



## Pesticides in wines from two major production regions in the North of Spain

V. Fernández-Fernández<sup>a</sup>, M. Ramil<sup>a</sup>, P. Blanco<sup>b</sup>, M. Soledad Andrades<sup>c</sup>, I. Rodríguez<sup>a,\*</sup>

<sup>a</sup> Department of Analytical Chemistry, Nutrition and Food Sciences, ARCUS – Aquatic One Health Research Centre, Universidade de Santiago de Compostela, R/ Constantino Candeira, 5, Santiago de Compostela 15782, Spain

<sup>b</sup> Estación de Viticultura e Enoloxía de Galicia (EVEGA-AGACAL), Ponte San Clodio s/n, Leiro, Ourense 32428, Spain

<sup>c</sup> Department of Agriculture and Food, University of La Rioja, Madre de Dios 51, Logroño 26006, Spain

### ARTICLE INFO

#### Keywords:

Wine  
Pesticides  
Occurrence  
Liquid chromatography mass spectrometry  
Chiral analysis  
Toxicological risk

### ABSTRACT

The occurrence of fungicides and insecticides in wines from two reputed production regions in the North of Spain (Galicia and La Rioja) was assessed by liquid chromatography tandem mass spectrometry, including achiral and chiral analysis of more than four hundred wines, elaborated in four consecutive vintages. Fungicides presented a higher prevalence than insecticides in all samples, particularly those employed for the treatment of mildew infections. Individual compounds showed different distribution patterns in wines from both geographic regions, likely related to the different pressure of diseases affecting their vineyards. Total residue varied significantly among wines from the five denominations of origin in Galicia, with higher levels in those closest to the Atlantic coast. Temporal trends point out to a slight reduction in total residues of pesticides in wine. On the other hand, chiral analysis reflected that metalaxyl, the top concentration pesticide in most of the processed wines, was applied to vines as a mixture of active and non-active enantiomers. Thus, a potential reduction in the residues of this fungicide in wine are expected after the future phase-out of racemic formulations. Maximum concentrations of pesticides in wine remained below 10 % of maximum residues authorized in vinification grapes. Moreover, independently of the production area, the moderate consumption of wine contributes less than 1 % to the acceptable daily intake of most often detected pesticides.

### 1. Introduction

Pesticide residues in grapes can affect the organoleptic quality of wine, through their interaction with yeasts involved in the fermentation process (Guo et al., 2023; Noguero-Pato et al., 2015); moreover, they might represent a toxicological risk for wine consumers (Dumitriu Gabur et al., 2022; Santana-Mayor et al., 2023). The intensive application of pesticides in vineyards also poses a hazard to the environment, as it has been proved through their persistence in vineyard soils (Pérez-Mayán et al., 2020; Pose-Juan et al., 2015); occurrence in surface (Pappalardo et al., 2022) and ground water (Herrero-Hernández et al., 2017); and drifts impacting the vicinity of vineyards (Teyseire et al., 2023). In the current situation of climate change, sudden variations of climate conditions (temperature and humidity) increase the pressure of pests affecting the productivity of vines. Thus, grape growers need to achieve a balance between the economic and the environmental sustainability of their activity. Nowadays, despite current efforts focussed on the use of bio-pesticides, and those aiming to assess the commercial potential of new generation, disease-resistant hybrid vines (Duley et al., 2023),

grapes from *Vitis vinifera*, managed under conventional agronomic practices, are still the main source of wine elaborated in the Southwest of Europe, and more specifically in Spain.

The European Food Safety Agency (EFSA) maintains an updated list of authorized pesticides, with maximum residue levels (MRLs) in different vegetable commodities, including vinification grapes (European pesticide database of MRLs in food commodities, n.d.). However, no MRLs have been set for wine, yet. Extrapolation of those values legislated for grapes to wine using the so-called processing factors (PFs) (Zincke et al., 2024) is not a straightforward process. PFs are still based on fragmented data, they might be not available for more recently authorized plant protection products, and their exact values are dependent on the steps involved in the elaboration of each wine (Liu et al., 2023; Pazzirota et al., 2013).

Therefore, although the control of pesticide residues in vinification grapes permits to verify their compliance with current legislation, it has a limited usefulness to predict their levels in wine, and to calculate exposure levels of wine consumers. Grape berries are a less homogeneous matrix than wine. In the latter case, a single sample is

\* Corresponding author.

E-mail address: [isaac.rodriguez@usc.es](mailto:isaac.rodriguez@usc.es) (I. Rodríguez).

<https://doi.org/10.1016/j.jfca.2025.107525>

Received 17 September 2024; Received in revised form 10 March 2025; Accepted 21 March 2025

Available online 29 March 2025

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representative for thousands of bottles proceeding from same tank. Despite these facts, systematic studies assessing the presence of pesticide residues in wines are still scarce. Some of them were conducted more than 10 years ago (Duca et al., 2012; Walorczyk et al., 2011) when different regulations were in force. In other cases, they consider few dozens of samples (Čuš et al., 2022; Pelajić et al., 2019; Zhang et al., 2019), which limits the representativeness of the results. In Spain, the most recent, larger study was focused on wines produced in the Canary Islands (Santana-Mayor et al., 2023), a region with a limited production and environmental conditions (climate and soil characteristics) different to those existing in other production areas of the country.

Herein, we present an assessment of fungicide and insecticide residues in wines elaborated in two main production regions in the North of Spain: La Rioja and Galicia, with different pedologic and climate characteristics. The denomination of origin (D.O.) Rioja covers a surface of 47000 ha, divided in three sub-zones. Together, they produce around 370 million Kg of grapes (85 % corresponding to red grapes). The region is characterised by basic soils, with low organic carbon content and continental climate conditions. Galicia hosts five different D.O., with a total surface of vineyards of 25000 ha. Most of the production in the region corresponds to white wines. Soils in this region are acidic, with moderate to high organic carbon contents. Climate conditions vary from continental to Atlantic, depending on the proximity of each D.O. in this region to the coast of the Atlantic Ocean, Fig. S1. The aims of this research were: (1) assessing total residues of pesticides in wine; (2) identifying potential differences depending on the production area; (3) evaluating the impact of regulatory policies on the residues of active ingredients, which lost the authorization of use, or that are candidates for substitution; and (4) investigating potential risks of human exposure to pesticides through a regular, moderate consumption of wine. To this, more than four hundred wines, produced after 2019 were analysed.

