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ORIGINAL ARTICLE

Research on the certification of the apple orchard pest and disease control program as an innovative strategy for the production of apples practically free of pesticide residues, i.e., below 0.01 mg · kg^{-1}

Stanisław Sadło[1], Magdalena Szczepanik[2], Paweł Krawiec[3], Bartosz Piechowicz[4,5]*

[1] Institute of Biotechnology, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland
[2] Bio Berry Polska sp. z o.o., Lublin, Poland
[3] Horti Team Paweł Krawiec, Lublin, Poland
[4] Institute of Biology, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland
[5] Interdisciplinary Center for Preclinical and Clinical Research, University of Rzeszów, Werynia, Poland

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*Corresponding address:
bpiechowicz@ur.edu.pl

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Abstract

The aim of this research was to prepare the basis for the certification of the apple orchard protection program by determining disappearance models for active ingredients (AIs) of plant protection products (PPPs) in fruits. Field trials were carried out in a conventional apple orchard protected with PPPs in accordance with the currently adopted program. Residues of their AIs were determined using Agilent GC-MS/MS 7000D and LC-MS/MS 6470 QQQ, and their decreases were expressed by the exponential formula: $R_t = R_0 \times e^{-k \times t}$. Of all the AIs found in mature fruits, captan disappeared at the fastest rate [$t_{(1/2)}$ in the range of 9 to 13 days], followed by fluopyram [$t_{(1/2)}$ = 13 days], tebuconazole [$t_{(1/2)}$ = 14 days] and carbendazim [$t_{(1/2)}$ in the range of 24 to 32 days]. With the exception of dithiocarbamates and some fungicides (e.g., Captan 80 WDG) based on captan and methyl thiophanate, other insecticides and fungicides currently recommended can be used up to 3 months before harvest practically with virtually no restrictions. From July 15 to August 15, the chemicals effective at application rates not exceeding 0.3 kg of AI per ha should be used. To protect apples against storage diseases, PPPs that are effective at a dose ≤ 0.1 kg AI per ha (e.g., certain triazoles or strobilurins) and applied not later than 1 month before harvest, should be used.

Keywords: apple orchard, pest and disease control, preharvest interval, residues below 0.01 mg · kg^{-1}, residue disappearance

Introduction

The term conventional farming (CF) refers to an agricultural system involving the use of genetically modified seeds, synthetic fertilizers, chemical plant protection products (PPPs: insecticides, fungicides, herbicides, and growth regulators), and intensive soil cultivation.

Organic farming (OF), on the other hand, is based on crop rotation, biological fertilizers mainly from animal and vegetable waste, and cultivation of nitrogen-fixing plants. Active ingredients (AIs) of biological PPPs, recommended now for pest and disease control, ensure only a short, if ever, protection period, therefore, they may require more frequent application (Jankowska *et al.* 2016; Sadło *et al.* 2017). Natural farming, also known as biological farming, excludes or strictly restricts the use of chemicals, though analytical practice shows that not all organic products are free of pesticide residues (Larsen *et al.* 2021).

Organic fruit and vegetables are increasingly in demand, though they still remain a luxury due to their high prices (Tasiopoulou *et al.* 2007; Barański *et al.* 2014; Geissen *et al.* 2021). This is because organic farming is much less productive than CF methods, with differences in yield of up to 20–25% under experimental conditions, and up to 50% under actual farming conditions (Seufert and Ramankutty 2017; Meemken and Qaim 2018).

The Integrated Farming System is based on Integrated Pest Management (IPM), which promotes the use of all available techniques and selected PPPs to control or to limit the development of pest and pathogen populations, is economically viable, and attempts to reduce or minimize the risk to the environment and the consumer's health. In general, none of these basic agricultural systems is perfect (Vereijken 1986).

On the European market, the majority of fresh fruit and vegetables come from CF systems. According to the European Food Safety Authority (EFSA) (2018), in 2016, 56.6% of apple samples contained PPPs residues, with as many as 41.8% of them containing residues of two or more substances (multiple residues). The same EFSA report noted that 16.9% of tested samples of certified organic products, which formally should be completely free of pesticides, contained residues, undoubtedly as a consequence of attempts to save the crop.

In Poland, the pesticide residue monitoring program was officially initiated by the Ministry of Agriculture in 2005. As Nowacka and Hołodyńska-Kulas (2020) report, pesticide residues were found in 74.9% of apple samples collected from conventional orchards, e.g., in 2016 in most cases, they were residues of fungicides: captan (30.1%), boscalid (17.7%), and tebuconazole (15.3%), and of insecticides: acetamiprid (25.4%), pirimicarb (12.9%) and thiacloprid (6.7%).

As a result, trends have emerged in crop protection (Jacquet *et al.* 2022) aiming at providing consumers with plant-based food free of pesticide residues through, for example, rigorous fruit and vegetable certification. Similarly to Certification Services (www.SCSglobalServices.com), such schemes involve a thorough assessment of well-documented pest and disease control, independent and professional sampling of mature fruit and vegetables directly from the field, and chemical analyses to confirm that no pesticide residues are present at levels exceeding the lower Limit of Quantification/Determination (LOQ/LOD = 0.01 mg · kg^{-1}), using the QuEChERS method in routine analyses. However, it can only be used to a limited extent, as certification services will never be able to perform several necessary analyses of, for example, ripe dessert strawberries in a sufficiently short time, and deliver a quality certificate to the producer.

