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MONITORING OF PESTICIDE RESIDUES IN GRAPEVINE LEAVES UNDER VARIANT FUNGAL DISEASE MANAGEMENT

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ABSTRACT

Food contamination is the result of over-reliance on fungicides in crop production. The presence of pesticide residues in agricultural commodities needs to be strategically solved by applying gentle agrotechnical procedures. The research aimed to evaluate the effect of the treatment of grapevine with fungicides on the content and mobility of 4 active substances in the leaves of the variety Hibernal. The experiment included a control variant treated with fungicides and an untreated variant. We focused on monitoring the active substances of fungicides used in the first half of the vegetation: azoxystrobin, cyflufenamid, difenoconazole, myclobutanil, and another 25 active substances, in terms of multi-residue analysis methodologies. Grape leaves for analysis were collected on two dates: in the phenological phase of berry development BBCH 73 and 14 days after grape harvest – BBCH 91. Samples were prepared according to STN EN 15662. For qualitative analyzes of residues, we used the QuEChERS method in combination with liquid (LC-MS/MS) and gas (GC-MS/MS) chromatography with tandem mass spectrometry. In the untreated variant, the contents of residues from both leaves collection were in undetectable values. In the leaf samples of the treated variant collected in BBCH 73, we established higher values of residues than the MRL determines, namely for azoxystrobin 0.083 mg/kg (MRL – 0.01 mg/kg). Azoxystrobin is the active substance of the Quadris max fungicide, and we applied this preparation 41 days before the first collection of leaves. In the analyzes of leaf sampling from the treated variant after grape harvest in BBCH 91, we found a decrease in the content of all monitored residues below detectable values. We consider the gradual degradation of residues in biological materials to be a very favorable result from the point of view of their impact on consumer health and the environment. Through visual observations of the vegetation, we found out that even the untreated variant had a perfect state of health. Hibernal is a variety suitable for biological production systems, and we assume that even in vintages with high pressure of fungal diseases, it is possible to maintain the health of the grapevine with ecological products. Using the appropriate grape variety combined with the right agrotechnical interventions makes it possible to use safer ecological practices to produce healthy food, maintaining sustainability and a healthy natural environment. The research will continue in the early Table grape varieties, where the vegetation period is shorter, and space for breaking down the residues is more difficult.

Keywords: *Vitis vinifera*, Hibernal, pesticides, residues

INTRODUCTION

The grapevine (*Vitis vinifera* L.) is a significant global crop both economically and nutritionally. Grapes are versatile, serving as fresh produce or as ingredients for various processed goods such as wine, jam, jelly, grape seed extract, vinegar, juice, raisins, grape seed oil, and pekmez. Grapes and wines are renowned for their abundance of phenolic compounds, which encompass hydroxybenzoic and hydroxycinnamic acids, phenolic alcohols, flavan-3-ol monomers, flavonols, stilbenes, anthocyanins, oligomeric, and polymeric procyanidins **(Cueva et al., 2017)**. In their chemical composition, we can find micronutrients, such as vitamins B1, B6, C, and minerals, such as manganese and potassium **(Dumitriu (Gabur) et al., 2021)**. Grapes are known to possess high amounts of carbohydrates, and this makes them very vulnerable to damage by diverse fungal pests and insects **(Golge and Kabak, 2018)**. The heightened vulnerability of grape varieties to biotic stressors can result in significant economic losses, diminished wine quality, and the development of undesirable sensory attributes. Grapevines and grapes are susceptible to numerous diseases, including downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), black rot (*Guignardia bidwellii*), Botrytis rot (*Botrytis cinerea*), Eutypa dieback (*Eutypa lata*), Phomopsis cane and leaf spot (*Phomopsis viticola*), sour rot (caused by *Aspergillus niger*, *Alternaria tenius*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus arrihizus*, and *Penicillium* spp.), and various others. The significant disease pressure and limited availability of genetically resistant cultivars have resulted in the widespread use of substantial quantities of pesticides in vineyards to ensure consistent yields and high-quality grapes **(Grimalt and Dehouck, 2016)**. Throughout the grape production season and later, in winemaking, growers have detected trace amounts of pesticides, referred to as residues. Annually, approximately 2 million tons of various pesticides are applied worldwide, and projections suggest that global pesticide usage in overall production will rise to approximately 3.5 million tons **(Sharma et al., 2019)**. Spraying of grapevines is necessary multiple times during various developmental stages, and the presence of pesticide residues has been documented in the literature by authors **(Baša Česnik et al., 2008)**.