## 2. Material and methods

### 2.1. Solvents, standards and sorbents

Trace analysis grade methanol (MeOH) and acetonitrile (ACN), for LC-MS, were purchased from Merck (Darmstadt, Germany). Ultrapure deionized water (18.2 MΩ cm<sup>-1</sup>) was obtained using a Geni-U system (Rephile, Shanghai, China). Standards for a selection of 54 pesticides (fungicides and insecticides) were provided by Sigma-Aldrich (Milwaukee, WI, USA), and Dr. Ehrenstorfer GmbH (Augsburg, Germany). Metalaxyl was acquired as racemate and as the pure enantiomers (R and S-forms) from Sigma-Aldrich. The choice of target compounds was made attending to information received from grape growers, and sales in the two investigated regions. Moreover, occurrence data available for wines elaborated in Canary Islands and other countries were considered during selection of analytes (Čuš et al., 2022; Santana-Mayor et al., 2023). Finally, those fungicides reported in soils from vineyards located in La Rioja were also included in this research (Pose-Juan et al., 2015). Isotopically labelled analogues (either deuterated or <sup>13</sup>C enriched molecules) of a suite of 16 pesticides were purchased either from Sigma-Aldrich, or from Toronto Research Chemicals (North York, ON, Canada). These compounds were employed as surrogate standards (SSs), added to wine samples before extraction. Individual stock solutions of each compound were prepared in methanol. Two mixtures of native pesticides and SSs, both at a concentration level of 10 µg mL<sup>-1</sup>, were made in the same solvent. Calibration standards were prepared in ACN:MeOH (80:20, v:v).

OASIS HLB, 60 mg solid-phase extraction cartridges were purchased from Waters (Milford, MA, USA).

### 2.2. Samples and sample preparation

Wine samples were received either directly from winemakers, or from winemaker associations responsible for organoleptic quality of

wines elaborated within each D.O. No information was available neither regarding agronomic practices, nor concerning the wine elaboration process, apart from production year and D.O., or sub-zone, within the two geographic regions. Two different sets of wines were employed. The first comprised an average of 8 wines per D.O. (or sub-zone), and year, corresponding to four consecutive vintages, from 2020 to 2023. Overall, 100 wines from the three sub-zones in La Rioja (*Rioja Alavesa*, R-AI; *Rioja Oriental*, R-E; and *Rioja Alta*, R-H), and 170 samples distributed among the five D.O. of Galicia (*Valdeorras*, G-VD; *Monterrei*, G-MO; *Ribeira Sacra*, G-RS; *Ribeiro*, G-RI; and *Rías Baixas*, G-RB), were included in this set of samples, Fig. S1. The second series of wines was obtained from same D.O. in Galicia (G-RI). It comprised 168 additional wines, elaborated between years 2019 and 2022. After reception, bottles were kept at room temperature (16 °C- 18 °C), in a dark room, for a maximum of one month before extraction and analysis.

Extraction of pesticides from wine was performed using a previously developed procedure (Castro et al., 2018). In brief, 2 mL of unfiltered wine were spiked with SSs (addition level 25 ng mL<sup>-1</sup>), diluted with the same volume of ultrapure water, and passed through SPE cartridges previously conditioned with MeOH and an ethanol: water (12:88, v:v) solution (3 mL each). Compounds were eluted with acetonitrile: methanol (ACN:MeOH, 80:20), adjusting the extract to a final volume of 2 mL. After filtration, extracts were analysed by LC-MS/MS under conditions described in Section 2.3.

### 2.3. Determination conditions

Pesticide residues in the extracts from wine samples were determined by LC-MS/MS. The employed equipment comprised a Waters Acquity UPLC instrument combined with a triple quadrupole MS system (XEVO TQD), from same supplier, through an electrospray ionization source (ESI). Compounds were separated in a C<sub>18</sub>-type column (Zorbax Eclipse Plus, 50 mm × 2.1 mm, 1.8 µm) acquired from Agilent Technologies (Wilmington, DE, USA). The column was connected to a C<sub>18</sub> 2.1 mm i.d. Security Guard™ cartridge, supplied from Phenomenex (Torrance, CA, USA). The mobile phase consisted of water (phase A) and ACN (phase B), both 0.1 % in formic acid (Castro et al., 2018). The MRM transitions of target compounds and SSs are compiled in Table S1. Levels of pesticides in extracts from wine samples were established by comparison with calibration standards (10 different concentration levels, from 0.5 ng mL<sup>-1</sup> to 200 ng mL<sup>-1</sup>), prepared in the same mixture of solvents employed for elution of SPE cartridges (ACN:MeOH, 80:20), containing the same concentration of SSs as that added to wine samples before extraction.

In addition to C<sub>18</sub>-based determinations, two chiral columns were tested for enantiomeric analysis of selected fungicides. They were a Cellulose-2 (50 mm × 3 mm, 2.5 µm, chiral selector tris(3-chloro-4-methylphenylcarbamate) and an Amylose-1 (150 mm × 3.0 mm, 3 µm, chiral selector tris(3,5-dimethylphenylcarbamate)) purchased from Waters and Phenomenex, respectively. The enantiomeric fractions (EFs) of the chiral fungicides mandipropamid, tebuconazole, metalaxyl and myclobutanil, often detected in wine samples, were calculated as the concentration of the earlier eluting enantiomer divided by the sum of concentrations of both isomers. Identities of enantiomers were not investigated, except in the case of metalaxyl. For this fungicide, the active enantiomer (Metalaxyl-M, corresponding to the R-form) displayed the longer retention time in both columns. Chiral separations were employed to identify potential enantioselective processes of those species marketed as racemates, during wine elaboration; as well as to investigate the form of metalaxyl used by grape growers. This fungicide is commercialized under two different formulations: as racemate and as the only active enantiomer, that is metalaxyl-M. While the racemate is a candidate for substitution by the end 2026, the use of metalaxyl-M is authorized until 2035 (PPDB, Pesticides Properties Database, n.d.).

Irrespectively of the employed LC column (achiral or chiral), identification of compounds in LC-MS/MS records of wine extracts was based

on retention times, and qualification (Q2) to quantification (Q1) ions response ratios matching those obtained for calibration standards within a range of values of  $\pm 0.1$  min and  $\pm 30$  %, respectively, Table S1.

## 2.4. Quality control

Quality control procedures involved checking the stability of calibrates by injection of two different standards, at concentrations of  $10 \text{ ng mL}^{-1}$  and  $50 \text{ ng mL}^{-1}$ , and analysis of procedural blanks (c.a., every 10 samples) to identify potential contamination problems. Moreover, accuracy of concentrations determined in wine samples was evaluated with analysis of a spiked sample ( $n = 2$  replicates) within each batch of wines (typically 20 samples). The responses of analytes in procedural blanks should stay below those corresponding to their LOQs, Table S1. Responses for calibration standards must remain between 90 % and 110 % of expected values, and recoveries for spiked samples within 70 % and 120 %. During evaluation of EFs, the sum of concentrations for enantiomers separated in any of both chiral columns was verified to agree with that determined with the  $C_{18}$ -type column, with a maximum variation of  $\pm 10$  %.

## 3. Results and discussion

### 3.1. Performance of achiral and chiral LC-MS/MS methods

Table S1 summarizes data related with the performance of the LC-MS/MS methodology based on the use of the  $C_{18}$  column. Procedural LOQs ranged from  $0.5 \text{ ng mL}^{-1}$  to  $10 \text{ ng mL}^{-1}$ , depending on the compound. The linear response range extended up to concentrations of  $200 \text{ ng mL}^{-1}$ . Accuracy was assessed with different samples of red and white wines, processed in triplicate, and spiked at three different levels: 10, 20 and  $50 \text{ ng mL}^{-1}$ . The percentage of pesticides with recoveries in the range of values from 70 % to 120 % accounted for 94 % except in case of red wines spiked at  $20 \text{ ng mL}^{-1}$ , with 89 % of the compounds rendering recoveries within the above range, Table S2.