However, to feed the growing human population, chemicals cannot be completely abandoned, due to their direct advantages in pest and disease control and, consequently, for crop quality (e.g., a lack of patulin or skin damage) and yield (Damalas 2009; Casida 2017), as well as the fact that farmers are accustomed to their use and are concerned about giving up this method of crop protection (Damalas 2021; Wyckhuys *et al.* 2022). It is also not true that residues found in mature fruit and vegetables pose a threat to the consumer's health (Hernández *et al.* 2013; Alengebawy *et al.* 2021), as evidenced by comparing the actual daily intake of residues with food to the Acceptable Daily Intake (ADI) (Sadło *et al.* 2015; Piechowicz *et al.* 2016; Jankowska *et al.* 2016), and the residue level to the Maximum Residue Level (MRL) (Łozowicka and Kaczyński 2011; Sadło *et al.* 2016; Podbielska *et al.* 2017; Sadło *et al.* 2018; Kowalska *et al.* 2022).

The aim of the field trials was to prepare the basis for the certification of an apple orchard protection program by defining disappearance models in/on fruit for AIs of PPPs currently recommended for pest and disease control in Poland. Thus, if the time required for residues in apples to decrease below the official standard level of 0.01 mg · kg^{-1} (preharvest interval – PHI), as well as to reduce their level to half of the initial value (half-life – $t_{½}$), this will provide a rational premise for reducing the application rates (dose – D) and determining the optimal sequence of treatments. In consequence, the health risk to the consumer caused by the consumption of apples containing residues below the LOQ = 0.01 mg · kg^{-1} can be reduced to virtually zero.

Materials and Methods

Field trials

The field trials were carried out in the same conventional apple orchard protected with PPPs according to the current standard program, resulting from the threat of agrophages that have occurred (pests) or may occur (pathogens) (Tab. 1), in late varieties of apples: Idared (objects: 1, 2, and 3) and Florina (objects: 4, 5, and 6). Until August 5, the same, standard PPPs had been used in all experimental objects.

As part of the field trial, captan (MerPlus 800 SC; prod.: Adama Polska Sp. z o.o, Poland) at a dose of 0.72 kg · ha^{-1} was applied in object 2 with the Idared variety, and in all Florina objects on August 15, followed, on September 1, with fluopyram + tebuconazole (Luna Experience 400 SC; prod.: Bayer SAS, France) at doses of 0.12 kg · ha^{-1} each, but exclusively on object 4 of the Florina variety (Tab. 1). All PPPs were applied in accordance with recommendations and directions in their label, using the Agrola Optimum 1500 V cross-flows sprayer with a 1500 l tank and 18 nozzles (9 on each side). Working solutions were applied at

Table 1. Idared and Florina apple tree protection program implemented by the orchard owner in 2021; BBCH – growth stage of apple trees

Application of plant protection product			Active ingredient						
Date	Trade name	D [kg, l · ha⁻¹]	Common name	D [kg · ha⁻¹]	AD [mg · ha⁻¹ b.w]	PTI	PHI[a] [day]	BBCH	Object number
April 10	Miedzian 50 WP, F[b]	1.5	copper oxychloride	0.75	n.a.[c]	–	190	51	
April 19	Miedzian 50 WP, F	1.5	copper oxychloride	0.75	n.a.	–	181	54	
	Siarkol 800 SC, F	5	sulphur	4	n.a.	–			
April 30	Delan 700 WG, F	0.5	dithianon	0.35	0.01	35.0	170	56	
	Siarkol 800 SC, F	5	sulphur	4	n.a.	–			
May 10	Score 250 EC, F	0.2	diphenoconazole	0.05	0.01	5.0	160	56	
	Cyperfor 100 EC, I[d]	0.3	cypermethrin	0.03	0.05	0.6			
May 15	Merpan 80 WDG, F	1.9	captan	1.52	0.1	15.2	155	56	
	Orius Extra 250 EW, F	0.6	tebuconazole	0.15	0.03	5.0			
May 21	Domark 100 EC, F	0.4	tetraconazole	0.04	0.004	10.0	149	61	
	Merpan 80 WDG, F	1.9	captan	1.52	0.1	15.2			1–6
May 26	Merpan 80 WDG, F	1.9	captan	1.52	0.1	15.2	144	65	
	Los Ovados 200 SE, I	0.125	acetamiprid	0.025	0.025	1.0			
June 5	Caldera 700 WG, F	0.75	dithianon	0.525	0.1	52.5	134	69	
June 7	Teppeki 50 WG, I	0.14	flonicamid	0.07	0.025	2.8	132	69	
June 12	Merpan 80 WDG, F	1.9	captan	1.52	0.1	15.2	127	71	
	Cyperfor 100 EC, I	0.3	cypermethrin	0.03	0.05	0.6			
June 21	Caldera 700 WG, F	0.75	dithianon	0.525	0.01	52.5	118	71	
June 26	Topsin M 500 SC, F	1.5	methyl thiophanate	0.75	0.08	9.4	113	75	
July 15	MerPLUS 800 SC, F	0.36	captan	0.72	0.1	7.2	94	79	
		0.657	potassium phosphonate	1.314	2.25	0.6	94		
July 15	MerPLUS 800 SC, F	0.36	captan	0.72	0.1	7.2	94	79	
Aug. 05	Indofil 80 WP, F	3.2	mancozeb	2.56	0.023	111.3	73	81	
Aug. 15	MerPLUS 800 SC, F	0.36	captan	0.72	0.1	7.2	63	81	2, 4, 5, 6
		0.657	potassiumn phosphonate	1.314	2.25	0.6	63		
Sept. 01	Luna Experience 400 SC, F	0.6	fluopyram	0.12	0.012	10.0	46	85	4
		0.6	tebuconazole	0.12	0.03	4.0	46		