The occurrence of harmful organisms depends mainly on climatic, agroecological, and anthropogenic factors. Appropriate conservation interventions with fungicides can prevent the spread of economically dangerous pests and diseases in good time. Fungicides are contaminants. They eliminate the development of fungi, but their effectiveness cannot be adjusted to target pathogens alone. They are harmful throughout the entire environmental chain. Their use must be targeted and controlled **(Mcgrath, 2004)**.

Peronospora is one of the most economically serious diseases of grapevines, caused by the fungus *Plasmopara viticola*. In our conditions, Peronospora causes an average yield loss of around 10 to 15%. It attacks leaves, inflorescences, berries, and yearling green shoots. Inflorescences and young berries are very susceptible. It spreads in rainy weather. It is important to choose the right product and to determine the appropriate date of application for chemical treatment. In the list of authorised plant protection products for 2021, published in the **Bulletin of the Ministry of Agriculture and Rural Development (2021)** of the Slovak Republic, the following products have been included for the protection of vines against *P. viticola*, the following active substances (and preparations) are authorised: ametoctradine + dimethomorph (Orvego), amisulbrom + folpet (Sanvino), azoxystrobin + folpet (Quadris max), benalaxyl-M + folpet (Fantic F), copper hydroxide (Cuprozin progress, Funguran progress, Champion 50 WG, Kocide 2000), copper hydroxide + copper oxychloride (Airone SC, Badge WG, Coprantol Duo), copper hydroxide + cymoxanil (Copforce Extra, Cupman), copper oxychloride (Cuprocaffaro Micro, Flowbrix), copper oxychloride + cymoxanil (Kupfer Fusilan WG, Mildicut), cyazofamid + folpet (Daimyo F, Videryo F, Vincya F), cymoxanil + famoxadone (Tanos 50 WG), cymoxanil + folpet + fosetyl (Afrasa Triple WG, Momentum Trio), dimethomorph + folpet (Forum FP).

The grapevine powdery mildew (*Erysiphe necator*) is an obligate surface parasitic fungus that is strictly subordinate to the genus *Vitis*. It attacks leaves, annual shoots, inflorescences, and young developing berries. It should be noted that its infestation is sometimes the cause of unpleasant wine flavors due to the lack of ripeness of the berries at the time of ripening. In addition, several fungal volatile compounds produced by *E. necator* give musts a mushroomy or fishy smell. After infections, faint pale green to yellowish-green dull spots appears on the face and underside of the leaves. Whitish, later greyish coatings form on the leaf cheeks affected leaves, are often deformed and chlorotic and may wilt and die if heavily infected. Significant symptoms appear after prolonged warm and dry weather **(Gadoury et al., 2011)**.

In the list of authorized plant protection products for 2021, the following active substances (and preparations) have been registered for the protection of vines against powdery mildew: Ampelomyces quisqualis strain AQ10 (AQ 10), azoxystrobin + folpet (Quadris max), azoxystrobin + tebuconazole (Azimut, Custodia), Bacillus amyloliquefaciens strain FZB24 (Taegro), boscalid + kresoxim-methyl (Collis), cyflufenamid + difenoconazole (Dynali), difenoconazole + spiroxamine (Spirox D), fluopyram + spiroxamine (Luna Max), fluopyram + tebuconazole (Luna Experience), fluxapyroxad (Sercadis), meptyldinocap (Enter, Karathane New), methyram + pyraclostrobin (Cabrio Top), metrafenone (Vivando), penconazole (Topas 100 EC), potassium hydrogen carbonate (Karma, Kumar, Vitisan), proquinazid (Talendo 20 EC), proquinazid + tetraconazole (Talendo Extra), Precautions should be taken when applying fungicides to prevent the development of resistance **(Bulletin of the Ministry of Agriculture and Rural Development, 2021)**.

Grey mold (*Botryotinia fuckeliana*) – the conidial stage (*Botrytis cinerea*) can infect all green parts of the vine. Of economic importance is the infestation of inflorescences, bunches, and berries. It usually infects after a long period of spring rains. Infected flowers turn brown and, in wet weather, may be overgrown with a grey coating of the fungus. The infected part of the inflorescence dries out. The danger from early infection is mainly that the pathogen settles on the remnants of the inflorescence. In the form of vegetative mycelium, it survives the hot summer period and is reactivated before or after the bunches have closed. In wet weather, it infects berries that are still immature **(Williamson et al., 2007)**.