Regarding chiral analysis, different resolution factors were obtained depending on the column and organic solvent (MeOH or ACN) included in the mobile phase. Separation of the enantiomers of benalaxyl was only possible using the amylose-1 column. On the other hand, this column failed to separate the isomers of myclobutanil and penconazole. The enantiomers of myclobutanil were partially separated in the Cellulose-2 column in combination with MeOH in the mobile phase, but not when using ACN; however, the resolution of the enantiomers metalaxyl and penconazole worsened when changing ACN by MeOH. Fig. S2 shows the LC-MS/MS chromatograms obtained for a selection of chiral fungicides, using both columns and different mobile phases. Considering that compounds most often found in wine were tebuconazole, mandipropamid, myclobutanil and metalaxyl, their EFs were recorded, in the same injection, with the Cellulose-2 column, using MeOH as the organic solvent in the mobile phase. For those wines containing just metalaxyl, as chiral pesticide, the amylose-1 column, in combination with  $\text{ACN:H}_2\text{O}$  (5 mM ammonium acetate) as mobile phase, was preferred.

### 3.2. Pesticides as function of wine origin

#### 3.2.1. Total residues

The concentrations of fungicides and insecticides in wines elaborated in the three sub-zones of La Rioja (codes R-Al, R-H and R-E) and the five D.O. of Galicia (codes G-VD, G-MO, G-RS, G-RI and G-RB), in the period 2020–2023 ( $n = 270$  samples), are provided as supplementary information, Tables S3 and S4, respectively. Residues in the three sub-zones of La Rioja showed a homogeneous distribution, with similar average and median values; however, significant differences are observed for wines from the different D.O. in Galicia, Fig. 1. Those elaborated in the East part of this region (codes G-MO and G-VD) present similar residues

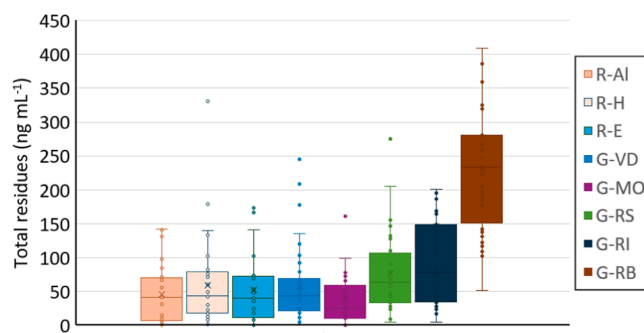


Fig. 1. Box-Whisker plots for total pesticide residues in wines elaborated between 2020 and 2023 in the three sub-zones of La Rioja, and the five D.O. of Galicia.

to wines from Rioja. For the other three D.O., median and average concentrations increased in the order of  $\text{G-RS} < \text{G-RI} < \text{G-RB}$ , Fig. 1. As a global statement, the closer to the Atlantic coast the D.O. (Fig. S1), the higher the residues found in wines from Galicia. Likely, this is the consequence of an increasing pressure of fungi infections in these vineyards due to a combination of higher humidity and mild temperatures. From data depicted in Fig. 1 it became evident that, considering the full set of samples, wines from Galicia showed higher average and median values of pesticide residues than those elaborated in La Rioja, Fig. S3. Out of the entire set of 270 wines, just seven samples did not contain residues of any of the determined compounds above the procedural LOQs, Tables S3 and S4.

#### 3.2.2. Occurrence of individual pesticides

Table 1 summarizes the maximum concentrations, detection frequencies and ratios between highest concentrations in wines and MRLs defined by the EU for vinification grapes (European pesticide database of MRLs in food commodities, n.d.), in both geographic regions. In wines from Galicia ( $n = 170$  samples), 32 different compounds were identified, whereas 22 different species were detected in those elaborated in La Rioja ( $n = 100$  samples). These figures are reduced to 23 and 14 pesticides with concentrations above  $10 \text{ ng mL}^{-1}$  in Galicia and La Rioja, respectively. This threshold was set as the procedural LOQ in some previous monitoring studies (Walorczyk et al., 2011); moreover, it corresponds to the MRL (usually given in  $\text{ng g}^{-1}$ ) of non-authorized phytosanitary chemicals in food commodities.

Most of the pesticides in wine corresponded to fungicides. The only insecticides above the LOQs of the analytical procedure were acetamiprid, chlorantraniliprole, methoxyfenozide and tebufenozide, Table 1. Their detection frequencies ranged from 2 % to 24 %, with a maximum concentration of  $101.2 \text{ ng mL}^{-1}$  for tebufenozide in wines elaborated in La Rioja. In Galicia, their detection frequencies varied between 1 % and 7 %, with maximum concentrations lower than, or equal to,  $10 \text{ ng mL}^{-1}$ .

In general, the upper residues of individual pesticides in wine samples remained below 10 % of its MRLs in vinification grapes, Table 1. The only exceptions corresponded to metalaxyl, with ratios around 11 % in wines from both geographic areas, and carbendazim, with maximum values of 32 % and 50 % for Galician and Rioja wines, respectively, Table 1. Carbendazim was reported at values 3 times higher than its MRL in vinification grapes ( $500 \text{ ng g}^{-1}$ ) in wines from other geographic regions (Santana-Mayor et al., 2023). Above findings point out to a high PF of this compound from grapes to wine. Iprodione was noticed in a small percentage of Galician wines (3 % of samples). This compound was authorized until 2017, thereafter, its MRL in vinification grapes was set at  $10 \text{ ng g}^{-1}$ . Four wines contained residues of iprodione in the range from  $19 \text{ ng mL}^{-1}$  to  $70 \text{ ng mL}^{-1}$ . Attending to the legislation in force during their date of elaboration, these samples should not be commercialized. Likely, grape growers have applied their stock of this fungicide

**Table 1**Maximum concentrations, detection frequencies (%) and ratio between maximum reported concentrations in wine ( $\text{ng mL}^{-1}$ ) and MRLs in vinification grapes ( $\text{ng g}^{-1}$ ).

