed that the average quantity of pyrimethanil (2 days after Chorus 50 WG application), and cyprodinil (on the day of Faban 500 SC application) on the flowers was, respectively, 11.51 and 8.64 μg per single flower, while on leaves – 1.45 and 1.35 μg per cm^2 (Table 3). Initial flower and leaf residue ratios were 7.9 : 1 for pyrimethanil and 6.4 : 1 for cyprodinil, respectively, but dropped to 2.0 : 1 and 1.5 : 1 on the last sampling date.

Similarly, the quantity of cyflufenamid (2 days after its application) on the flowers was 0.27 μg per single flower and on the leaves 0.064 μg per cm^2 (flower-to-leaf residue ratio 4.2 : 1).

The residues of all tested pesticides were found in the soil at a level close to the limit of quantification: $<LOQ$ -0.01 $\mu\text{g} \cdot \text{g}^{-1}$ for cyflufenamid, $<LOQ$ -0.03 $\mu\text{g} \cdot \text{g}^{-1}$ for cyprodinil and 0.02–0.13 $\mu\text{g} \cdot \text{g}^{-1}$ for pyrimethanil. Such small residues in the soil and high amounts on flowers significantly differ from our previous studies in which we detected the residues of captan and

fluopyram (Piechowicz *et al.* 2018) and captan and penthiopyrad (Piechowicz *et al.* 2021b), which, despite the high doses used in cultivation, were less concentrated on the leaves and more concentrated in the soil. It is most likely related to the rainfall occurring during the research. Such rainfall during these tests (oral information obtained from the owner of the orchard) took place only between the penultimate and last sampling. This resulted in a more than sixfold increase in the level of pyrimethanil residue in the soil (from 0.02 to 0.13 $\mu\text{g} \cdot \text{g}^{-1}$) and a threefold increase in cyprodinil (from 0.01 to 0.03 $\mu\text{g} \cdot \text{g}^{-1}$). The greater increase in pyrimethanil residues in soil after rainfall may be related to the fact that it has the highest solubility among the analyzed pesticides (Table 1; PPDB 2022).

A slow disappearance of pesticide in soil is a natural phenomenon. Pyrimethanil and cyprodinil are characterized by moderate persistence in soil, and the half-life in soil of cyflufenamid, the fastest-disappearing of all tested compounds, is 25.3 days under aerobic conditions (Table 1; PPDB 2022).

Based on the results of analyses of all samples of leaves, apple flowers and pollen (Table 3), exponential Equation 1 was determined. Except for cyflufenamid in pollen, the average residues had a strong negative correlation with time t (r^2 from 0.8025 to 0.9864; Eq. 1) (Table 4).

Two days after, and on the day of spraying, the quantities of residues on leaves and flowers of apple trees and pollen were as follows: pyrimethanil 1.45 μg

Table 3. Pyrimethanil, cyprodinil and cyflufenamid residues on leaves, apple blossoms, pollen and soil

Sampling date	Pyrimethanil	Cyprodinil	Cyflufenamid
Leaves [μg per cm^2]			
May 02	1.453 \pm 0.105	1.347 \pm 0.115	0.064 \pm 0.006
May 06	0.577 \pm 0.160	0.573 \pm 0.083	0.026 \pm 0.005
May 09	0.351 \pm 0.101	0.439 \pm 0.043	0.026 \pm 0.015
May 13	0.188 \pm 0.051	0.183 \pm 0.019	0.006 \pm 0.002
May 20	0.093 \pm 0.007	0.130 \pm 0.010	<LOQ
Flowers [μg per single flower]			
May 02	11.511 \pm 2.711	8.636 \pm 5.617	0.266 \pm 0.052
May 06	2.450 \pm 0.913	1.959 \pm 0.459	0.098 \pm 0.072
May 09	1.311 \pm 0.290	1.039 \pm 0.073	0.072 \pm 0.013
May 13	0.471 \pm 0.083	0.338 \pm 0.006	0.015 \pm 0.004
May 20	0.183 \pm 0.021	0.197 \pm 0.006	<LOQ
Pollen [$\mu\text{g} \cdot \text{g}^{-1}$]			
May 02	7.18 \pm 0.26	7.94 \pm 1.21	0.11 \pm 0.03
May 06	2.30 \pm 0.80	0.74 \pm 0.51	0.09 \pm 0.01
May 09	1.48 \pm 0.20	0.83 \pm 0.10	0.17 \pm 0.03
May 13	0.95 \pm 0.15	0.51 \pm 0.14	0.11 \pm 0.01
May 20	0.35 \pm 0.07	0.31 \pm 0.06	<LOQ
Soil [$\mu\text{g} \cdot \text{g}^{-1}$]			
May 02	0.04 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.00
May 06	0.03 \pm 0.00	0.01 \pm 0.00	<LOQ
May 09	0.02 \pm 0.00	<LOQ	<LOQ
May 13	0.02 \pm 0.02	0.01 \pm 0.00	0.01 \pm 0.00
May 20	0.13 \pm 0.04	0.03 \pm 0.01	0.01 \pm 0.00

Table 4. Parameters for the exponential disappearance of pyrimethanil, cyprodinil and cyflufenamid residues