The infected berries turn brown and develop a grey, mold growth which later covers the whole berry. No synthesis of sugars takes place. The infected bunches have an unpleasant fungal smell and become a suitable substrate for infection by secondary fungi, which can significantly impair the quality of the raw material for wine production. In blue varieties, grey mold also breaks down the coloring agents in the skin **(Ellis, 2016)**.

To protect the vines against gray mold it was possible to use the fungicides Botector (active ingredient Aureobasidium pullulans), Quadris max (azoxystrobin + folpet), Taegro (Bacillus amyloliquefaciens strain FZB24), Serenade ASO (Bacillus subtilis str. QST 713), Cantus (boscalid), Switch 62.5 WG (cyprodinil + fludioxonil), Teldor 500 SC (fenhexamid), Prolectus (fenpyrazamine), Cassiopee 79 WG (folpet + fosetyl + iprovalicarb), Melody Combi WG (folpet + iprovalicarb), Mythos 30 SC, Pyranil-I, Pyrus 400 SC, Scala (pyrimethanil), Magnicur Core, Zato 50 WG (trifloxystrobin) **(Bulletin of the Ministry of Agriculture and Rural Development, 2021)**.

Multi-residue analytical methods, are developed with a focus on enhancing their analysis efficiency based on appropriate international standards. The developed multi-residue methods are particularly suitable for residual pesticide detection, which involves the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and the use of liquid chromatography (LC), gas chromatography (GC) and tandem mass spectrometry **(Yin et al., 2022)**. Concisely, QuEChERS extraction involves two processes of extraction, making it a simple pretreatment method. It also allows the extraction and purification of various pesticide compounds. Although its purification efficiency is typically low, QuEChERS extraction is currently the most commonly used method when combined with mass spectrometry, which ensures high selectivity and sensitivity **(Pang et al. 2021)**.

The primary objective of the research was to evaluate the impact of fungicide treatments on grapevines on the concentration and dispersion of four active substances in the leaves of the Hibernal variety. Our focus was on examining the active ingredients of fungicides applied during the early stages of growth, specifically azoxystrobin, cyflufenamid, difenoconazole, and myclobutanil, utilizing multiresidue analysis methodologies.

MATERIAL AND METHODS

Characteristics of the habitat climatic conditions of the experimental plot

The research was carried out in the Nitra wine-growing region. BPEJ of the vineyard: 0013004.

Climatic region – 00: very warm, very dry lowland. The average air temperature for the growing season (IV-IX) is 16-17 °C.

Soil grain size – 4: very heavy soils (clays and loams, fractional content < 0.01 mm → clays 60-75%, clays over 75%).

Climatic characteristics of the 2021 growing year

The specific weather patterns with precipitation and average air temperatures in individual days and months during the 2021 growing season are given in Figure 1.

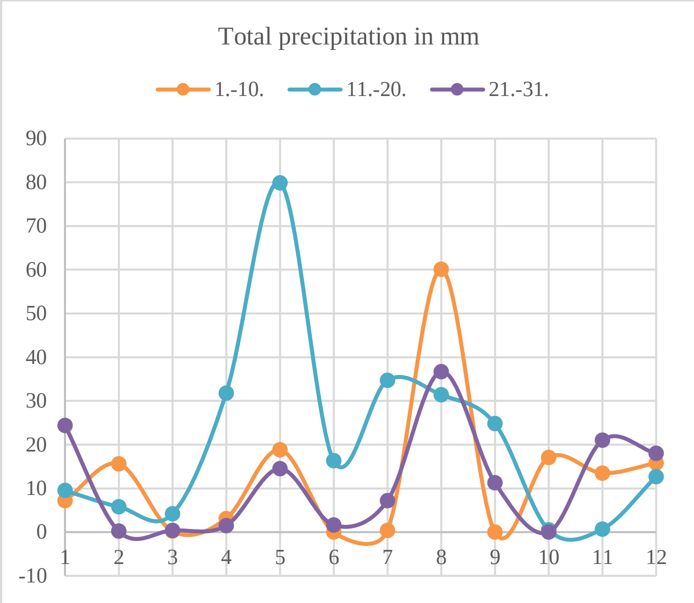

Figure 1 Total precipitation in mm within days in Nitra wine-growing region in 2021