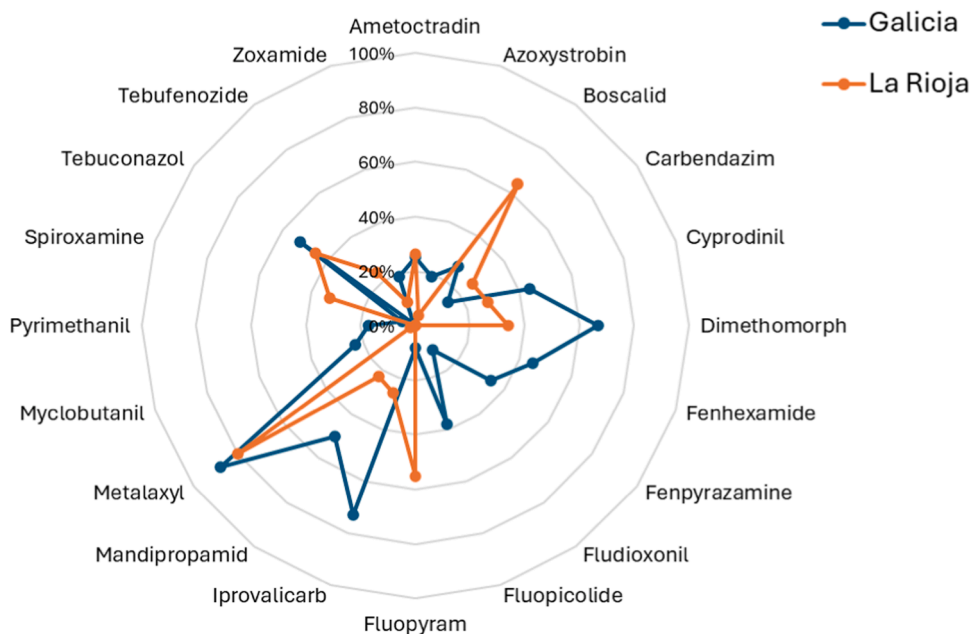
Compound	GALICIA			LA RIOJA		
	Max ( $\text{ng mL}^{-1}$ )	Frequency	Max/ MRL	Max ( $\text{ng mL}^{-1}$ )	Frequency	Max/ MRL
Acetamiprid	3.5	8 %	0.7 %	21.45	3 %	4.3 %
Ametoctradin	70.4	25 %	1.2 %	33.9	26 %	0.6 %
Azoxystrobin	31.9	19 %	1.1 %	22.8	4 %	0.8 %
Benalaxyl	1.6	2 %	0.5 %	4.1	23 %	1.4 %
Boscalid	41.7	27 %	0.8 %	73.5	64 %	1.5 %
Carbendazim	157.7	15 %	31.5 %	248.6	26 %	49.7 %
Chlorantraniliprole	3.6	1 %	0.4 %			
Cyflufenamid				1	1 %	0.5 %
Cyprodinil	73.0	44 %	2.4 %	82.1	28 %	2.7 %
Dimethomorph	86.1	67 %	2.9 %	65.9	34 %	2.2 %
Fenhexamid	185.0	45 %	1.2 %			
Fenpyrazamine	111.0	34 %	3.7 %			
Fludioxonil	89.0	11 %	2.2 %			
Fluopicolide	39.0	38 %	2.0 %			
Fluopyram	11.0	8 %	0.7 %	31.0	55 %	2.1 %
Iprodione	70.0	3 %	700 %			
Iprovalicarb	136.4	73 %	6.8 %	55.0	26 %	2.8 %
Isofetamid	122.4	9 %	3.1 %			
Kresoxim-methyl	9.4	1 %	0.6 %			
Mandipropamid	72.9	50 %	3.6 %	15.0	23 %	0.8 %
Metalaxyl	118.0	88 %	11.8 %	108.0	80 %	10.8 %
Methoxyfenozide	5.9	2 %	0.6 %	6	2 %	0.6 %
Metrafenone	4.2	5 %	0.1 %	4.8	8 %	0.1 %
Myclobutanil	14.2	23 %	0.9 %	2.9	2 %	0.3 %
Pyrimethanil	158.0	17 %	3.2 %			
Pyriofenone	4.2	5 %	0.5 %	23.5	8 %	2.9 %
Spiroxamine	12.0	5 %	2.4 %	7.8	33 %	1.6 %
Tebuconazol	39.6	52 %	4.0 %	17.5	45 %	1.8 %
Tebuconazole	10.1	1 %	0.3 %	101.2	24 %	2.5 %
Tetraconazole	1.3	1 %	1.9 %	3.3	3 %	4.7 %
Thiophanate methyl	37.9	4 %	1.3 %			
Trifloxystrobin	1.8	1 %	0.1 %			
Zoxamide	19.0	19 %	0.4 %	7	9 %	0.1 %

to vines. Before the phase-out of the fungicide, 40 % of wines produced in the period from 2012 to 2017 contained residues of iprodione (Santana-Mayor et al., 2020).

Fig. 2 illustrates the distribution of pesticides (compounds with maximum residues higher than  $10 \text{ ng mL}^{-1}$  and detection frequencies above 10 % in at least one of the two regions) attending to the

geographic origin of wine samples. Boscalid and fluopyram were more often detected in wines elaborated in La Rioja versus those produced in Galicia. The opposite trend was noticed for cyprodinil, dimethomorph, fenhexamid, fenpyrazamine, fluopicolide and iprovalicarb.

Fig. 3 displays detection frequencies per sub-zone, or D.O. The plot also includes the median of their concentrations during the four years



**Fig. 2.** Detection frequencies of compounds found in at least one wine from both geographic regions at levels above  $10 \text{ ng mL}^{-1}$ , and detection frequencies higher than 10 % in one or both regions.