[a]Preharvest interval defined as the amount of time, in days, between the time at which the fruit was sprayed with a pesticide and at which it was harvested; [b]fungicide; [c]not applicable; [d]insecticide

400–500 l per ha, using TXA 80015 VK hollow cone nozzles for fungicides, and at 700–750 l per ha, using TXA 8003 VK hollow cone nozzles for insecticides.

The protection program for apple trees of the Idared and Florina varieties

On the basis of information obtained from the grower (trade names of PPPs, their application rates and dates of application; Tab. 1), application rates of individual AIs (D_i), and their preharvest intervals (PHIs), were calculated. The predicted levels of their residues in the fruit immediately after treatments (R_0) were calculated using the formula established in other studies (Sadło *et al.* 2016):

$$R_{0(i)} = 1.259 \times D_i. \quad (1)$$

Furthermore, pesticide toxicity indices (PTIs) were calculated for individual AIs of applied PPPs according to the formula:

$$PTI_i = D_i/ADI_i \text{ (Sadło et al. 2015)}, \quad (2)$$

where: ADI_i – the acceptable daily intake of i-th AI per 1 kg of body weight (b.w.) per 1 day. On the basis of the values of these indices, it is possible to identify the PPPs currently recommended for disease and pest control which, according to current knowledge, pose the greatest risk to the consumers' health (Tab. 1).

Sampling and residue extraction

The average weight of an apple was 135 g for Idared and 130 g for Florina. A total of six representative laboratory samples of mature apples (fruit ripe for picking: BBCH 87) were collected (one sample contained 10–12 apples), one from each object, which were transported to an accredited laboratory, where analytical portions of 10 g were taken, frozen at –20°C, and stored until analyzed. Residues of applied pesticides were extracted with the standard QuEChERS method, and determined using gas and liquid chromatography techniques. The lower LOQ for all analytes was 0.005 mg · kg^{-1}, with 50% expanded uncertainty. Recoveries of individual analytes were within the range of 75–110%, therefore, they were within the assumed range of 70–120% (SANTE 2022).

Apparatus and operating conditions

The obtained extracts were analyzed using the following chromatographs:

Agilent GC-MS/MS 7000D EI; column: HP-5MS Ul 30 m, 0.25 mm, 0.25 µm; the temperature program: 60°C (1 min), 60–170°C (a gradient of 40°C · min^{-1}), 170–310°C (a gradient of 10°C · min^{-1}), 310°C (3 min); the flow rate: 1 ml · min^{-1}; carrier gas: helium.

Agilent LC-MS/MS 6470 QQQ; column: InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7-micron; the gradient program: Phase B: 0% (0 min), 6% (0.75 min), 95% (18–20 min), 96% (21 min); the flow rate: 0.5 ml · min^{-1}; the thermostat temperature: 50°C.

The course of changes in the levels of residues of applied PPPs

The pesticide residue declines were expressed by the following general exponential formula:

$$R_t = R_0 \times e^{-k \times t}, \quad (3)$$

where: R_0, and R_t represent the residue levels on the next day (t = 0) and t days after treatment, t – PHI (day), k – disappearance constant per day, e – Euler's constant.

Results and Discussion

General comments on the implemented program for the protection of apple trees of the Idared and Florina varieties

A review of the implemented program for protection of Idared and Florina indicates that fungicides: Indofil 80 WP (prod.: Indofil Industries Limited, Italy; AI: mancozeb, 2.56 kg · ha^{-1}), Merpan 80 WG (prod.: Adama Polska Sp. z o.o., Poland; AI: captan, 1.52 kg · ha^{-1}), and Caldera 700 WG (prod.: Globachem N.V, Belgium; AI: dithianon, 0.525 kg · ha^{-1}) were used at the highest doses, and their AIs were characterized by the highest toxicity indices, of 111.3, 15.2 and 52.5, respectively. Therefore, they should be used judiciously.