Parameter	Pyrimethanil	Cyprodinil	Cyflufenamid
	Leaves		
R_0 [μg per cm^2]	1.5628	1.0847	0.1014
k [day^{-1}]	0.150	0.131	0.2
$t_{1/2}$ [day]	4.6	5.3	3.5
r^2	0.9694	0.9613	0.9452
Flowers			
R_0 [μg per single flower]	11.896	5.439	0.4765
k	0.224	0.208	0.25
$t_{1/2}$	3.1	3.3	2.8
r^2	0.9615	0.9549	0.9864
Pollen			
R_0 [$\mu\text{g} \cdot \text{g}^{-1}$]	7.4311	3.2076	n. d.
k [day^{-1}]	0.159	0.152	–
$t_{1/2}$ [day]	4.4	4.6	–
r^2	0.9458	0.8025	–

R_0 – initial concentration of the pesticide, k – rate constant (day^{-1}); $t_{1/2}$ – dissipation half-life (day^{-1}); r^2 – coefficient of determination
n.d. – not determined

per cm², 11.51 µg per single flower and 7.18 µg · g⁻¹ in pollen, cyprodinil 1.35 µg per cm², 8.64 µg per single flower and 7.94 µg · g⁻¹, and cyflufenamid 0.064 µg per cm², 0.266 µg per single flower and 0.11 µg · g⁻¹, respectively. All of them subsequently disappeared exponentially (Table 3) (constant rates: pyrimethanil: $k = 0.150, 0.224, 0.159 \text{ day}^{-1}$; cyprodinil: $k = 0.131, 0.208, 0.152$ and cyflufenamid: $k = 0.2, 0.25, \text{n.d.}$; Table 4, Eq. 1).

A comparison of the exponential disappearance constants, k (Table 4), showed that the residues of pyrimethanil, cyprodinil and cyflufenamid decreased by far the fastest on flowers ($t_{1/2}$: 3.1, 3.3 and 2.8 days; Eq. 2). Of these three pesticides, cyprodinil residues decreased the slowest in all types of plant material ($t_{1/2} = 5.3$ – leaves, 3.3 – apple flowers and 4.6 days – pollen; Eq. 2), pyrimethanil residues dropped slightly faster and the cyflufenamid on leaves the fastest. These values were similar to those obtained in other studies, in which the half-life of cyprodinil on tomato leaves was 7 days (Szpyrka and Sadło 2009), and of pyrimethanil it was 5.7 days (Sadło 2002). Similarly, in our research, pyrimethanil disappeared on leaves for 4.6 days (Table 4), and on the leaves of raspberry of Laszka variety – 6 days (Sadło *et al.* 2014). Faster disappearance on flowers than on leaves is related to their progressive development.

Flowers collected at later dates were often not fully developed at the time of pesticide application, therefore their surfaces which had contact with the preparation were smaller, and the ongoing development of the generative organ was conducive to the dilution of the pesticide. Such a phenomenon was observed by Sadło *et al.* (2017) who compared the dissipation of captan, boscalid and trifloxystrobin in developing apple fruit. Cyflufenamid residues in pollen ranged from 0.09 to 0.17 µg · g⁻¹, and changed irregularly, i.e., randomly (no exponential correlation; $r^2 = 0.0572$), therefore the disappearance parameters were not determined.

Intoxication of the worker bee population in an apple orchard as a result of the application of fungicides during the flowering period

The occurrence of pyrimethanil, cyprodinil and cyflufenamid residues in/on worker bees

Sampling began on May 2nd, i.e., 2 days after Faban 500 SC and Kendo 50 EW application, that is on the day of Chorus 50 WG application. so the worker bee population was exposed to pyrimethanil and cyflufenamid for 2 days while to cyprodinil for several hours. Hence, the initial concentration of pyrimethanil of 1.81 µg · g⁻¹ (Table 5) found in honeybees taken for analysis 2 days after treatment, is the equilibrium mean value of the intoxication degree of the worker

bee population after multiple orchard visits by the bees. It is therefore understandable why even the highest ($0.55 \pm 0.09 \text{ µg} \cdot \text{g}^{-1}$) content of cyprodinil on the day of treatment (May 2nd) was so significantly lower than the content of pyrimethanil, even though the application rates differed only 2.5 times (Table 3).

To determine the unknown dynamics of intoxication of the bee population just after treatment (at the beginning of our field trial), i.e., when worker bees are free of applied PPP, it was decided to collect samples every 2 h just after the application of Chorus 50 WG (Table 6).

Worker bee intoxication with cyprodinil started from zero-level and within the first 3 hours after spraying increased at the rate of $0.1149 \text{ µg} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. Starting from the 5th day after spraying the residue level decreased exponentially ($R_t = 2.6993e^{-0.149}$). This was also found for the content of pyrimethanil from the 3rd day after the spraying ($R_t = 2.411e^{-0.141}$).

The results of the analysis of the bee samples taken for tests on May 2nd (Table 5) showed that 15 min. after treatment of Chorus 50 WG, the worker bees contained small amounts of cyprodinil ($0.24 \pm 0.07 \text{ µg} \cdot \text{g}^{-1}$), and then in about 4 h its level increased by $0.1149 \text{ µg} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ i.e., according to the approximate value of the slope of the straight line $R_t = 0.1149t + 0.108$. After approx. 4 h (point of intersection of two lines,

Table 5. Pyrimethanil, cyprodinil and cyflufenamid residues in worker bees [µg · g⁻¹]

Sampling date	Pyrimethanil	Cyprodinil	Cyflufenamid
May 02	1.81 ± 1.28	0.55 ± 0.09*	0.04 ± 0.04
May 06	1.67 ± 0.21	1.52 ± 0.13	0.04 ± 0.02
May 09	0.53 ± 0.14	0.97 ± 0.08	<LOQ
May 13	0.22 ± 0.07	0.48 ± 0.15	<LOQ
May 20	0.20 ± 0.15	0.19 ± 0.04	<LOQ

*the highest mean residue level of cyprodinil in worker bee samples collected on the day of treatment; not used to determine the exponential equation

Table 6. Intoxication of the worker bee population by cyprodinil in the course of the day on May 2

Time after application [h]	Cyprodinil contents [µg · g ⁻¹]
0	0
0.25	0.24 ± 0.07
2	0.36 ± 0.05
4	0.55 ± 0.16
6	0.48 ± 0.06
8	0.55 ± 0.09
10	0.47 ± 0.15

P (2.94, 0.45)) the accumulation rate decreased as indicated by the approximate linear function $R_t = 0.011t + 0.416 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, i.e., approximately $0.011 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ (Fig. 1).