Characteristics of the biological material (Hibernal variety under study)

The variety was bred at the Research Institute in Geisenheim, Germany. It was created by crossing the varieties (Seibel 7053 x Riesling 239 Gm) F2. It includes genes from *Vitis labrusca* (5.1%), *Vitis lincecumii* (0.8%), *Vitis rupestris* (13.3%), and *Vitis vinifera* (78.9%, from species to which noble varieties such as Chardonnay belong). The hybrid was crossed in 1944 by Heinrich Birk (Figure 2). The berries are small and round, with firm skin and flesh, which results in a lower yield of juice from the grapes. The basic color is greenish yellow, with a pinkish to purplish color at full aromatic maturity, the flesh is colorless, with a sweet, neutral to slightly spicy flavor **(Sedlo, 2022)**.

Figure 3 Hibernal variety

Description of the Experiment

In the experiment, we used the white interspecific variety Hibernal grafted onto the rootstock Kober 5 BB. We investigated 2 variants (Table 1): a pesticide-treated (Table 2) and an untreated control. Each variant had 3 replications of 12 tillers.

Number of plant samples: 36

Year of planting: 2007

Planting spacing: 1 x 3 m.

Cultivation form: Rhine-Hessian line with 1 stem

Cutting method: Guyot's cut

Bud load: 8-10 bud mast

Soil treatment method: all-over black eel

Green work: clearing stems, tucking leaf litter into double wire, defoliation in the bunch zone, removing tops of leaf litter, removing leaf litter in the bunch zone.

Leaf collection: 2 dates – 3/8/2021 (BBCH 73): in full vegetation and 2/11/2021 (BBCH 91): 14 days after the grape harvest. Leaf samples were taken randomly from the entire leaf wall from both sides of the bush.

Table 1 Experiment variant codes

Date of leaves collection		Date of leaves collection	
3.8.2021		2.11.2021	
variant with pesticide use	variant with untreated control	variant with pesticide use	variant with untreated control
Sample no. 8672	Sample no. 8673	Sample no. 8674	Sample no. 8671

Table 2 Protection and auxiliary substances used in the treated variant of the year 2021

Day of application	Preparation	Active substance
1.4.2021	Sulka K 0.5%	N: 3%, K_2O: 14%, CaO: 2%, S: 19%
8.6.2021	IQ Crystal 0.02%	quinoxyfen 250 g/l
	Shavit 72WG 0.2%	triadimenol 20 g/kg, folpet 700 g/kg
	Harmavit Special 0.3%	N: 10%, P_2O: 9%, K_2O_5: 10.5%, Cu: 5 mg/kg, Zn: 5 mg/kg, Mn: 23 mg/kg, B: 5 mg/kg, Mo: 10 mg/kg. Fe: 150 mg/kg
	Silwet Star 0.05%	polyalkyleneoxid heptamethyl trisiloxane 80%, allyloxypolyethyleneglycol 20%
24.6.2021	Quadris Max 0.2%	azoxystrobin 93.5 g/l, folpet 500 g/l
	Talent 0.02%	myclobutanil 200 g/l
	Samppi 0.2%	N: 8%, P_2O_5: 3%, K_2O: 3%, MgO: 2%, CaO: 1%, MnO: 1%, B_2O_3: 0.5%, Fe: 0.4%, Mo: 0.1%, Cu a Zn: 0.05%
	Silwet Star 0.05%	polyalkyleneoxid heptamethyl trisiloxane 80%, allyloxypolyethyleneglycol 20%
22.7.2021	Ampexio 0.5 kg/ha	mandipropamid 250 g/kg, zoxamide 240 g/kg
	Dynali 0.065%	difenoconazole 60 g/l, cyflufenamid 30 g/l
	Citowett 0.015%	octoxynol 60-90%, poly(oxy-1,2-ethanediyl), alpha -[(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxyl 10-30%
6.8.2021	Folpan 80 WDG 0.2%	folpet 800 g/kg
	Karathane New 0.05%	meptyldinocap 350 g/kg
	Citowett 0.015%	octoxynol 60-90%, poly(oxy-1,2-ethanediyl), alpha -[(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxyl 10-30%
25.8.2021	Teldor 500SC 0.1%	fenhexamid 500 g/l