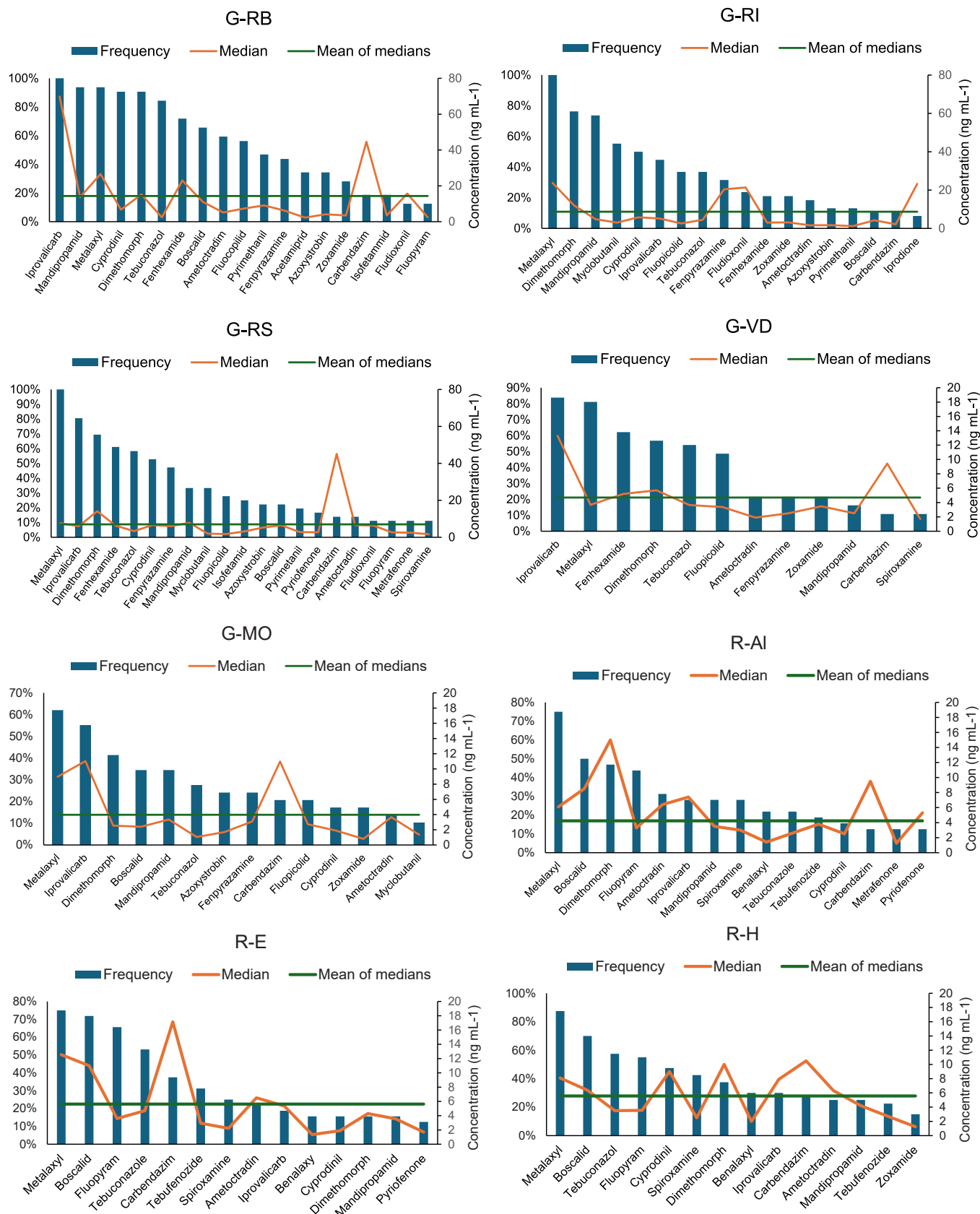


Fig. 3. Detection frequencies (%), median and mean of medians (ng mL<sup>-1</sup>) for compounds detected in more than 10 % of samples from each D.O., or sub-zone.

sampling campaign and the mean of medians, within each sub-zone or D.O. Metalaxyl showed the highest detection frequency in 6 out of 8 sub-zones and iprovalicarb in the remaining two. Median concentrations included in Fig. 3 are related to application rates of compounds to vines, and with the risk of being transferred from grapes to wine. Metalaxyl, dimethomorph and iprovalicarb combine high detection frequencies with median residues above the overall mean in wines from most of the sub-zones. Carbendazim showed a moderate detection frequency, but high median concentrations, again suggesting a significant risk of being transferred to wine. Other fungicides applied to vineyards close to harvest time, displayed also median concentrations above the mean in each sub-zone or D.O. This is the case of fenpyrazamine, fenhexamide and cyprodinil. The three species are used for the prevention of botrytis, a disease whose incidence increases due to rain events close to harvest time.

Fig. 4 shows the normalized cumulative concentrations of fungicides, attending to the infection they are designed to treat in vineyards. The lowest contribution to total residues in wines from La Rioja (R-AL, R-H, R-E) and the D.O. G-MO corresponded to species used in the prevention of botrytis, followed by those used against oidium and mildew. The normalized residues of anti-botrytis agents increased in the rest of D.O., overpassing that of anti-oidium fungicides. Finally, in G-RI main residues of fungicides corresponded to anti-botrytis agents.

### 3.3. Inter-year trends

Supranational policies pursue a significant reduction in the application rates of pesticides to crops to protect the environment, the health of consumers, and the fertility of agricultural soils. Inter-year variations in the residues of pesticides in wine, independently of geographic patterns, were investigated using wines from G-RI, which was identified as the second most problematic zone attending to the content of pesticides, Figs. 1 and 3. In addition to samples compiled in Table S4, a second set of 168 wines from this D.O. were processed. Residues found in these samples are summarized in Table S5. Combining data from Tables S4 and S5, we assessed the evolution of residues in wines produced between 2019 and 2022. The minimum and maximum number of samples per year was 29 (year 2022) and 85 (year 2020). From year 2023, only 8 wines were available; thus, it was excluded from the study.

The average, median an inter-quartile values of total pesticide residues reached a maximum in wines elaborated in 2020, with a progressive reduction in consecutive years, Fig. 5A. To assess the impact of climate conditions in this trend, we recovered the mean values of rainfall ( $L m^{-2}$ ) and the number of rain days during May and June (the most problematic months as regards fungal infections affecting vineyards), from three meteorological stations in the area, between 2019 and 2022 (Meteogalicia- Meteorological historical data, n.d.). Climate conditions were more favourable to fungi attacks in the campaigns of 2021 and

2022 than in years 2019 and 2020, Fig. 5B. Thus, data depicted in Fig. 5A and B point out to a slight reduction in the use of fungicides by grape growers in G-RI, a fact reflected in lower residues in the commercialized wines. Obviously, this trend requires confirmation through the systematic surveillance of wines from coming vintages, including also data for the most problematic D.O., in particular those elaborated in G-RB.

Fig. 5C summarizes the detection frequencies of myclobutanil, thiophanate methyl and carbendazim (the main degradation product of thiophanate methyl) in wines from D.O. G-RI. Commercialization of myclobutanil and thiophanate methyl was not allowed after May 2021 and October 2020, respectively (European pesticide database of MRLs in food commodities, n.d.). As observed in Fig. 5C, their detection frequencies decreased for wines produced in the campaign of 2022. Another compound whose detection frequency was reduced in wines elaborated in 2022 was dimethomorph. Despite dimethomorph is still an authorized fungicide, its application is controversial due to environmental persistence in agricultural soils (Pérez-Mayán et al., 2020) and high detection frequencies in wines, Fig. 3. Eventually, dimethomorph has been considered a candidate for substitution (PPDB, Pesticides Properties Database, n.d.), and all formulations including this molecule were not authorized after November 2024. Data in Fig. 5C suggests that farmers in this region have anticipated the phase-out of dimethomorph. In fact, this fungicide was hardly found (above  $10 ng mL^{-1}$ ) in any wine produced in the campaign of 2023, Tables S3 and S4.

### 3.4. Enantiomeric analysis of chiral compounds

Very often, the activity of chiral pesticides differs significantly between enantiomers (Carrão et al., 2020). Despite this fact, most of the chiral fungicides detected in wines from Galicia and La Rioja are only commercialized as racemates. This is the case of myclobutanil, tebuconazole, and mandipropamid. Metalaxyl is available under both formulations: the racemate and the active isomer (R-form, usually termed as metalaxyl-M). In this case, the S-form has not antifungal activity. Fig. 6 shows the distribution of their EF values in wines containing concentrations above  $10 ng mL^{-1}$ . EFs of tebuconazole and mandipropamid ( $n = 15$  samples) presented a low dispersion, with average values close to 0.5. Thus, their transfer from grapes to wine was not enantioselective. Wines were slightly enriched in the less retained enantiomer of myclobutanil, leading to an average EF of 0.58 ( $n = 14$  samples). Finally, the EFs of metalaxyl varied in the range of 0.1–0.7 ( $n = 181$  wines). From these values two conclusions are drawn. First, although racemic metalaxyl is a candidate for substitution (PPDB, Pesticides Properties Database, n.d.), this formulation is preferred by grape growers to metalaxyl-M, given the average EF close to 0.5, Fig. 6. Second, a relevant percentage of wines showed EFs above 0.5 (the EF of metalaxyl was higher than 0.58 in 25 % of wines) meaning they contained higher residues of metalaxyl-S than of metalaxyl-R, Fig. 6. Given that, total concentrations of metalaxyl obtained using achiral and chiral LC columns were in excellent agreement (see Fig. S4), these figures (EF values above 0.5) cannot be considered as artifacts due to different matrix effects for both forms. Furthermore, the analysis of wines elaborated from grapes containing only the R-form confirmed the absence of racemization reactions during the vinification process. Thus, we concluded that, under certain vinification conditions, the PF of S-metalaxyl is higher than that of R form (Metalaxyl-M).