Disappearance of pyrimethanil and cyprodinil in the worker bee population 2 and 4 days after application of Faban 500 SC and Chorus 50 WG

Based on the average residues found in/on worker bee 2 (pyrimethanil) and 4 (cyprodinil) days after treatments, Equation 1 was determined (Table 7).

According to the correlation coefficient, cyprodinil and pyrimethanil residues had a strong negative correlation with time t ($r^2 = -0.998$; $r^2 = 0.8371$) (Fig. 2, Eq. 1). A comparison of the exponential disappearance constants showed that cyprodinil and pyrimethanil residues decreased at similar rates ($t_{1/2} = 4.7$ and 4.9 days, Eq. 2) as on leaves and pollen and was significantly slower than on flowers.

Initial quantities (R_0) of pyrimethanil and cyprodinil versus their application rates (D)

Derived from Equation 1 (Fig. 2), initial residues (R_0) of pyrimethanil and cyprodinil found in worker bee populations were 1.82 and $1.48 \mu\text{g} \cdot \text{g}^{-1}$. The ratios of R_0

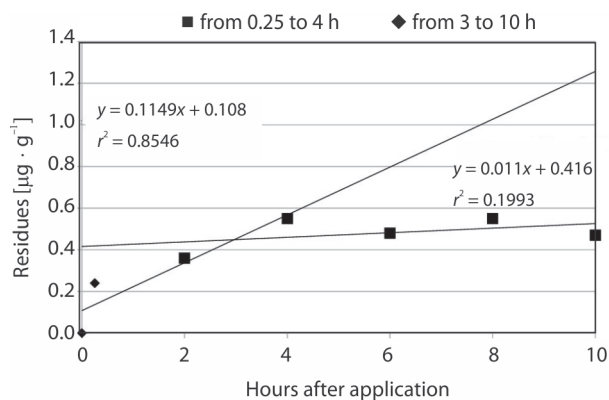


Fig. 1. Approximate estimation of the rate of changes in cyprodinil content in the honeybee population on the day of treatment

Table 7. Parameters of the exponential disappearance of pyrimethanil and cyprodinil residues in the worker bee population from May 2nd to 20th (i.e., from the third day after Faban 500 SC application) and from May 6th to 20th (i.e., from the 5th day after Chorus 50 WG application)

Parameter	Pyrimethanil	Cyprodinil
R_0 [$\mu\text{g} \cdot \text{g}^{-1}$]	2.411	2.6993
k [day^{-1}]	0.141	0.149
$t_{1/2}$ [day]	4.9	4.7
r^2	0.8371	0.998

R_0 – initial concentration of the pesticide, k – rate constant (day^{-1}); $t_{1/2}$ – dissipation half-life (day^{-1}); r^2 – coefficient of determination

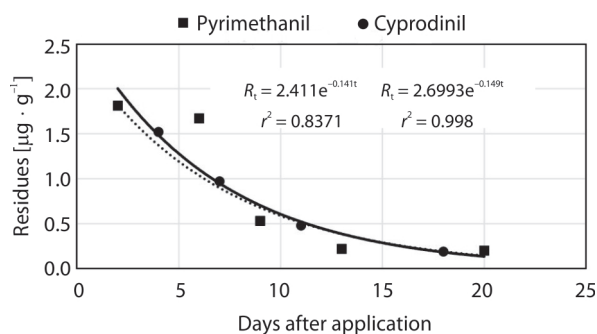


Fig. 2. The course of intoxication of the worker bee population in the apple orchard with pyrimethanil (exponential disappearance; beginning: the third day after treatment) and cyprodinil (residue accumulation on the day of treatment and exponential disappearance; beginning: the fifth day after treatment)

to D , estimated for pyrimethanil ($D = 0.375 \text{ kg} \cdot \text{ha}^{-1}$) and cyprodinil ($D = 0.150 \text{ kg} \cdot \text{ha}^{-1}$) were 4.8 and $9.9 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{kg}^{-1}$. Their average value was $7.4 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{kg}^{-1}$, hence, the residue of any other pesticides disappearing at a constant rate $k = 0.145 \text{ day}^{-1}$, at a given t -time after spraying may be estimated using the approximate formula:

$$R_t = 7.4 \times D e^{-0.145t} \mu\text{g} \cdot \text{g}^{-1}.$$

External factors affecting the amount of pyrimethanil, cyprodinil and cyflufenamid transferred from an apple tree of Idared variety to a hive

Relationship between paired average residues of pyrimethanil, cyprodinil and cyflufenamid

Spearman's correlation coefficient (r_s), a statistical measure of the strength and direction of the monotonic relationship between two paired variables, made it possible to find the correlations between residues of pesticides transported to the hive. Therefore, the r_s was calculated for comparison of paired residues of pyrimethanil vs. cyprodinil and cyflufenamid, and of cyprodinil vs. cyflufenamid on leaves and flowers and in pollen (the result of spraying blooming apple trees), in/on worker bees (contact with pesticide deposits) and in broods, honey and wax (the result of transport of pesticides to the hive by worker bees).