Legend: fungicides – IQ Crystal, Shawit 72WG, Quadris Max, Talent, Ampexio, Dynali, Folpan 80 WDG, Karathane New, Teldor; fertilizers – Sulka K, Harmavit Special, Samppi; wetting agents – Silwet Star, Citowett

Methods of analysis of the content of active substances

Analyzes were performed according to **STN EN 15662 (2018)**. For the extraction of pesticides from leaves, we used a simple solvent extraction with acetonitrile in a centrifuge tube, water desalination with salts including magnesium sulfate ($MgSO_4$), and purification based on dispersive solid-phase extraction with dSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine), in which common matrix components were retained by the sorbents and the analytes were retained in the extract. In mass spectrometry, matrix effects negatively affect the accuracy and precision of the method. After extraction with acetonitrile and after purification of the analytes from water and unwanted co-extractants present in the samples, all residues were amenable to LC-MS/MS (liquid chromatography with tandem mass spectrometry) and GC-MS/MS (gas chromatography with tandem mass spectrometry) analysis. The equipment used for liquid chromatography with tandem mass spectrometry was an Agilent HPLC 1290 system and an Agilent 6460 Triple Quadrupole Mass Spectrometer system. For GC, an Agilent 7890B gas chromatograph with a 7010B triple quadrupole mass spectrometer was used. Multiresidue analysis with GC-MS/MS or LC-MS/MS output – analysis of 29 active substances:

- active substances analyzed by the GC-MS/MS method: azoxystrobin, boscalid, cyprodinil, difenoconazole, fludioxonil, fluopicolide, metalaxyl-M, myclobutanil, penconazole, propiconazole, pyrimethanil, spiroxamine, tebuconazole, tetraconazole, triadimenol.
- active substances analyzed by the LC-MS/MS method: cyflufenamide, cymoxanil, dimethomorph, famoxadone, fenpyroximate, fenpyrazamine, fluopyram, hexythiazox, indoxacarb, iprovalicarb, metrafenone, proquinazid, thiophanate-methy.
- active substances analyzed by the combined method of GC-MS/MS and LC-MS/MS: fenhexamide.

The SANTE document is currently used for pesticide analysis. According to this document, the LOQ is set as a practical value, fortified, and measured.

Statistical analyses

Data distribution was verified using the Shapiro-Wilk test. The variance was examined using one-way ANOVA and the Tukey post hoc test (least significant difference test, $p \leq 0.01$). It is used to test a well-defined number of hypotheses. To statistically treat obtained results, we used XLSTAT v. 2021.4.1 (Addinsoft, France) software.

RESULTS AND DISCUSSION

We applied Talent (myclobutanil) and Quadris max (azoxystrobin) 41 days before the first leaf collection and Dynali (difenoconazole, cyflufenamid) 12 days before the first leaf collection. Experimental results from leaf sampling on 3.8.2021 showed that several samples with the observed active substances had elevated concentration levels above the maximum residue limits for vine leaves defined by European legislation. According to **Commission Regulation No 396/2005**, maximum residue levels (MRLs) have been established for various active substances in vine leaves and grapes (Table 3). For difenoconazole, MRLs are set at 0.005 mg/kg in vine leaves and 3 mg/kg in grapes. Cyflufenamid is permitted at levels of 0.01 mg/kg in vine leaves and 0.2 mg/kg in grapes. Myclobutanil has MRLs of 0.05 mg/kg in vine leaves and 1.5 mg/kg in grapes. Additionally, the EU pesticide database maintained by the European Commission lists MRLs for azoxystrobin at 0.01 mg/kg in vine leaves and 3 mg/kg in grapes. The results from the leaf sampling on 2.11.2021 showed that fungicide residue levels at the time of grape harvesting had fallen below detectable limits, which we consider to be a positive phenomenon in terms of their impact on the environment and consumer health (Table 4).

