Multiresidue procedure to assess the occurrence and dissipation of fungicides and insecticides in vineyard soils from Northwest Spain

L. Pérez-Mayán, M. Ramil, R. Cela, I. Rodríguez*

Department of Analytical Chemistry, Nutrition and Food Sciences. Research Institute on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, 15782-Santiago de Compostela, Spain.

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Abstract

The presence of fungicide and insecticide residues in wine has been largely investigated. However, few studies have addressed the persistence of these compounds in vineyard soils. In this research, we investigate the residues of a relevant number of fungicides and insecticides in vineyard soils, obtained in the Northwest of Spain, at the beginning of each agriculture campaign. Moreover, the dissipation of species showing high concentrations were monitored during the non-vegetative period of vines, in order to understand their soil evolution between application campaigns. To this end, a multiresidue analytical procedure based on pressurized liquid extraction (PLE) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) determination was first optimized. Under final working conditions, absolute recoveries in the range from 70 to 130% were achieved for 44 out of 51 selected compounds. The method LOQs remained at the low ng g^{-1} level (0.2 to 13 ng g^{-1}) with a linear response range up to 500 ng g^{-1}. Analysis of vineyard soils, collected during a 2-year period, from a geographic area with a high incidence of fungal diseases, demonstrated the presence of relevant concentrations of several fungicides and the insecticide imidacloprid (IMI) in this compartment. Most compounds detected at the end of the application season remained in soil at the beginning of the next year campaign. Among them, six fungicides (dimethomorph, boscalid, myclobutanil, penconazole, pyraclostrobin and pyrimethanil) and IMI showed average dissipation efficiencies below 50%, so they pose a potential to accumulate in this kind of soils.

Keywords: fungicides; insecticides; vineyard soil; occurrence; liquid chromatography-mass spectrometry

*corresponding author
e-mail: isaac.rodriguez@usc.es

1. Introduction

Presence of pesticide residues in arable fields has been recognized as a concerning problem in intensive agriculture areas (Silva et al., 2019). Vineyards are regarded as the permanent crops receiving the largest amount of organic fungicides per hectare (Endure, 2010). The application rates of these compounds are modulated by climate conditions, being most frequent in template and high humidity areas, as it is the case of Galicia, in the Northwest of Spain. This region produces around 40 million Kg of vinification grapes per year and gathers several production areas (Designations of Origin) with different features as regards climate characteristics. Existence of residues from organic pesticides in vineyard soils in this area was highlighted more than 10 years ago (Bérmudez-Couso et al., 2007); however, this pioneer study addressed the determination of a small number of active ingredients. Moreover, since that date new active ingredients have been marketed. More recent data, obtained in other production areas in Spain, reflect concerning soil pollution levels despite having more favorable climate conditions, which should turn in lower fungicide application rates in vineyards (Pose-Juan et al. 2015; Vallejo et al., 2019).

The potential accumulation of organic pesticides in vineyard soils does not only represent an environmental problem (species might be filtered to ground and/or surface water and affect wildlife in this areas, i.e. invertebrates placed at the lowest levels of food web) (Herrero-Hernández et al., 2016; Schreck et al. 2008a; Zhang and Zhou, 2019), but also impacts the enzymatic activity and the microbial community of soil (Marinozzi et al., 2013; Wang et al., 2017). Nowadays, it is accepted that this community affects also to yeast involved in must fermentation and, thus, to the range of volatile organic compounds generated during wine production (Belda et al., 2017). Another serious problem associated to the presence of low fungicide and insecticide residues in vineyard soils is the development of resistant fungal strains (Leroux et al., 2000; Walker et al., 2017) limiting the efficiency of the applied treatments.

Studies under controlled laboratory conditions have been carried out in order to obtain an estimation of half-lives for most marketed pesticides (Marín-Benito et al., 2012; PPDB: Pesticide properties database, 2020). However, such conditions do not necessarily mimic those existing in vineyards, containing variable levels of organic matter, exposed to changing environmental conditions, suffering regular soil movements and treated, often at short intervals, with commercial formulations containing pooled mixtures of organic pesticides in combination with copper salts and complexes of other metal ions, such as zinc and manganese. Consequently, there is a need to investigate the levels of pesticide (fungicides and insecticides) residues in vineyard soils and to understand their dissipation during the non-vegetative period of vines. So far, the number of studies dealing with this topic is still limited (Bermúdez-Couso et al., 2007; Pose-Juan et al., 2015; Schreck et al. 2008b; Vallejoet al., 2019); moreover, some of them cover a reduced number of compounds. Thus, there is also a demand of validated methodologies covering the largest possible set of pesticides.