There was a very strong, almost perfect, positive monotonic correlation between pyrimethanil and cyprodinil ($r_s = 1.00$ and 1.00), pyrimethanil and cyflufenamid ($r_s = 0.97$ and 0.98) and cyprodinil and cyflufenamid ($r_s = 0.99$ and 0.98) residues found in/on leaves and flowers. It is understandable, because the content of these pesticides in flowers and leaves reached the

highest value (R_0) during the treatment (for $t = 0$) and then declined according to exponential decreasing function at a very similar rate.

The process of changes in the content of test pesticides in pollen is slightly different than in flowers and leaves or in worker bees, as it follows the processes of real disappearance and of biological dilution caused by the production of new pollen without pesticides. Nevertheless, a very strong correlation between residue levels of pyrimethanil and cyprodinil ($r_s = 0.98$) was noted.

In the case of worker bees, a very strong Spearman's correlation between pyrimethanil and cyflufenamid ($r_s = 0.98$) was found but there was no correlation between residues of pyrimethanil and cyprodinil. However, it should be stated that the residues of cyprodinil began to gather from zero level (an increasing part of the function) and when the rate of accumulation (the contact, e.g., with flowers) was equal to the rate of real disappearance, i.e., after no more than 2 days, the change in residues followed in accordance with an exponential decreasing function, so, in total, did not meet the condition for monotonicity of the function.

Bees that have contact with pesticides carry them to the hive (Piechowicz *et al.* 2018; Piechowicz *et al.* 2021b). Pesticides, along with food (Böhme *et al.* 2017), enter the larvae and can be found in honey and wax (Calatayud-Vernich *et al.* 2018). Their presence in the hive and brood is also confirmed by our research (Table 8). However, only a moderately strong correlation was found between the residues of these pesticides in broods and honey and their lack in wax ($r_s = 0.54$, 0.51 and 0.31, respectively).

The relationship between the average residues found in samples of different types

Spearman's correlation coefficients of a relationship between the average residues of pyrimethanil, cyprodinil and cyflufenamid found in samples of different types were also calculated. The residues of pyrimethanil and cyprodinil in flowers were very strongly, almost perfectly, correlated with their residues in leaves ($r_s = 0.99$ and 0.98) and pollen ($r_s = 1.00$ and 0.95), and the residues of cyflufenamid with its residues in leaves ($r_s = 0.99$). There was also a strong or very strong correlation between the content of pyrimethanil and cyflufenamid in bees vs. flowers ($r_s = 0.78$ and 0.79) and leaves ($r_s = 0.86$ and 0.75) and of pyrimethanil in bees vs. pollen ($r_s = 0.81$). These relationships indicate that the residues found in the hive come from the surrounding cultivation, and not from other areas where the same pesticides would be used, but according to a different treatment calendar. What is particularly noteworthy is the strong correlation of the remains of deep and contact-acting pyrimethanil and systemic cyflufenamid in bees and leaves. From the leaves, bees obtain guttation

water for drinking and cooling the nest. Such water may also contain systemic or even deep-seated PPPs (Sadło *et al.* 2014), or residues of PPPs (Hrynko *et al.* 2021) which were also transported together with the water into the plant. Water is obtained by the oldest collectors, who, due to their age, are no longer capable of long flights. This may mean that they are largely responsible for the residues observed in the samples from the hives.

The Kruskal-Wallis test showed that there was very strong evidence of statistically significant differences between the concentrations of fungicide residues found in different types of samples:

1) pyrimethanil:

– brood and honey vs. leaves (p -value = 0.0035 and 0.0019), flowers (p -value = 0.0 and 0.0) and bees (p -value = 0.0001 and 0.0000),

– wax and pollen vs. brood (p -value = 0.0007 and 0.0000) and honey (p -value = 0.0004 and 0.0000);

2) cyprodinil:

– brood and honey vs. leaves (p -value = 0.0062 and 0.0012), flowers (p -value = 0.0000 and 0.0000) and bees (p -value = 0.0015 and 0.0003),

– wax and pollen vs. brood (p -value = 0.0006 and 0.0000) and honey (p -value = 0.0001 and 0.0000);

3) cyflufenamid:

– worker bees vs. flowers (p -value = 0.0169),

– brood, honey and wax vs. leaves (p -value = 0.0022, 0.0177 and 0.0042) and flowers (p -value = 0.0000, 0.0002 and 0.0000),

– pollen vs. bees (p -value = 0.0014), brood (p -value = 0.0000), honey (p -value = 0.0000) and wax (p -value = 0.0000).

The assessment of exposure of honeybees to pyrimethanil, cyprodinil and cyflufenamid used in the orchard during the flowering period of Idared

Pyrimethanil, cyprodinil and cyflufenamid residues were found in all samples of worker bees, broods, honey and wax. Probably, those samples also contained other, unidentified pesticides that were applied immediately before and especially during flowering, e.g., dithianon (Faban 500 SC).