### 3.5. Toxicological considerations

Table 2 summarizes the normalized ratio between the estimated exposure and the acceptable daily intake (ADI) for compounds found in wines at concentrations above  $10 ng mL^{-1}$ . ADI values ( $mg Kg^{-1} day^{-1}$ ) for chronic exposure were obtained from the European Food Safety Agency (EFSA) (European pesticide database of MRLs in food commodities, n.d.). Values for body mass and wine consumption were set at

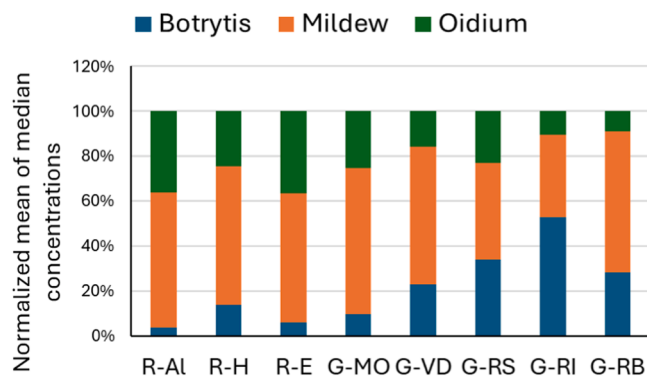
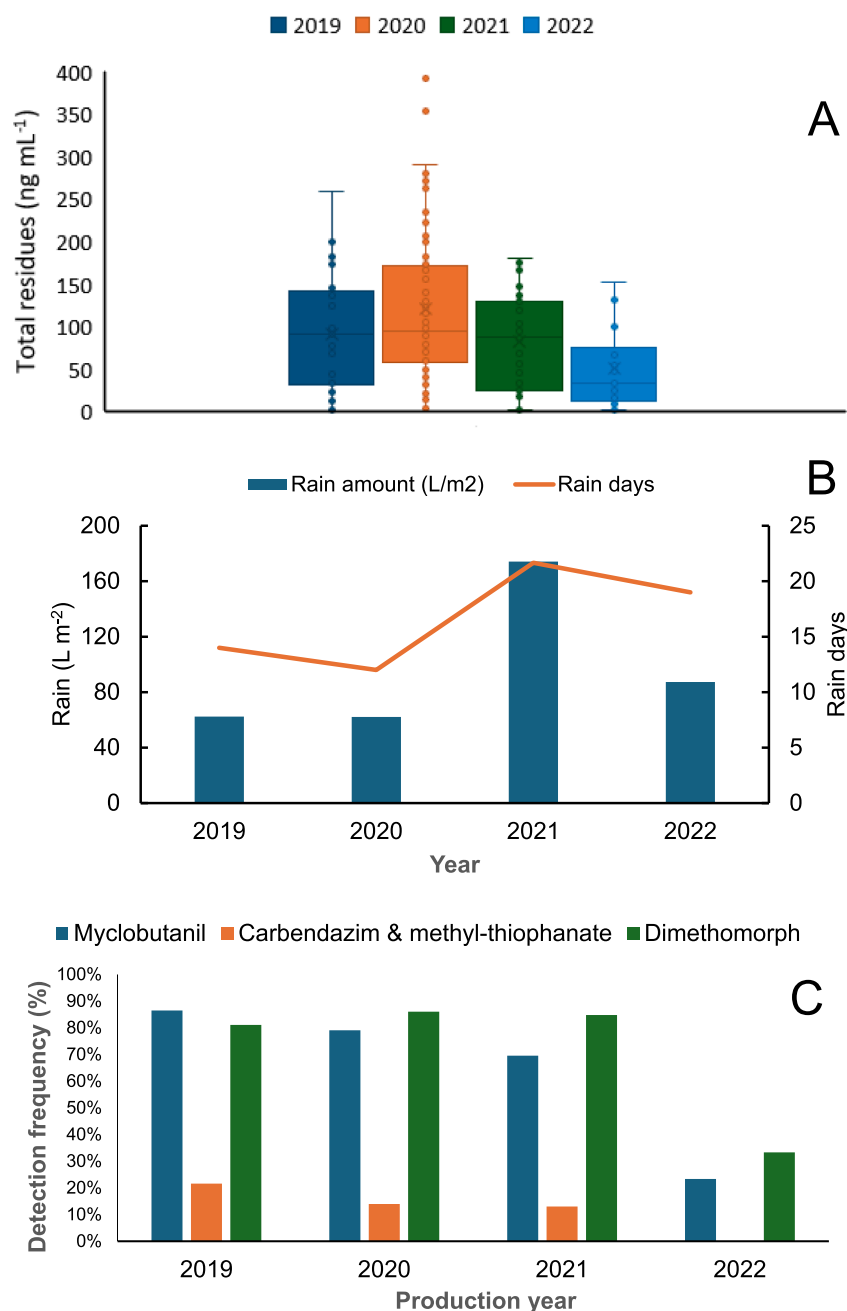


Fig. 4. Normalized contribution of fungicides employed in the treatment of the three major diseases affecting vines to total residues in wines from different sub-zones or D.O.



**Fig. 5.** Summary of data for wines elaborated in Galician D.O. Ribeiro (G-RI) in consecutive years. A, Box-Whisker plots of total residues as function of production year. B, Total rain and number of rainy days in the four considered years. C, detection frequencies of fungicides no longer authorized (myclobutanil, thiophanate-methyl and its degradation product), or candidates to substitution (dimethomorph).

70 Kg and 70 mL, respectively (Santana-Mayor et al., 2020). Normalized ratios below 1 % were noticed, except in case of carbendazim (ratio 1.2 %), Table 2. Among currently authorized compounds, iprovalicarb showed the highest normalized ratio between estimated consumption and acceptable intake (0.91 %). From values in Table 2, considering the simultaneous presence of the five most often detected fungicides (metaxyl, iprovalicarb, dimethomorph, boscalid and spiroxamine, Fig. 2), at their maximum concentrations in the same wine, the cumulative risk accounts for 1.46 % of the ADI. Even assuming the larger wine volume consumption reported by the OIV (250 mL of wine for a 70 Kg man), the cumulative ratio between exposure and ADI stays below 6 %.

Despite above comments, it is important noting that current knowledge on the chronic effects and human toxicity of fungicides is limited. In this vein, in-vitro studies have reflected that some fungicides

(i.e. cyprodinil, boscalid and fenhexamide) are active as endocrine disruptors, affecting estrogenic receptors linked to the survival of breast cancer model cells (Go et al., 2021; Jabłońska-Trypuć et al., 2023). Consequently, the MRLs of certain compounds and their acceptable ADI values might be revised, and probably reduced, in the future.