Captan was applied five times: four times as Merpan 80 WDG and once as MerPLUS 800 SC, at 6.80 kg · ha^{-1} in total. Despite such a large quantity of this substance applied by July 15, no residues exceeding LOQ were found in samples of mature Idared apples (objects: 1 and 3), and its total level, predicted on the basis of parameters of its exponential disappearance reported earlier (Piechowicz *et al.* 2016; Sadło *et al.* 2016), did not exceed 0.0043 mg · kg^{-1} (Tab. 2).

Out of 10 different AIs (3 insecticides and 7 fungicides) applied during the growth season 2021, only residues of fungicides were found in mature apples, and they included: carbendazim (0.024–0.027 kg · ha^{-1}, the Idared variety), an exceptionally stable metabolite of methyl thiophanate (< 0.005 kg · ha^{-1} in both varieties), and captan (0.009 kg · ha^{-1} in Idared, and 0.017–0.032 kg · ha^{-1} in Florina), as well as fluopyram (0.013 kg · ha^{-1}, the Florina variety), and tebuconazole (0.015 kg · ha^{-1}, the Florina variety), which were used as the last treatment in the experiment. It is highly probable that mancozeb residues in mature apples were at a level of ca. 0.035 kg · ha^{-1} (Tab. 2). However, for technical reasons their presence was not confirmed analytically, as at that time the analytical laboratory did not have a specific method for implementing dithiocarbamates determination.

The total foreseen residues of all AIs applied by July 15 did not exceed the level of 0.005 kg · ha^{-1}, i.e., below LOQ for an individual compound. The total residues of substances found in the sample from object 2 amounted to 0.033 kg · ha^{-1}, of which 0.024 kg · ha^{-1} were carbendazim residues, while in the sample from object 4, in which the fungicide, Luna Experience 400 SC, was applied for experimental purposes, amounted to 0.088 kg · ha^{-1}, of which carbendazim residues represented 50%. Finally, after the estimated mancozeb residues were added, the total amount of residues in sample 4, creating a so-called cocktail effect, reached 0.123 kg · ha^{-1}.

Occurrence of residues of AIs of the applied PPPs in mature apples

Predicted residue levels of AIs applied in field experiments, but not found in collected samples of mature apples [$R_{t\,=\,\mathrm{PHI}}$ < 0.005 kg · ha^{-1}]. Apart from carbendazim derived from methyl thiophanate (AI of Topsin M 500 SC, prod.: Nisso Chemical Europe GmbH,

Table 2. Residue levels of applied fungicides and insecticides: predicted from previous studies for k = 0.062 per day, R_0 = 1.259 × D_i (Piechowicz *et al.* 2016; Sadło *et al.* 2016) and respective PHIs, determined by chemical analysis (LOQ = 0.005 mg · kg⁻¹), as well as exponential disappearance parameters determined for residues of carbendazim (thiophanate-methyl), captan, fluopyram and tebuconazole found in mature Idared and Florina apples; sampling date – September 17, BBCH 87

Application date	Active ingredient Common name	R_0 [mg · kg^{-1}]	$t_{(R=0.01)}$ [day]	k [day^{-1}]	PHI [day]	$R_{t=PHI}$ [mg · kg^{-1}]	$t_{1/2}$ [day]	Object number
April 30	Dithianon	0.44	61	0.062	170	0.0000	11	1–6
May 10	Diphenoconazole	0.06	29	0.062	160	0.0000	11	1–6
	Cypermethrin	0.04	22	0.062	160	0.0000	11	1–6
May 15	Captan	1.91	85	0.062	155	0.0001	11	1–6
	Tebuconazole	0.19	47	0.062	155	0.0000	11	1–6
May 21	Tetraconazole	0.05	26	0.062	149	0.0000	11	1–6
	Captan	1.91	85	0.062	149	0.0002	11	1–6
May 26	Captan	1.91	85	0.062	144	0.0003	11	1–6
	Acetamiprid	0.03	18	0.062	144	0.0000	11	1–6
June 05	Dithianon	0.66	68	0.062	134	0.0002	11	1–6
June 07	Flonicamid	0.09	35	0.062	132	0.0000	11	1–6
June 12	Captan	1.91	85	0.062	127	0.0007	11	1–6
	Cypermethrin	0.04	22	0.062	127	0.0000	11	1–6
June 21	Dithianon	0.66	68	0.062	118	0.0004	11	1–6
June 26	Carbendazim	0.53[a]	151	0.026	113	0.027	27	1
			146	0.027		0.024	26	2
			145	0.027		0.024	26	3
			147	0.027		0.025	26	The mean of 1-3
			179	0.022	113	0.043	32	4
			156	0.025		0.030	28	5
			139	0.029		0.021	24	6
			158	0.025		0.031	27	The mean of 4-6
July 15	Captan	0.91	73	0.062	94	0.003	11	1–6
August 05	Mancozeb	3.22	93	0.062	73	0.035	11	1–6
August 15	Captan	(0.00)[b]		0.062		(0.018)		1
		0.91	62	0.073	63	0.009	9	2
		(0.00)[b]		0.062		(0.018)		3
		0.91	71	0.063	63	0.017	11	4
		0.91	77	0.058	63	0.023	12	5
		0.91	85	0.053	63	0.032	13	6
			74	0.058	63	0.024		The mean of 4–6
September 01	Fluopyram	(0.00)[b]		0.062	46	(0.009)		1, 2, 3
	Tebuconazole	(0.00)[b]		0.062	46	(0.009)		1, 2, 3
		0.15	51	0.053	46	0.013	13	4
		0.15	54	0.050	46	0.015	14	4
		(0.00)[b]		0.062	46	(0.009)		5, 6
		(0.00)[b]		0.062	46	(0.009)		5, 6