Pyrimethanil and cyprodinil (anilinopyrimidine group) used in the orchard against Gray mold *Botrytis cinerea*, are inhibitors of many fungal enzymes, including polygalacturonase, cellulase, proteinase and laccase (Milling and Richardson 1995). They also disturb the synthesis of ergosterol – the main sterol of fungal cell membrane (Aleksić *et al.* 2019), and methionine (Fritz *et al.* 2003). Pyrimethanil is a compound that also has a strong cytotoxic effect on Animalia cells (Aleksić *et al.* 2019). It disrupts the normal development of organisms (Bernabò *et al.* 2016), increases reactive oxygen

stress levels and heightens the activity of superoxide dismutase and catalase (Meng *et al.* 2020).

Phenylacetamides (represented in these studies by cyflufenamid) are compounds whose main mechanism of action is the disruption of ergosterol synthesis in fungal cell membranes. They are also inhibitors of DNA synthesis through the inhibition of thymidylate synthase (Ferreira *et al.* 2021). In addition to fungicidal properties, some of them also have strong bactericidal properties (Yele *et al.* 2021). Cyflufenamide also interferes with the function of the thyroid gland, heart, kidneys and brain, and is an inducer of microsomal enzymes, causing liver damage (Stavnichenko *et al.* 2016).

Despite the presence of residues of pyrimethanil, cyprodinil and cyflufenamid in wax (the main material of the hive), in honey (the only source of energy for overwintering bees) and in pollen (the source of protein for developing broods), during nearly a month of research, we observed no changes in the condition of the studied bee colonies. This may be related to the low level of insect intoxication, respectively 0.22% LD_{50} (after food and contact intoxication) for pyrimethanil, <0.01% LD_{50} for cyflufenamid (after food and contact intoxication) and 0.24% LD_{50} after contact intoxication and 0.16% after food intoxication LD_{50} for cyprodinil (Eq. 3) (PPDB 2022). Also, Wu *et al.* (2011) and Smith *et al.* (2020) indicated that small doses of pesticides do not significantly affect the work of future generations of workers. Grassl *et al.* (2018) and Doublet *et al.* (2015) did not observe an increase of susceptibility to diseases in bees subjected to low doses of pesticides, and according to Pohorecka *et al.* (2017) and Almasri *et al.* (2020) the ability of their families to wintering was not weakened. The families used in our research are still alive (their mothers were replaced in 2021).

The assessment of exposure of honey and pollen consumers to pyrimethanil, cyprodinil and cyflufenamid

Bee honey

Honey is considered to be a natural health-promoting product and is recommended in various diets (Bogdanov 2006; Meo *et al.* 2017). For this reason, the standards set for pesticide residues are very rigorous (EU Pesticide Database 2022). Meeting such standards is a serious problem for producers of honey obtained from crops that are intensively protected with chemicals during flowering against fungal diseases (Piechowicz *et al.* 2018; Piechowicz *et al.* 2019).

In honey, residues of all three pesticides (Table 8) were detected, with pyrimethanil and cyprodinil being found in all samples, with the largest amounts of 0.09 and 0.23 $\mu\text{g} \cdot \text{g}^{-1}$, respectively, representing

180 and 460% *MRL* (Eq. 4) of the *MRL*. Residues of cyflufenamid were only found in samples taken on May 6 (0.02 $\mu\text{g} \cdot \text{g}^{-1}$; 40% *MRL*). Exceeding the permissible levels means that the tested honey cannot be sold. However, assuming an average daily honey consumption of 0.00129 kg per person, the daily intake of test pesticides, calculated according to Equation 5, in any case did not exceed 0.02% of the *ADI*. Safe consumption of honey containing the highest amounts of pyrimethanil, cyprodinil and cyflufenamid, calculated in accordance with Equation 6, amounted to 143.6, 9.9 and 152.0 kg, respectively, which means that despite significant *MRL* violations and the probable occurrence of other pesticides, such honey is completely safe for consumption.

Pollen

Bee pollen brought into the beehive by gatherers is considered to be a bee product and therefore the same *MRLs* apply to it as for honey (EU Pesticide Database 2022). The residues of pyrimethanil and cyprodinil ranged, respectively, 0.35–7.18 and 0.35–7.94 $\mu\text{g} \cdot \text{g}^{-1}$, while cyflufenamid ranged 0.09–0.17 $\mu\text{g} \cdot \text{g}^{-1}$ and only in samples collected from May 2 to 13 (Table 8). Their highest levels were, respectively, 14360, 15880 and 340% of the *MRL* (Eq. 4) and, as a result, this product is not suitable for sale. Such a high concentration of residues is related to the structure of anthers in apple flowers. They are exposed high above the calyx, and thus strongly exposed to protective treatments. Additionally, the complex structure of the pollen surface (Evrenosoğlu and Mısırlı 2009) increases its contact with the applied chemicals. The nectar that is dedicated to bees for pollination is produced in the structures hidden deep inside the flower calyx. Therefore, much less of protective preparations get into it than settle on the surface of the pollen. As a result, the residues detected in the honey made from nectar are also significantly smaller.

Due to the lack of information on annual bee pollen production, the %*ADI* and C_{safe} were not estimated.

Conclusions

1. Two days after Faban 500 SC and Kendo 50 EW, and on the day of Chorus 50 WG application, pyrimethanil residues on leaves, flowers of apple trees and pollen were as follows: 1.45 μg per cm^2 , 11.51 μg per single flower and 7.18 $\mu\text{g} \cdot \text{g}^{-1}$, cyprodinil: 1.35 μg per cm^2 , 8.64 μg per single flower and 7.94 $\mu\text{g} \cdot \text{g}^{-1}$, and cyflufenamid: 0.064 μg per cm^2 , 0.266 μg per single flower and 0.11 $\mu\text{g} \cdot \text{g}^{-1}$, respectively. All of them disappeared exponentially (constant rates: pyrimethanil – 0.150, 0.224

and 0.159 day^{-1} ; cyprodinil – 0.131, 0.208 and 0.152 day^{-1} and cyflufenamid – 0.2, and 0.25 day^{-1} .