Table 3 Maximum residue levels (MRLs) established for four active substances in vine leaves and grapes

Active substances			
Azoxystrobin (mg/kg)	Cyflufenamide (mg/kg)	Difenoconazole (mg/kg)	Myclobutanil (mg/kg)
MRL (for vine leaves) according to EU Pesticides Data			
0.01	0.01	0.005	0.05
Limit of Detection (LOD)			
0.002	0.003	0.002	0.002
Limit of Quantification (LOQ)			
0.005	0.01	0.005	0.005

From the results in Table 4, there was a statistically significant difference in the content of the four positively detected residues of active substances between the samples of Hibernal leaves and those that were not treated (control sample). In the second leaf sampling in November, the values of these residues were below the detection limit. The decomposition of chemicals used to spray plants can be sunlight, microorganisms in the soil, or other environmental factors. This process can lead to the gradual removal of spray residues from the surface of the leaves. Another factor may be the washing away of leaf residues by rain or precipitation, which may have happened between August and November. Plants also can clean and regenerate themselves. New cells produced on the leaves and their regeneration can contribute to residues from the previous spray. Residue values of dimethomorph, cymoxanil, metalaxyl-M, penconazole, propiconazole, tebuconazole, fluopyram, spiroxamine, tetraconazole, proquinazid, triadimenol, famoxadone, fluopicolide, iprovalicarb, metrafenone, thiophanate-methyl, indoxacarb, fenpyroximate, hexythiazox, boscalid, cyprodinil, fludioxonil, fenhexamide, fenpyrazamine, pyrimethanil were below the limit of detection throughout the research.

Table 4 Results of residual analyzes (mean ± SD) with GC-MS/MS and LC-MS/MS output

Sample No. / Method	Active substances			
	Azoxystrobin (mg/kg)	**Cyflufenamide (mg/kg)**	**Difenoconazole (mg/kg)**	**Myclobutanil (mg/kg)**
variety Hibernal - leaves, treated with pesticides 3.8.2021				
8672 / LC-MS/MS		0.177 ± 0.006^{a}		
8672 / GC-MS/MS	0.083 ± 0.002^{a}		1.407 ± 0.035^{a}	0.012 ± 0.001^{a}
variety Hibernal - leaves, untreated control 3.8.2021				
8673 / LC-MS/MS		0.013 ± 0.002^{b}		
8673 / GC-MS/MS	<0.002^{b}		0.031 ± 0.001^{b}	<0.002^{b}
variety Hibernal - leaves, treated with pesticides 2.11.2021				
8674 / LC-MS/MS		<0.003		
8674 / GC-MS/MS	<0.002		<0.002	<0.002
variety Hibernal - leaves, untreated control 2.11.2021				
8671 / LC-MS/MS		<0.003		
8671 / GC-MS/MS	<0.002		<0.002	<0.002

Legend: LC-MS/MS – liquid chromatography with tandem mass spectrometry, GC-MS/MS – gas chromatography with tandem mass spectrometry, a, b means rows with different letters are statistically different (Tukey test, p <0.01).

Difenoconazole is an active substance from the group of triazoles belonging to the group of demethylating inhibitors. It can control a wide range of fungi – Including members of the *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* classes **(Syngenta, 2022)**. The fungicidal substance has toxic effects on human health. Hazardous to mammals, birds, and most aquatic organisms. Was identified as an environmental contaminant, xenobiotic (sterol 14alpha-demethylase) inhibitor, and an antifungal agrochemical **(Commission Regulation No 396/2005)**.

Cyflufenamide is the (trifluoromethyl) benzene class and belongs to the amide group. Preparations with this active substance act as both protective and therapeutic fungicides used against powdery mildew. They are formulated as suspension concentrates and are applied postemergently by spraying on the leaf. These synthetic pesticides are toxic to fish and aquatic invertebrates **(Syngenta, 2022)**.

Myclobutanil is a triazole chemical used as a fungicide. It is an inhibitor of demethylation of mycosterols, specifically inhibiting ergosterol biosynthesis, thereby killing fungi **(Syngenta, 2022)**.

Azoxystrobin belongs to the chemical group of B-methoxyacrylates (strobilurin derivatives). The mechanism of action is the inhibition of electron transport during the respiration of fungal mitochondria. The active substance penetrates the plexus (translaminar and systemic action). The effect of azoxystrobin is primarily preventive and must, therefore, be applied before or at the beginning of infection. It has a long-lasting effect and may thus prevent the development of a later infection. Treated stands are green for a longer period (green effect) **(Syngenta, 2022)**.