[a]The carbendazim dose was calculated in accordance with stoichiometry of methyl thiophanate, D = 0.94 kg · ha⁻¹, transformation;
[b]The indicated PPPs were not applied on this object, thus, predicted residue levels of captan, fluopyram and tebuconazole for their doses 0.91, 0.15 and 0.15 kg · ha⁻¹ were calculated;
MRLs: mancozeb – 5.0 kg · ha⁻¹; carbendazim – 0.2 kg · ha⁻¹; captan – 10.0 kg · ha⁻¹; fluopyram – 0.8 kg · ha⁻¹; tebuconazole – 0.3 kg · ha⁻¹

Germany), no residues of AIs of any PPPs applied before July 15 were found in mature apples of both varieties at a level exceeding 0.005 kg · ha⁻¹ (LOQ).

Therefore, to determine at least their approximate levels in fruit after t = PHI, we used the formula:

$$R_{t\,=\,\mathrm{PHI}} = R_0 \times e^{-k \times t\,=\,\mathrm{PHI}}, \qquad (4)$$

for which parameters of exponential disappearance, k = 0.062 per day and R_0 = 1.259 × D, were taken from our previous studies published in peer-reviewed journals (Piechowicz *et al.* 2016; Sadło *et al.* 2016).

The results of those predictions indicate that, in fruit, residues of captan, applied at the lowest dose recommended for this fungicide, of 0.72 kg · ha⁻¹, on July 15, i.e., 73 days before harvest, could have been at a level of 0.003 mg · kg⁻¹ (Tab. 2). Therefore, except for methyl thiophanate, dithiocarbamates, and, possibly, captan when applied at its highest dose of 1.52 kg · ha⁻¹, all PPPs applied before July 15 (including fungicide MerPlus 800 SC), as well as other PPPs currently recommended for apple orchard protection, regardless of their dose (Tab. 3), should not generate residues at levels even approaching LOQ of the analytical method, set at 0.005 mg · kg⁻¹ in an accredited laboratory.

Furthermore, the possible compatibility of fluopyram and tebuconazole (AIs of Luna Experience 400 SC applied on September 1), as well as captan

Table 3. Application rates (D) and approximate preharvest intervals (PHI), estimated for the other currently recommended fungicides active ingredients, meeting the requirements of the innovative strategy to produce apples virtually free of pesticide residues, i.e., ≤ 0.01 mg · kg⁻¹

Fungicide	Active ingredient						
Chemical group	Common name	solubility in water [mg · l⁻¹]	MRL [mg · kg⁻¹]	ADI [mg · kg⁻¹ b.w.]	D [kg · ha⁻¹]	PTI	PHI [day]
1. Dithiocarbamate	mancozeb*	6.2	5.0	0.023	2.4	104.3	92
	metiram	2	5.0	0.03	1.75	58.3	87
2. Phthalimide	captan	5.2	10.0	0.1	1.52[a]	15.2	85
					1.11[b]	11.1	80
					0.72[c]	7.2	73
	folpet	0.8	0.3	0.1	1.4	14.0	84
3. Organophosphate	fosetyl-AL	111300	150	1.0	1.332	1.3	83
4. Guanidine fungicide	dodine	930	0.9	0.1	0.65	6.5	71
5. Anilinopyrimidine	pyrimethanil	110	15.0	0.17	0.45	2.65	65
	cyprodinil	13	2.0	0.03	0.15	5.0	47
6. Quinone	dithianon	0.22	3.0	0.01	0.3	30.0	59
7. Anilide	boscalid	4.6	2.0	0.04	0.25	6.3	56
8. Phenylpyrrole	fludioxonil	1.8	5.0	0.37	0.225	0.6	54
9. Pyrimidine	bupirimate	13.06	0.3	0.05	0.225	4.5	54
10. Carboxamide	penthiopyrad	1.375	0.5	0.1	0.15	1.5	47
	fluxapyroxad	3.44	0.9	0.02	0.075	3.8	36
11. Pyrazole	isopyrazam*	0.55	0.7	0.03	0.15	5.0	47
12. Benzamide	fluopyram	16.0	0.8	0.012	0.15	12.5	47
13. Triazole	tebuconazole	36	0.3	0.03	0.15	5.0	47
	mefentrifluconazole	0.81	0.4	0.035	0.15	4.3	47
	difenoconazole	15	0.8	0.01	0.06	6.0	33
	tetraconazole	156.6	0.3	0.004	0.04	10.0	26
	penconazole	73	0.15	0.03	0.0375	1.3	25
14. Strobilurin	pyraclostrobin	1.9	0.5	0.03	0.1	3.3	41
	kresoxim-methyl	2.0	0.2	0.4	0.1	0.3	41
	trifloxystrobin	0.61	0.7	0.1	0.074	0.7	36
15. Quinazoline	proquinazid	0.93	0.08	0.01	0.06	6.0	33
16. Amide	cyflufenamid	4.6	2.0	0.04	0.025	0.6	18