2. In the honeybee workers, the residues of pyrimethanil, cyprodinil and cyflufenamid immediately after the treatment amounted to, respectively, $1.81 \mu\text{g} \cdot \text{g}^{-1}$, up to $0.55 \mu\text{g} \cdot \text{g}^{-1}$ and $0.04 \mu\text{g} \cdot \text{g}^{-1}$.
3. The intoxication of worker honeybees by cyprodinil started from a zero level and then within about 4 h its content increased at the rate of $0.1149 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$.
4. From the 5th day after application of cyprodinil, its residues decreased exponentially ($R_t = 2.6993e^{-0.149t}$), as did the pyrimethanil content from the 3rd day after spraying ($R_t = 2.411e^{-0.141t}$).
5. The residues of all chemicals used were found in brood samples, honey and wax.
6. In no case did the residues of pyrimethanil, cyprodinil and cyflufenamid in worker bees exceed 0.3% of the LD_{50} .
7. The residues of pyrimethanil, cyprodinil and cyflufenamid in honey accounted for 180, 460 and 40% of the *MRL*.
8. The daily intake of pyrimethanil, cyprodinil and cyflufenamid did not exceed 0.02% of the Acceptable Daily Intake (*ADI*) established for honey and the safe consumption level by an adult consumer of such honey was not less than 9.9 kg.

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References

- Aleksić M., Stanisavljević D., Smiljković M., Vasiljević P., Stevanović M., Soković M., Stojković D. 2019. Pyrimethanil: Between efficient fungicide against *Aspergillus* rot on cherry tomato and cytotoxic agent on human cell lines. *Annals of Applied Biology* 175 (2): 228–235. DOI: 10.1111/aab.12532
- Almasri H., Tavares D.A., Pioz M., Sené D., Tchamitchian S., Cousin M., Brunet J.L., Belzunces L.P. 2020. Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter *Apis mellifera* honey bees. *Ecotoxicology and Environmental Safety* 203: 111013. DOI: 10.1016/j.ecoenv.2020.111013
- Bakour M., Fernandes Â., Barros L., Soković M., Ferreira I.C.F.R., Iyousi B. 2019. Bee bread as a functional product: Chemical composition and bioactive properties. *Lebensmittel-Wissenschaft und Technologie* 109: 276–282. DOI: 10.1016/j.lwt.2019.02.008
- Batista A.C., Domingues C.E., Costa M.J., Silva-Zacarin E.C. 2020. Is a strobilurin fungicide capable of inducing histopathological effects on the midgut and Malpighian tubules of honey bees? *Journal of Apicultural Research* 59: 834–843. DOI: 10.1080/00218839.2020.1724678
- Bernabò I., Guardia A., Macirella R., Sesti S., Crescente A., Brunelli E. 2016. Effects of long-term exposure to two fungicides, pyrimethanil and tebuconazole, on survival and life history traits of Italian tree frog (*Hyla intermedia*). *Aquatic Toxicology* 172: 56–66. DOI: 10.1016/j.aquatox.2015.12.017
- Bogdanov S. 2006. Contaminants of bee products. *Apidologie* 37 (1): 1–18. DOI: 10.1051/apido:2005043
- Böhme F., Bischoff G., Zebitz C.P., Rosenkranz P., Wallner K. 2017. From field to food—will pesticide-contaminated pollen diet lead to a contamination of royal jelly? *Apidologie* 49: 112–119. DOI: 10.1007/s13592-017-0533-3
- Calatayud-Vernich P., Calatayud F., Simó E., Picó Y. 2018. Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. *Environmental Pollution* 241: 106–114. DOI: 10.1016/j.envpol.2018.05.062
- Costa Domingues da C.E., Tadei R., Inoue L.V.B., Silva-Zacarin da E.C.M., Malaspina O. 2021. Effects of larval exposure to the fungicide pyraclostrobin on the post-embryonic development of Africanized *Apis mellifera* workers. *Environmental Advances* 4: 100069. DOI: 10.1016/j.envadv.2021.100069
- Doublet V., Labarussias M., de Miranda J.R., Moritz R.F., Paxton R.J. 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environmental Microbiology* 17 (4): 969–983. DOI: 10.1111/1462-2920.12426
- Eeraerts M., Vanderhaegen R., Smagghe G., Meeus I. 2019. Pollination efficiency and foraging behaviour of honey bees and non-*Apis* bees to sweet cherry. *Agricultural and Forest Entomology* 22 (1): 75–82. DOI: 10.1111/afe.12363
- EU Pesticides Database (2022). Available on: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en. [Accessed: 18 January 2022]
- Everich R., Schiller C., Whitehead J., Beavers M., Barrett K. 2009. Effects of captan on *Apis mellifera* brood development under field conditions in California almond orchards. *Journal of Economic Entomology* 102 (1): 20–29. DOI: 10.1603/029.102.0104
- Evrenosoğlu Y., Mısırlı A. 2009. Investigations on the pollen morphology of some fruit species. *Turkish Journal of Agriculture and Forestry* 33 (2): 181–190. DOI: 10.3906/tar-0801-47
- Ferreira E.S., Cordeiro L.V., Silva D.F., Souza H.D.S., Athayde-Filho P.F., Barbosa-Filho J.M., Scotti L., Lima E.O., Castro R.D. 2021. Antifungal activity and mechanism of action of 2-chloro-N-phenylacetamide: a new molecule with activity against strains of *Aspergillus flavus*. *Anais da Academia Brasileira de Ciências* 93 (3): e20200997. DOI: 10.1590/0001-3765202120200997
- Fritz R., Lanen C., Chapeland-Leclerc F., Leroux P. 2003. Effect of the anilinopyrimidine fungicide pyrimethanil on the cystathionine β -lyase of *Botrytis cinerea*. *Pesticide Biochemistry and Physiology* 77 (2): 54–65. DOI: 10.1016/S0048-3575(03)00094-4
- Gallai N., Salles J.-M., Settele J., Vaissiere B.E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* 68 (3): 810–821. DOI: 10.1016/j.ecolecon.2008.06.014
- Grassl J., Holt S., Cremen N., Peso M., Hahne D., Baer B. 2018. Synergistic effects of pathogen and pesticide exposure on honey bee (*Apis mellifera*) survival and immunity. *Journal of Invertebrate Pathology* 159: 78–86. DOI: 10.1016/j.jip.2018.10.005
- GUS. 2020. Produkcja i handel zagraniczny produktami rolnymi w 2019 r. Available on: https://stat.gov.pl/files/gfx/portalinformacyjny/pl/defaultaktualnosci/5509/1/16/1/produkcja_i_handel_zagraniczny_produkami_rolnymi_2019.pdf [Accessed: 18 January 2022]