Liquid chromatography (LC) tandem mass spectrometry (MS/MS) is the most resorted technique for the selective determination of pesticide residues. State-of-art LC-ESI-MS/MS instruments provide limits of quantification (LOQs) scarcely affected by the number of transitions simultaneously recorded. Thus, total compounds which can be covered in the same analysis is mainly controlled by the efficiency of the sample preparation process. The most often followed approaches range from techniques using mild conditions, such as solid-liquid extraction (Pose-Juan et al., 2015), QuEChERS (Silva et al., 2019) and matrix solid-phase dispersion (Zhao et al., 2018), to those supplying relevant amounts of energy to the solvent-sample mixture, allowing to accelerate the kinetics and the thermodynamic of the extraction process. Apart from Soxhlet, ultrasound assisted extraction (Tadeo et al., 2012), use of microwave energy (Merdassa et al., 2013) and pressurized solvents (Barbieri et al. 2019; Masiá et al., 2015) have been successfully employed for the extraction of pesticides from agriculture soils and similar matrices, such as sediments. Among them, pressurized liquid extraction (PLE) offers a favorable balance among solvent consumption, extraction efficiency and automation capabilities.

The aim of this study was to obtain an overview of the presence and the persistence of organic pesticides, particularly fungicides and insecticides, in vineyard soils obtained from a geographic region with a high incidence of fungal diseases in Galicia, Northwest of Spain. Soils in this area are characterized by acidic pHs, and relatively high organic carbon contents, which might affect to the dissipation rate of pesticide residues. To this end, a multi-analyte procedure, covering the largest possible set of compounds and based on PLE extraction and LC-ESI-MS/MS was first optimized. Thereafter, the method was applied to the analysis of soil samples obtained from several vineyards (belonging to three different Designations of Origin) at different dates, in order to draw some conclusions regarding detection frequencies, residues in soil at the beginning of each campaign, and time-course trends of compounds found at higher concentrations during the non-vegetative period of vines.

2. Material and methods

2.1. Chemicals and solvents

Standards of fungicides and insecticides, authorized for treatment of vineyards, were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Some known transformation products, such as carbendazim (CAR) and two suspected soil metabolites of metalaxyl (MET), corresponding to hydrolysis of ester group (CGA 62826) and further oxidation to a benzoic acid derivative (CGA 108906), were purchased from the same supplier. The list of internal surrogates (ISs), employed to compensate for non-quantitative extraction yields and/or changes in the efficiency of electrospray ionization, includes the following compounds: carbendazim-d_3 (CAR-d_3), cyprodinil-d_5 (CYP-d_5), pyrimethanil-d_5 (PYR-d_5), imidacloprid-d_4 (IMI-d_4), metalaxyl $^{13}C_6$ (MET-$^{13}C_6$), thiamethoxam-d_4 (THM-d_4), clothianidin-d_3 (CLO-d_3), dimethomorph-d_6 (DIM-d_6) and fenhexamide-d_3 (FENH-d_3). All of them were obtained from Sigma-Aldrich. Selection of ISs was done attending to availability, non-quantitative extraction yields and/or significant matrix effects (MEs) observed for native species during method development, and application rates of native compounds inferred from residues in commercial wines elaborated in the same geographic area (Castro et al., 2018). The list of compounds involved in the study is provided as supplementary information, Table S1.

Individual solutions of each compound and the ISs were prepared in methanol (MeOH). Further dilutions were made in the same solvent. Calibration standards, concentration range 1-200 ng mL^{-1}, were prepared in MeOH: acetonitrile (ACN): formic acid (FA), 65:30:5. A mixture of ISs (1 μg mL^{-1}) was made in MeOH. The concentration of ISs in calibration standards and final extracts obtained from soil samples was 25 ng mL^{-1}.

MeOH and ACN, both LC-MS grade purity, and FA (98 %) were supplied by Merck (Darmstadt, Germany). Ultra-pure deionized water (18.2 MΩ cm^{-1}) was obtained from a Milli-Q Gradient A-10 system (Millipore, Billerica, MA, USA).

2.2. Samples and sample preparation

Sample preparation conditions were optimized using soil from an experimental vineyard managed by a regional research institution (EVEGA, Galicia). Samples from 9 different vineyards, located in three different Designations of Origin in Galicia, were taken (or provided by owners) between March 2017 and March 2019. Fig. S1 shows a map with sampling points. A sampling area (c.a. 4 square meters) was selected in each vineyard. Top soil (0-15 cm depth) was taken in 5 random points, within selected areas, to prepare a composite sample with a size around 2 Kg from each vineyard. Composite samples were transported to the laboratory in plastic bags, at room temperature, the same day of sampling. During the sampling period (in some points 2 years) no restrictions were imposed to mechanic soil movements (tilling) by vineyard owners.