*active ingredient recommended for use during the research period, and currently not recommended for use in the EU; MRL – maximum residue limit; ADI – acceptable daily intake; D – application rate; PTI – pesticide toxicity index; PHI – preharvest interval; b.w. – body weight (application of MerPLUS 800 SC on August 15) residues, found in mature apples using the best analytical techniques and predicted on a basis of the above-mentioned disappearance parameters, was also evaluated.

According to the calculations made, the predicted residues of fluopyram and tebuconazole (D = 0.15 kg · ha^{-1}) in mature fruit of the Florina variety, i.e., 46 days after application, should be at a level of ca. 0.009 mg · kg^{-1}, while they were found to be 0.013 and 0.015 mg · kg^{-1}, and residues of captan (D = 0.72 kg · ha^{-1}), 63 days after treatment, should amount to 0.018 mg · kg^{-1}, while they were actually at a level of 0.009 mg · kg^{-1} (the Idared variety), and of 0.024 mg · kg^{-1}, on average (the Florina variety).

Considering the above, and also assuming the same disappearance parameters, the predicted residue level was also estimated for mancozeb (not determined analytically), which could amount to 0.035 mg · kg^{-1} (0.7% MRL) 73 days after treatment with Indofil 80 WP.

It should also be emphasized that all insecticides (Cyperfor 100 EC, prod.: SBM Développement SAS, France; Teppeki 50 WG, prod.: ISK Biosciences Europe N.V, Belgium; and Los Ovados 200 SE, prod.: Innvigo Sp. z o.o., Poland) were applied before July 15, at relatively low doses (mean: 0.04 kg · ha^{-1}) and their residues were not detected. Predicted residues of their AIs in mature apples did not even reach the level of 0.0007 mg · kg^{-1} (Tab. 2).

Residue levels of AIs applied in field experiments and found in collected samples of mature apples ($R_{t = \mathrm{PHI}} \geq 0.005$ mg · kg^{-1}). PPPs were applied according to the Idared and Florina apple tree protection program implemented by the orchard owner in 2021 (Tab. 1). Additionally, this program was to serve as a basis for certification of the apple orchard's pest and disease protection scheme, according to the course of disappearance of their active ingredients in the fruit.

The residues of their AIs found in mature apples included captan, 0.024 mg · kg^{-1} (0.24% MRL), on average, fluopyram and tebuconazole, 0.013 mg · kg^{-1} (1.6% MRL) and 0.015 mg · kg^{-1} (0.5% MRL), respectively, and carbendazim, 0.025 mg · kg^{-1} (12.5% MRL; Idared) and 0.031 mg · kg^{-1} (15.5% MRL; Florina), on average. Therefore, apples from orchards protected in this way met the EU standards (MRLs) by a wide margin, but the program tested could only be used to produce apples free of pesticide residues (<0.01 mg · kg^{-1}) after its significant modification, especially in the final period of fruit ripening.

The carbendazim residues above the LOQ (0.005 mg · kg^{-1}) found in all six samples of mature apples, even though 113 days had passed since the application of Topsin M 500 SC (AI: methyl thiophanate), were highly surprising. Two reasons for the occurrence of residues of this systemic fungicide can be identified: its high persistence (Sadło *et al.* 2016), and possible redistribution after the treatment, i.e., its transport from leaves, where photosynthesis takes place, to fruit, together with assimilates. However, it should be stressed that the deadline for compliance with the Regulation was October 19, 2021, but the knowledge gained could be useful in other countries.

Establishing the model of carbendazim disappearance requires thorough research, especially since this fungicide could potentially protect fruit against infection together with fungicides applied at a later date (a possible synergistic or additive action, i.e., a so-called cocktail effect), and Indofil 80 WP, MerPlus 800 SC and Luna Experience 400 SC applications at their full application rates was not necessary.

Disappearance of AIs of PPPs in apples

Assessment of disappearance of AIs of PPPs used in field experiments, performed on the basis of previous experiments. To determine the approximate time, t, that should elapse for levels of residues, R_t, of pesticides applied before July 15 to decrease to $R_t = 0.01$ mg · kg^{-1}, the exponential disappearance parameters of $k = 0.062$/day and $R_0 = 1.259 \times \mathrm{D}$ were assumed, in accordance with previous studies published in peer-reviewed journals (Piechowicz *et al.* 2016; Sadło *et al.* 2016), using a formula derived from a general exponential formula, $R_t = R_0 \times e^{-k \times t}$, i.e.:

$$t_{(R = 0.01\ \mathrm{mg \cdot kg^{-1}})} = (\ln(0.01) - \ln(1.259 \times \mathrm{D_i}))/(-k). \quad (5)$$

Values of this parameter, which are of importance for the "apples free of pesticide residues" program, indicate that regardless of the dose size and the number of applications (e.g., captan), residues of pesticides applied in the experiments will drop to the level of 0.01 mg · kg^{-1} in a period much shorter than actual PHI, and after that time, they will not exceed even 0.0007 mg · kg^{-1} (captan; D = 1.52 kg · ha^{-1}). As it has already been mentioned, residues of mancozeb, not determined for technical reasons, could be at a level of ca. 0.035 mg · kg^{-1}.