- GUS. 2021a. Stan i struktura ludności według wieku w latach 1989–2019. Available on: <https://stat.gov.pl/obszary-tematyczne/ludnosc/ludnosc/struktura-ludnosc,16,1.html> [Accessed: 18 January 2022]
- GUS. 2021b. Wynikowy szacunek głównych ziemioplodów rolnych i ogrodnich w 2021 roku. Available on: https://stat.gov.pl/download/gfx/portalinformacyjny/pl/defaultaktualnosci/5509/5/20/1/wynikowy_szacunek_glownych_ziemioplodow_rolnych_i_ogrodnich_w_2021_r..pdf [Accessed: 18 January 2022]
- Hrynkó I., Łozowicka B., Kaczyński P. 2021. Development of precise micro analytical tool to identify potential insecticide hazards to bees in guttation fluid using LC-ESI-MS/MS. *Chemosphere* 263: 128143. DOI: 10.1016/j.chemosphere.2020.128143
- Jankowska M., Łozowicka B., Kaczyński P. 2019. Comprehensive toxicological study over 160 processing factors of pesticides in selected fruit and vegetables after water, mechanical and thermal processing treatments and their application to human health risk assessment. *Science of the Total Environment* 652: 1156–1167. DOI: 10.1016/j.scitotenv.2018.10.324
- Johnson R.M., Dahlgren L., Siegfried B.D., Ellis M.D. 2013. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLoS One* 8 (1): e54092. DOI: 10.1371/journal.pone.0054092
- Kapsi M., Tsoutsis C., Paschalidou A., Albanis T. 2019. Environmental monitoring and risk assessment of pesticide residues in surface waters of the Louros River (N.W. Greece). *Science of The Total Environment* 650 (2): 2188–2198. DOI: 10.1016/j.scitotenv.2018.09.185
- Kobylińska M. 2021. Regionalne zróżnicowanie pszczelarstwa w Polsce. In: *The Polish Statistician, Statistics Poland* 66 (2). Available on: https://ws.stat.gov.pl/WS/2021/2/gus_ws_2021_02.pdf [Accessed: 18 January 2022] DOI: 10.5604/01.3001.0014.7388
- Majewski J. 2011. The value of crops pollination in Poland. *Prace Naukowe Uniwersytetu Ekonomicznego we Wrocławiu* 166: 426–435.
- Mayer D.F., Lunden J.D. 1986. Toxicity of fungicides and an acaricide to honey bees (Hymenoptera: Apidae) and their effects on bee foraging behavior and pollen viability on blooming apples and pears. *Environmental Entomology* 15 (5): 1047–1049. DOI: 10.1093/ee/15.5.1047
- McKerchar M., Potts S.G., Fountain M.T., Garratt M.P.D., Westbury D.B. 2020. The potential for wildflower interventions to enhance natural enemies and pollinators in commercial apple orchards is limited by other management practices. *Agriculture, Ecosystems and Environment* 301: 107034. DOI: 10.1016/j.agee.2020.107034
- Meng Y., Zhong K., Xiao J., Huang Y., Wei Y., Tang L., Chen S., Wu J., Ma J., Cao Z., Liao X., Lu H. 2020. Exposure to pyrimethanil induces developmental toxicity and cardiotoxicity in zebrafish. *Chemosphere* 255: 126889. DOI: 10.1016/j.chemosphere.2020.126889
- Meo S.A., Al-Asiri S.A., Mahesar A.L., Ansari M.J. 2017. Role of honey in modern medicine. *Saudi Journal of Biological Sciences* 24 (5): 975–978. DOI: 10.1016/j.sjbs.2016.12.010
- Milling R., Richardson C.J. 1995. Mode of action of the anilopyrimidine fungicide pyrimethanil. 2. Effects on enzyme secretion in *Botrytis cinerea*. *Pesticide Science* 45 (1): 43–48. DOI: 10.1002/ps.2780450107
- Mussen E.C., Lopez J.E., Peng C.Y.S. 2004. Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environmental Entomology* 33 (5): 1151–1154. DOI: 10.1603/0046-225X-33.5.1151
- NIK. 2017. NIK o wsparciu pszczelarstwa. Available on: <https://www.nik.gov.pl/aktualnosci/nik-o-wsparciu-pszczelarstwa.html> [Accessed: 18 January 2022].
- Piechowicz B., Kobielska M., Koziorowska A., Podbielska M., Szpyrka E., Pieniżek M., Potocki L. 2021a. Dynamics of λ -cyhalothrin disappearance and expression of selected P450 genes in bees depending on the ambient temperature. *Open Chemistry* 19 (1): 1242–1249. DOI: 10.1515/chem-2021-0104
- Piechowicz B., Sadło S., Piechowicz I., Czawa M. 2019. Formal and legal aspects of obtaining honey from beehives located in apple orchard during blossoming and intensive chemical protection (in Polish). *Research Papers of the University of Work Protection Management in Katowice* 1 (15): 125–134. DOI: 10.32039/WSZOP/1895-3794-2019-09