Course materials (large stones, grass and pieces of vine canes) were manually removed and the remaining sample was freeze-dried within a period of 48 h after reception. Lyophilized soils were sieved and the fraction below 0.2 mm was selected for analysis. Before extraction, sieved samples were stored at room temperature in amber glass vessels. The organic carbon (OC) content in the freeze-dried soils varied between 1.5% and 8.3 %, depending on the vineyard. Main characteristics of soil at each sampling points, including pH, composition and texture are summarized in Table S2. In all cases, soils showed acid properties (pH values were comprised between 3.8 and 5.7) and relatively high OC levels (up to 8.3%). Their textures corresponded to sandy loam (7 vineyards) and loam (2 vineyards) soils.

Extraction (2 g of soil, previously lyophilized and sieved) was carried out using an ASE200 pressurized liquid extractor (Dionex, Sunnyvale, CA, USA) equipped with 11 mL cells. Spiked samples, employed during optimization of extraction conditions, were prepared by mixing an accurately weighted mass of soil with a standard solution of compounds prepared in MeOH (c.a. 0.5 mL of solvent per g of sample). The mixture was homogenized and left in the hood for 48 hours to allow the evaporation of the solvent. Thereafter, the spiked soils, employed in the earlier stages of this research, were aged for one week before extraction. Due to stability problems detected for Cymoxanil (CYM), the aging period of spiked soils was reduced to 12 h (overnight) in further experiments. PLE cells were packed using a layer of diatomaceous earth, followed by 2 g of soil spiked with the mixture of ISs. The remaining free volume was filled using again diatomaceous earth. Cellulose and glass fibre filters were placed at the bottom of each PLE cell; moreover, a cellulose filter was introduced on top.

Optimization of extraction conditions was performed with the help of experimental factorial designs, and following an univariant approach. Under final conditions, PLE cells were extracted twice. In first term, extraction was performed using a mixture of MeOH:ACN (70:30) at 80 ºC in a single cycle of 5 min. Purge time was 1 min. The 2nd extraction was carried out with MeOH:ACN:FA (65:30:5) at 120 ºC, in 2 static cycles of 5 min, with a flush volume of 70% and a purge time of 1 min. In both extractions, PLE cells were pressurized at 1500 psi. Extracts were combined and evaporated, using a gentle stream of nitrogen and adjusted to a final volume of 5 mL in volumetric flasks. The concentrated extracts were stored at 4 ºC and filtered (0.22 µm syringe filter) before injection in the LC-ESI-MS/MS system.

Dissipation of compounds during the non-vegetative period of vines (vineyards did not receive any fungicide or insecticide application from beginning of October to end of March) was evaluated comparing soil concentrations measured in the above months of consecutive years. Time-course of species noticed at higher concentrations in October was evaluated through analysis of soil taken at shorter intervals (until March next year) in two consecutive campaigns (2017/18 and 2018/19) in five vineyards from two Designations of Origin.

2.3. LC-MS/MS determination

Compounds were determined using a LC-MS/MS XEVO TQD, triple quadrupole mass spectrometer, acquired from Waters (Milford, MA, USA) and furnished with a Z spray ESI source. Separations were carried out with an Agilent (Wilmington, DE, USA) UPLC Zorbax Eclipse Plus C_{18} rapid resolution column (50 mm × 2.1 mm, 1.8 µm particle size) connected to a C_{18}, 2.1 mm i.d. guard cartridge from Phenomenex (Torrance, CA, USA). Acetonitrile (B) and ultrapure water (A), both containing 0.1% of FA, were used as mobile phases at 0.4 mL min^{-1}. The temperature of the column was 40 ºC and the injection volume 1.5 µL. The mobile phase gradient and ESI source parameters were adopted from a previous study dealing with the determination of pesticide residues in wine samples (Castro et al., 2018). Table S1 summarizes the list of retention times, capillary voltage and collision energy values in the MS/MS method. Except fludioxonil (FLUD), the rest of compounds were ionized in positive mode (ESI+).