#### 4. Conclusions

Total concentrations and distribution of individual pesticides showed relevant differences between wines elaborated in La Rioja and Galicia. Within the second geographic area, the residues of pesticides increased from D.O. located in the interior to those closer to the Atlantic coast. Compounds employed in the prevention and treatment of mildew infections were prevalent in all sub-zones, but G-RI. In this area, the

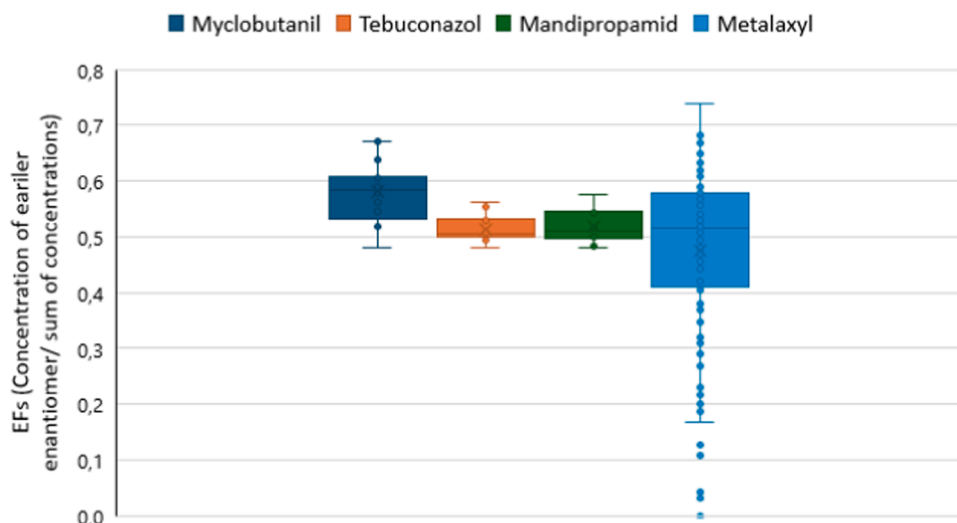


Fig. 6. Enantiomeric fractions (EFs) of chiral fungicides in wine samples.

Table 2

Assessment of toxicological risks derived from chronic exposure to pesticides based on acceptable daily intake (ADI) doses and average wine consumption data (70 mL day<sup>-1</sup>) for a 70 Kg adult.

Compound	Maximum residue (ng mL <sup>-1</sup> )	ADI (mg Kg <sup>-1</sup> body weight day <sup>-1</sup> )	Estimated daily intake (mg Kg <sup>-1</sup> )	Ratio Daily intake vs. ADI (%)
Acetamidrid	21.5	0.025	0.0000215	0.086 %
Ametotradin	70.4	10	0.0000704	0.001 %
Azoxystrobin	31.9	0.2	0.0000319	0.02 %
Boscalid	73.5	0.04	0.0000735	0.18 %
Carbendazim	248.5	0.02	0.0002485	1.2 %
Cyprodinil	82.1	0.03	0.0000821	0.27 %
Dimethomorph	86.1	0.05	0.0000861	0.17 %
Fenhexamid	185	0.2	0.000185	0.09 %
Fenpyrazamine	111	0.13	0.000111	0.09 %
Fludioxonil	89	0.37	0.000089	0.02 %
Fluopicolide	39	0.08	0.000039	0.05 %
Fluopyram	31	0.012	0.000031	0.26 %
Iprodione	70	0.02	0.00007	0.35 %
Iprovalicarb	136.4	0.015	0.0001364	0.91 %
Isofetamid	122.4	0.02	0.0001224	0.61 %
Mandipropamid	72.9	0.15	0.0000729	0.05 %
Metalaxyl	118	0.08	0.000118	0.15 %
Myclobutanil	14.2	0.025	0.0000142	0.06 %
Pyrimethanil	158	0.17	0.000158	0.09 %
Pyriofenone	23.5	0.07	0.0000235	0.03 %
Spiroxamine	12	0.025	0.000012	0.05 %
Tebuconazol	39.6	0.03	0.0000396	0.13 %
Tebufenozide	101.2	0.02	0.0001012	0.51 %
Thiophanate methyl	37.9	0.02	0.0000379	0.19 %
Zoxamide	19	0.5	0.000019	0.004 %

most important contribution to residues in wine corresponded fungicides used to prevent botrytis infections. Likely, the environmental conditions existing in the different production areas are the driven force determining the selection of fungicides applied to vines and thus, their presence in wine. Irrespectively of the considered sub-zone, or D.O., levels of pesticides measured in wine remained far below their MRLs in vinification grapes; moreover, risk assessment for a moderate, continuous consumption of wine, did not reflect relevant health hazards. The future phase-out of racemic metalaxyl, in favour of metalaxyl-M, will contribute to reduce the levels of the fungicide most often noticed in wine. The statement is based on the lower concentrations of metalaxyl in formulations containing only the active enantiomer, added to a

potentially lower PF of R-metalaxyl versus that of the S-form.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

Economic support from projects TED2021–129962B-C42, funded by the Spanish Ministry of Science and Innovation, through the UE Next Generation program; ED431C2021/06, from Xunta de Galicia, co-financed the EU FEDER program; and AC2021E-02, co-supported by European Agricultural Fund for Rural Development (FEADER), Xunta de Galicia and the Spanish Ministry of Agriculture, Fish and Food, is acknowledged.

#### Submission declaration

Authors declare that manuscript ref. JFCA-D-24–02967 is not under revision elsewhere. The content of the study is new, and it has not been published anywhere. Furthermore, all authors agree to submit the revision of the work to Journal of Food Composition and Analysis.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2025.107525](https://doi.org/10.1016/j.jfca.2025.107525).

#### Data availability

Data will be made available on request.

#### References

- Carrão, D.B., Perovani, I.S., de Albuquerque, N.C.P., de Oliveira, A.R.M., 2020. Enantioseparation of pesticides: a critical review. *TrAC - Trends in Analytical Chemistry* (Vol. 122, p. 115719). Elsevier B.V <https://doi.org/10.1016/j.trac.2019.115719>.
- Castro, G., Pérez-Mayán, L., Rodríguez-Cabo, T., Rodríguez, I., Ramil, M., Cela, R., 2018. Multianalyte, high-throughput liquid chromatography tandem mass spectrometry method for the sensitive determination of fungicides and insecticides in wine. *Anal. Bioanal. Chem.* 410 (3), 1139–1150. <https://doi.org/10.1007/s00216-017-0724-9>.