- Piechowicz B., Sieńko J., Mytych J., Grodzicki P., Podbielska M., Szpyrka E., Zaręba L., Piechowicz I., Sadło S. 2021b. Assessment of risk to honey bees and honey consumers resulting from the insect exposure to captan, thiacloprid, penthiopyrad, and λ -cyhalothrin used in a commercial apple orchard. *Environmental Monitoring and Assessment* 193 (3): 129. DOI: 10.1007/s10661-021-08913-6
- Piechowicz B., Woś I., Podbielska M., Grodzicki P. 2018. The transfer of active ingredients of insecticides and fungicides from an orchard to beehives. *Journal of Environmental Science and Health B* 53 (1): 18–24.
- Pohorecka K., Szczęśna T., Witek M., Miszczak A., Sikorski P. 2017. The exposure of honey bees to pesticide residues in the hive environment with regard to winter colony losses. *Journal of Apicultural Science* 61 (1): 105–125. DOI: 10.1515/jas-2017-0013
- Potts S.G., Biesmeijer J.C., Kremen C., Neumann P., Schweiger O., Kunin W.E. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution* 25 (6): 345–353. DOI: 10.1016/j.tree.2010.01.007
- PPDB. Pesticide Properties Database (2022). Available on: <http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm> [Accessed: 18 January 2022]
- Sad Sło. 2002. Disappearance of pyrimethanil residues on tomato plants. *Journal of Agricultural and Food Chemistry* 50 (5): 1089–1091. DOI: 10.1021/jf010570y
- Sadło S., Grodzicki P., Piechowicz B. 2017. Dissipation of captan, boscalid and trifloxystrobin residues in apples of four varieties within 2 months before their harvest. *Journal of Plant Diseases and Protection* 124: 177–184. DOI: 10.1007/s41348-016-0069-1
- Sadło S., Piechowicz B., Podbielska M., Szpyrka E. 2018. A study on residue levels of fungicides and insecticides applied according to the program of raspberry protection. *Environmental Science and Pollution Research* 25 (8): 8057–8068. DOI: 10.1007/s11356-017-1098-4
- Sadło S., Szpyrka E., Stawarczyk M., Piechowicz B. 2014. Behavior of pyrimethanil, pyraclostrobin, boscalid, cypermethrin and chlorpyrifos residues on raspberry fruit and leaves of Laszka variety. *Journal of Environmental Science and Health B* 49 (3): 159–168. DOI: 10.1080/03601234.2014.858005
- SANTE 2017. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE/2017/11813. Available on: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11813_2017-fin.pdf [Accessed: 18 January 2022]
- Silva V., Mol H.G.J., Zomer P., Tienstra M., Ritsema C.J., Geissen V. 2019. Pesticide residues in European agricultural soils – A hidden reality unfolded. *Science of the Total Environment* 653: 1532–1545. DOI: 10.1016/j.scitotenv.2018.10.441
- Smith D.B., Arce A.N., Ramos R.A., Bischoff P.H., Burris D., Ahmed F., Gill R.J. 2020. Insecticide exposure during brood or early-adult development reduces brain growth and impairs adult learning in bumblebees. *Proceedings of the Royal Society B: Biological Sciences* 287: 20192442. DOI: 10.1098/rspb.2019.2442
- Stavnichenko P.V., Antonenko A.M., Korshun M.M., Bardov V.G. 2016. Toxicological and hygienic regulation and assessment of danger of the new fungicide-cyflufenamid.

- Medical Perspectives 21 (2): 106–111. DOI: 10.26641/2307-0404.2016.2.72279
- Szpyrka E., Sadło S. 2009. Disappearance of azoxystrobin, cyprodinil, and fludioxonil residues on tomato leaves in a greenhouse. *Journal of Plant Protection Research* 49 (2): 204–208. DOI: 10.2478/v10045-009-0030-4
- Traynor K.S., van Engelsdorp D., Lamas Z.S. 2021. Social disruption: Sublethal pesticides in pollen lead to *Apis mellifera* queen events and brood loss. *Ecotoxicology and Environmental Safety* 214: 112105. DOI: 10.1016/j.ecoenv.2021.112105
- Wu J.Y., Anelli C.M., Sheppard W.S. 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PLoS One* 6 (2): e14720. DOI: 10.1371/journal.pone.0014720
- Yele V., Sanapalli B.K.R., Wadhvani A.D., Mohammed A.A. 2021. Benzohydrazide and phenylacetamide scaffolds: New putative ParE inhibitors. *Frontiers in Bioengineering and Biotechnology* 17(9): 669728. DOI: 10.3389/fbioe.2021.669728