Manal et al. (2009) monitored the mobility of azoxystrobin residues on grape leaves after 15 days of treatment. The initial concentration was dissipated by 99.38% respectively (4.48 to 0.03 mg/kg.). The results correspond with our findings. In samples from November, the values of azoxystrobin in Hibernal leaves were undetectable (0.083 to ND). **Baša Česnik et al. (2008)** presented the results of pesticide monitoring in 47 samples of wine grapes (*Vitis vinifera* L.) of the 2006 vintage. Folpet (97.9%), cyprodinil (51.1%), dithiocarbamates (44.7%), chlorothalonil (23.4%), chlorpyriphos (19.1%), and pyrimethanil (14.9%) were the most frequently found pesticides in grapes.

In 2021, 419 food samples were analyzed by the **SVFA SR (2021)** using the same method. In 288 food samples (68.7%), one or more pesticide residue species were detected, of which 18 samples (4.3%) were non-compliant. No pesticide residues (values below the limit of quantification of analytical methods – values below LOQ) were detected in 131 samples, representing 31.3%. The number of grape samples checked was 17. Of these, one was unsatisfactory – an exceedance of 0.03 mg/kg of the active substance chlorpyrifos in a sample of table grapes from Italy. It is an insecticide that we did not use in our research. The active substances, cyflufenamid and difenoconazole were found in the control variant sample taken on 3.8.21 above the MRL, but in a statistically significantly lower concentration than in the treated one. Probably this may be due to drift during routine chemical protection.

At the second leaf sampling, these leaf values decreased below the LOD. We do not expect the residues to move into the woody plant tissue, but they will be degraded to undetectable values, which is also the principle of the protection periods of active substances from the point of view of consumer health safety. In the case of inappropriate agrotechnics of pesticide application, active substances may escape to non-target areas.

Maestroni et al. (2019) monitored the kinetics of chlorpyrifos, diazinon, and dimethoate residues in vine leaves, considering the shelf life of the active substances. They found that Chlorpyrifos, the most apolar compound, has insufficient mobility, and its residues persist for a long time (more than 90 days) in biological material. **Zhang et al. (2019)**, in their study, report the risks of consuming fruits and vegetables treated with insecticides with the active ingredient neonicotinoid in children. The European Union publishes an annual report on the status of pesticide residues in food **(Carrasco Cabrera and Medina Pastor, 2022)**. **Mahdavi et al. (2022)** evaluate pesticide residues and risk assessment in apples and grapes from western Azerbaijan Province. Health risk assessment associated with pesticide residues was estimated by hazard quotient (HQ) and hazard index (HI), which indicated that the HI value was lower than 1 in adults and children due to apple consumption. HI in adults and children were 0.012 and 0.054 in apples; and 0.001 and 0.003 in grape samples, respectively. Although the health risk assessment showed that the consumers are not at considerable risk due to pesticide residues, implementing control plans to manage the proper application of this pesticide or replacing it with safer alternatives in apples and grapes is required.

CONCLUSION

Pesticide pollution is a major problem in all wine-growing areas. Consequently, reducing pesticide use requires the implementation of new agricultural practices aimed at sustainable development and consumer health protection. Given the risk of pesticide residues, it is important to monitor them regularly and to comply with the maximum permitted levels (MRLs) defined by the EU, the Codex Alimentarius, the European Environment Agency, and legislation, to minimize as far as possible the risks of their harmful effects. It is positive, that after the grape harvest in BBCH 91, we found a decrease in the content of all monitored residues below detectable values. In the leaf samples of the treated variant collected in BBCH 73, we established higher values of residues than the MRL determines, namely for azoxystrobin 0.083 mg/kg (MRL - 0.01 mg/kg). Azoxystrobin was applied 41 days before the first collection of leaves. Therefore, it is necessary to observe the protection periods of active substances and to consider the agrotechnical dates of grape harvesting with a sufficient reserve when applying fungicides. Integrated and ecological disease and pest management can be an option to minimize the use of pesticides, thereby reducing the environmental burden as well as the risk to present and future generations, and thus protecting the health of the Earth and the population. Hibernal is a variety suitable for IP and organic cultivation, and we anticipate that even in years with high fungal disease pressure, it is possible to preserve health with organic products. By applying nature-friendly agrotechnical practices in combination with the variety, we can eliminate residues in both the grapes and the wines produced and target the composition of the products in favor of health-promoting substances.

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