- Čuš, F., Česnik, H.B., Bolta, Š.V., 2022. Pesticide residues, copper and biogenic amines in conventional and organic wines. *Food Control* 132, 108534. <https://doi.org/10.1016/j.foodcont.2021.108534>.
- Duca, G., Sturza, R., Siretanu, L., 2012. Estimation of organic pesticide residues in wines of Moldova. *Clean. - Soil, Air, Water* 40 (6), 661–666. <https://doi.org/10.1002/clen.201100081>.
- Duley, G., Ceci, A.T., Longo, E., Boselli, E., 2023. Oenological potential of wines produced from disease-resistant grape cultivars. *Comprehensive Reviews in Food Science and Food Safety*. John Wiley and Sons Inc, pp. 2591–2610. Vol. 22, Issue 4, <https://doi.org/10.1111/1541-4337.13155>.
- Dumitriu Gabur, G.D., Gabur, I., Cucolea, E.I., Costache, T., Rambu, D., Cotea, V.V., Teodosiu, C., 2022. Investigating six common pesticides residues and dietary risk assessment of Romanian wine varieties. *Foods* 11 (15), 2225. <https://doi.org/10.3390/foods11152225>.
- European pesticide database of MRLs in food commodities. (n.d.). Retrieved June 3, 2024, from <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/products/details/38>.
- Go, R.E., Kim, C.W., Lee, S.M., Lee, H.K., Choi, K.C., 2021. Fenhexamid induces cancer growth and survival via estrogen receptor-dependent and PI3K-dependent pathways in breast cancer models. *Food Chem. Toxicol.* 149. <https://doi.org/10.1016/j.fct.2021.112000>.
- Guo, L., Li, R., Chen, W., Dong, F., Zheng, Y., Li, Y., 2023. The interaction effects of pesticides with *Saccharomyces cerevisiae* and their fate during wine-making process. *Chemosphere* 328, 138577. <https://doi.org/10.1016/j.chemosphere.2023.138577>.
- Herrero-Hernández, E., Rodríguez-Cruz, M.S., Pose-Juan, E., Sánchez-González, S., Andrades, M.S., Sánchez-Martín, M.J., 2017. Seasonal distribution of herbicide and insecticide residues in the water resources of the vineyard region of La Rioja (Spain). *Sci. Total Environ.* 609, 161–171. <https://doi.org/10.1016/j.scitotenv.2017.07.113>.
- Jabłońska-Trypuć, A., Wydro, U., Wolejko, E., Makula, M., Krętowski, R., Naumowicz, M., Sokolowska, G., Serra-Majem, L., Cechowska-Pasko, M., Łozowicka, B., Kaczyński, P., Wiater, J., 2023. Selected Fungicides as Potential EDC Estrogenic Micropollutants in the Environment. *Molecules* 28 (21). <https://doi.org/10.3390/molecules28217437>.
- Liu, C., Chen, R., Liu, F., Gao, Z., Li, X., Wang, Y., Wang, S., Li, Y., 2023. Distribution pattern, removal effect, transfer behavior of ten pesticides and one metabolite during the processing of grapes. *Food Res. Int.* 164, 112398. <https://doi.org/10.1016/j.foodres.2022.112398>.
- Meteogalicia- Meteorological historical data. (n.d.). Retrieved June 15, 2024, from [https://www.meteogalicia.gal/observacion/estacions/estacions.action?request\\_locale=gl](https://www.meteogalicia.gal/observacion/estacions/estacions.action?request_locale=gl).
- Noguerol-Pato, R., Siero-Sampedro, T., González-Barreiro, C., Cancho-Grande, B., Simal-Gándara, J., 2015. Evaluation of the effect of fenhexamid and mepanipyrin in the volatile composition of Tempranillo and Graciano wines. *Food Res. Int.* 71, 108–117. <https://doi.org/10.1016/j.foodres.2015.02.025>.
- Pappalardo, S.E., Ferrarese, F., Pizarro, P.M., Loddo, D., De Marchi, M., 2022. Pesticide contamination of freshwater ecosystems: mapping vulnerable areas and mitigation scenarios in the Prosecco DOCG wine production area. *Environ. Res. Lett.* 17 (10), 104037. <https://doi.org/10.1088/1748-9326/ac939e>.
- Pazzirota, T., Martin, L., Mezcuca, M., Ferrer, C., Fernandez-Alba, A.R., 2013. Processing factor for a selected group of pesticides in a wine-making process: distribution of pesticides during grape processing. *Food Addit. Contam. - Part A* 30 (10), 1752–1760. <https://doi.org/10.1080/19440049.2013.815806>.
- Pelajić, M., Pelajić, I., Pavlović, D.M., Čepo, D.V., 2019. GC-MS modified QuEChERS method for multiresidue pesticide determination in red wine. *Croat. Chem. Acta* 92 (3), 419–428. <https://doi.org/10.5562/cca3552>.
- Pérez-Mayán, L., Ramil, M., Cela, R., Rodríguez, I., 2020. Multiresidue procedure to assess the occurrence and dissipation of fungicides and insecticides in vineyard soils from Northwest Spain. *Chemosphere* 261, 127696. <https://doi.org/10.1016/j.chemosphere.2020.127696>.
- Pose-Juan, E., Sánchez-Martín, M.J., Andrades, M.S., Rodríguez-Cruz, M.S., Herrero-Hernández, E., 2015. Pesticide residues in vineyard soils from Spain: Spatial and temporal distributions. *Sci. Total Environ.* 514, 351–358. <https://doi.org/10.1016/j.scitotenv.2015.01.076>.
- PPDB, Pesticides Properties Database. (n.d.). Retrieved September 16, 2024, from <https://sitem.herts.ac.uk/aeru/ppdb/>.
- Santana-Mayor, Á., Rodríguez-Ramos, R., Herrera-Herrera, A.V., Conde-González, J.E., Socas-Rodríguez, B., 2023. Food safety assessment of wines commercialised in the Canary Islands by monitoring of pesticide residues from 2017 to 2019. *Food Control* 153, 109957. <https://doi.org/10.1016/j.foodcont.2023.109957>.
- Santana-Mayor, Á., Rodríguez-Ramos, R., Socas-Rodríguez, B., Díaz-Romero, C., Rodríguez-Delgado, M.Á., 2020. Comparison of pesticide residue levels in red wines from Canary Islands, Iberian Peninsula, and Cape Verde. *Foods* 9 (11), 1555. <https://doi.org/10.3390/foods9111555>.
- Teyssiere, R., Barron, E., Baldi, I., Bedos, C., Chazeaubeny, A., Le Menach, K., Roudil, A., Budzinski, H., Delva, F., 2023. Pesticide exposure of residents living in wine regions: protocol and first results of the pestprev study. *Int. J. Environ. Res. Public Health* 20 (5), 3882. <https://doi.org/10.3390/ijerph20053882>.
- Walorczyk, S., Drodziński, D., Gnusowski, B., 2011. Multiresidue determination of 160 pesticides in wines employing mixed-mode dispersive-solid phase extraction and gas chromatography-tandem mass spectrometry. *Talanta* 85 (4), 1856–1870. <https://doi.org/10.1016/j.talanta.2011.07.029>.
- Zhang, H., Li, Y., Zhu, J., Li, H., Li, D., Liu, Z., Sun, X., Wang, B., Wang, Q., Gao, Y., 2019. Disposable Pipette Extraction (DPX) Coupled with Liquid Chromatography–Tandem Mass Spectrometry for the Simultaneous Determination of Pesticide Residues in Wine Samples. *Food Anal. Methods* 12 (10), 2262–2272. <https://doi.org/10.1007/s12161-019-01569-6>.
- Zincke, F., Arno, K., Scholz, R., Michalski, B., & Tietz, E. (2024). European database of processing factors for pesticides residues in food. Version v3. Zenodo Database. <https://zenodo.org/records/10984823>.