Assessment of disappearance of AIs of PPPs used in field experiments, performed on the basis of previous experiments and on residues found by chemical analysis to be above 0.005 mg · kg^{-1}. Models for the disappearance of carbendazim and captan, as well as fluopyram and tebuconazole, were derived from a general exponential equation:

$$R_t = R_0 \times e^{-k \times t}, \text{ where } R_0 = 1.259 \times \mathrm{D_i}. \quad (6)$$

When the residues $R_{t = \mathrm{PHI}}$ for these compounds, found in mature apples after t = PHI, was used in this equation, their exponential disappearance constants (k) were calculated using the formula:

$$k_i = [\ln(R_{t = \mathrm{PHI}}) - \ln(1.259 \times \mathrm{D_i})]/(-t_{\mathrm{PHI}}), \quad (7)$$

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which, after transformation, was used to estimate the number of days, $t_{(R = 0.01 \text{ mg} \cdot \text{kg}^{-1})}$, after which the residue level will reach the value of $R_{t = \text{PHI}} = 0.01 \text{ mg} \cdot \text{kg}^{-1}$:

$$t_{i(R = 0.01 \text{ mg} \cdot \text{kg}^{-1})} = [\ln(0.01) - \ln(1.259 \times \text{D}_i)]/(-k_i). \quad (8)$$

Of all the AIs found in mature apples, captan residues disappeared at the fastest rate in Idared (object 2: k = 0.073 per day) and Florina (objects: 4, 5, 6; k = 0.063, 0.058, and 0.053 per day, respectively) varieties, i.e., similar to earlier studies (k = 0.062 per day; Piechowicz *et al.* 2016), followed by fluopyram (k = 0.053 per day), tebuconazole (k = 0.050 per day) and carbendazim (mean: 0.026 per day), which is formed from thiophanate methyl and is characterized by exceptional stability in the inert atmosphere of professional storage (Su *et al.* 2003). The time after which its residues will reach the level of 0.01 mg · kg^{-1} ($t_{(R = 0.01 \text{ mg} \cdot \text{kg}^{-1})}$) is ca. 152 days, and, therefore, PPPs based on methyl thiophanate, benomyl or carbendazim itself may be used only to a limited extent in the disease and pest control programs for an apple orchard certified as "apples free of pesticide residues".

It should be emphasized, however, that all these exponential disappearance constants, k, were calculated on the basis of the initial content, $R_0 = 1.259 \times \text{D}_i$, determined for any AI applied in the apple orchard at a dose D onto apples of near-ripe size. These, therefore, represent limiting values for the disappearance rate, because during the growth of the fruit, the concentrations of the applied AIs decrease, not only by actual disappearance, as it takes place in fully formed fruit, but also by biological dilution caused by their growth.

Thus, to eliminate the influence of this factor and, in consequence, to determine actual disappearance parameters for individual pesticides in fruit, their residue values provided by analytical laboratories should be expressed as mg per apple. Therefore, knowing the weight of one apple (Idared: 0.135 kg), the quantity of a given substance that may still be found in fruit can be calculated, to ensure that its levels do not exceed the standard of 0.01 mg · kg^{-1}. For the Idared variety, that limit value amounted to 0.00135 mg (0.135 kg × 0.01 mg · kg^{-1}).

Certification of the pest and disease control program for an apple orchard as an innovative strategy for producing apples practically free of pesticide residues

PPPs do not belong to substances that are harmless. They are also not neutral to the health and life of humans (Ferrer 2003; Zaller 2020), and to their direct surroundings (Caloni *et al.* 2016; Bertero *et al.* 2020), as well as to the environment (Glavan and Božič 2013; Zaller 2020; Piechowicz *et al.* 2022). Nevertheless, there is currently no practical or scientific basis for discontinuing the use of chemical PPPs for pest and pathogen control, mainly, due to the lack of equally effective alternatives in this area. This is particularly true for fungicides used primarily for preventive treatments, as they do not treat the plant, but only limit disease progression. Abandoning the use of pesticides in agriculture is not only objectively unjustified, but also may lead to significant losses in crops and pose a threat to food security of the human population. Thus, at the moment such an approach is doomed to failure.

However, because pesticides have unjustifiably been given a bad name (Saleh *et al.* 2021), consumers, and thus also European authorities, are calling for a drastic reduction in the use of chemical pesticides, by 50% by 2030. This ambitious target requires extensive and reliable research in the field of agroecology, as well as thorough analyses of significant changes in agricultural systems, including those in which crop protection against pest and diseases is not based solely on the use of chemical pesticides. Above all, however, they are not justified and can lead to a sudden breakdown in food production.

The Pesticide Residue Free Certification Program certificate is awarded on the basis of inspection reports, sampling and analyses. Targeted inspection protocols allow SCS (www.SCSglobalServices.com) to collect samples from those areas of a field or a cultivation environment where residues are most likely to be found. The tests are based on the actual use of pesticides, ensuring the highest possible accuracy of the results. The course of the analytical quality assurance process is supervised by SCS chemists.

Therefore, to meet the trend towards pesticide residue-free food production, we are proposing a solution in the form of certified crop protection programs, involving the study and implementation of knowledge on the disappearance of pesticide residues in crops. The scientific basis for the certification of an apple pest and disease control program will involve the determination of the parameters of actual disappearance (the initial residue R_0, the disappearance rate constant, k, and time, $t_R = 0.01 \text{ mg} \cdot \text{kg}^{-1}$) of the currently used AI of PPPs. In this way, fruit and vegetable growers will be provided with a tool for rational determination of the application rate of individual fungicides and/or reducing their application rate and frequency, in order to reduce residue levels to the minimum necessary.

Bearing in mind consumers' concerns related to health and environmental hazards, as well as to the food security, we carried out initial research on certification of programs for apple orchard protection without abandoning protective and curative treatments. In Poland, the list of fungicides approved for use is still long and currently covers 26 AIs belonging to 16 chemical groups having specific properties (Tab. 3), and residues of which are determined in apples.

The disappearance of pesticide residues, in fact, means a change in their concentration, involving processes of their chemical decomposition, evaporation from the plant surface (also, with water vapor in the process of evapotranspiration), washing off, penetration into the plant and potential redistribution, and biological dilution and biodegradation. From the mathematical point of view, it is a complex function of multiple variables. Estimating the effect of physical and chemical properties in this process would require the use of sophisticated analytical techniques and methods, and studies in many subjects and different locations, however, without any clear conclusions guaranteed. Eventually, the significance of differences found would be established by statistical methods. The discussion and evaluation of the obtained results of the chemical analyses, as well as the use of available scientific reports, clearly indicate that insecticides and fungicides, apart from dithiocarbamates, and some PPPs based on captan (phthalimide) and thiophanate-methyl, can be used until mid-July practically without any restrictions. From that date onwards, PPPs effective at lower doses should be selected.

Conclusions

The field studies were conducted in the same orchard, therefore, it can be assumed that changes in the weather conditions had the same influence on the disappearance rate of six experimental objects. The observed differences in disappearance constants estimated for the same substance resulted from the natural variability associated with sample collection, taking of analytical portions, and the analytical method (extraction and determination). The variability in the disappearance rates of different substances, e.g., fluopyram versus carbendazim, resulted from differences in their physical and chemical properties.

Of all AIs found in mature apples, captan disappeared at the fastest rate (Idared: $k = 0.073$ per day; $t_{(1/2)} = 9$ days, and Florina: $k = 0.063$, 0.058 and 0.053 per day; $t_{(1/2)}$, ranging from 11 to 13 days), followed by fluopyram ($k = 0.053$ per day; $t_{(1/2)} = 13$ days), tebuconazole ($k = 0.050$ per day; $t_{(1/2)} = 14$ days), and carbendazim (mean $k = 0.026$ per day; $t_{(1/2)}$, ranging from 24 to 32 days). In conclusion, after the application of any of these AIs, the next fungicide should be applied no earlier than after the time $t_{(1/2)}$, using half of its recommended dose (a possible additive effect).

In accordance with the principles of Good Agricultural Practice (GAP), insecticides and other fungicides can be used up to 3 months before harvest (in Poland until mid-July; PHI = 94 days) practically without any restrictions, with the exception of dithiocarbamates and some PPPs (e.g., Captan 80 WDG) based on captan (phthalimide fungicide) and thiophanate methyl (benzimidazole fungicide). According to our estimates, their total residues in mature apples will not even exceed 0.0007 mg · kg^{-1}.

After July 15, the selected PPPs should ensure that the treatment foreseen for August 15 is carried out with PPP at a dose not exceeding 0.3 kg of AI per ha.

To protect apples against storage diseases, PPPs effective at a dose below 0.1 kg AI per ha and applied no later than 1 month before harvest should be used. These conditions are met, for example, by certain fungicides from the triazole and strobilurin chemical groups.

In general, however, the only scientific basis for certification of the apple orchard protection program are the parameters of real AI disappearance established in field trials. For this purpose, the residues of individual pesticides expressed as mg · kg^{-1} should be multiplied by the average weight of the apple, and then the parameters of their (linear/exponential) changes in one apple should be determined. The orchard owner selects a specific PPP on the basis of the time, $t_{(R\ =\ 0.01\ \mathrm{mg\ \cdot\ kg^{-1}})}$, needed to reduce the residue level to 0.01 mg · kg^{-1}, which corresponds to a level of 0.00135 mg in one 0.135 kg apple (Idared).

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