2.4. PLE extraction efficiencies, matrix effects and overall method accuracy

Optimization of PLE conditions was carried out using soil samples, from an experimental vineyard, characterized by the presence of low pesticide residues and containing 1.5 % of OC, in the typical range of values reported in vineyard soils (Bermúdez-Couso et al., 2007; Vallejo et al., 2019). After setting the extraction conditions, the efficiency of the sample preparation process (PLE extraction followed by extracts concentration to 5 mL) was evaluated by spiking increasing amounts (n=5 concentration levels from 10 to 200 ng g^{-1}) of target compounds to sample extracts. Extraction efficiencies (EEs), as percentages, were defined as the difference between concentrations found in spiked and non-spiked samples of the same soil matrix, divided by the added amount and multiplied by 100. MEs during ESI ionization were defined as the ratio between the slopes of calibration curves obtained for spiked soil extracts (compounds were added to extracts concentrated to 5 mL) and solvent-based standards. In both cases (EEs and MEs evaluation), peak areas for the most intense MRM transition of each pesticide (Table S1), without ISs correction, was used as variable response. Both parameters were assessed in soils with different OC contents.

The accuracy of the method was investigated in two soil samples spiked at two different concentration levels. Responses for each compound were corrected with those observed for the assigned IS, Table S1, and compared with calibration curves for solvent-based standards. The overall recoveries are defined as the ratios between measured and spiked concentrations, multiplied by 100.

3. Results and discussion

3.1. Sample preparation conditions

Table S1 summarizes the determination coefficients (R^2) corresponding to standards, at seven concentration levels, in the range from 1 to 200 ng mL^{-1}. The plots of peak areas (measured for the Q1 transition) versus concentration fitted a linear model with R^2 values above 0.99. The instrument limits of quantification (ILOQ) were defined as the lowest concentration providing a signal to noise ratio (S/N) of 10 for the Q1 transition, whilst the Q2/Q1 ratio matched the average value (± 30%) obtained within the calibration range. ILOQs varied between 0.1 and 10 ng mL^{-1}, with 80% of the compounds showing values below 1 ng mL^{-1}.

Different parameters related to PLE extraction were evaluated in order to identify common conditions rendering the highest EEs for most compounds. The effects of temperature (80, 110 and 140 ºC), composition of the extraction solution (consisting on MeOH with percentages of ACN of 10, 30 and 50%), and number of static extraction cycles (1, 2, 3) were simultaneously investigated using a Box-Behnken experimental factorial design, with a triplicate of the central point. Pressure was fixed at 1500 psi and the extraction time per cycle was 5 min. Flush volume and purge time were 100% of the cell volume and 60 s, respectively. A preliminary assessment of MEs, evaluated under extraction conditions corresponding to the central point of the above design (2 cycles at 110 ºC using a 70:30 MeOH:ACN extraction solution), reflected minor variations in the efficiency of compounds ionization between sample extracts and solvent-based standards, data not shown. Thus, responses obtained for each compound in extracts from the spiked soil matrix (addition level 125 ng g^{-1}) were employed as a direct estimate of the EEs under each of the evaluated experimental conditions.

Fig. 1 summarizes representative trends observed for the set of investigated compounds. In most cases, only the quadratic term associated to the extraction temperature was statistically significant, at the 95% confidence level, or close to the significance limit. As shown for MET, the highest extraction yield was achieved at the intermediate level of this variable: 110 ºC, Fig. 1A. Maximum extraction yields at medium temperatures had been previously reported for PLE extraction of MET and MYC from soil with acetone (Schreck et al. 2008b). For seven compounds, including the 5 neonicotinoid insecticides (ACE, CLO, IMI, THC and THM) and the fungicides ZOX and CHLORA, the extraction temperature showed a significant, negative effect in the extraction yield, Fig. 1B. Thiabendazole (THIAB) was the only compound whose extraction efficiency increased significantly with the temperature and also with the number of cycles, Fig. 1C. That is, the extraction of THIAB requires harder conditions than the rest of target species, Fig. 1C. The percentage of ACN did not affect the extraction efficiency of any compound except CGA 62826, which preferred low percentages of this solvent, Fig. 1D. Finally, four compounds could be hardly detected under any of the assayed conditions. They were THIOM, methiocarb (METHI), CYM and CGA 108906. When the domain of the design was shifted to lower temperatures (50, 70, 90 ºC), maintaining the other two variables at same levels as in the first design, METHI was the only of the above problematic compounds found in soil extracts. In this case, the quadratic term of temperature showed a maximum extraction yield at 70 ºC. Very likely, METHI is degraded when extraction was carried out at higher temperatures. Such assumption agrees with data published for microwave assisted extraction of soil (Sun and Lee, 2002). For most compounds, within the domain of the 2nd design, temperature did not affect significantly to the yield of the extraction, except THIAB (positive effect).

Additional extraction experiments were carried out in order to understand the null responses observed from CYM, THIOM and CGA 108906. CYM could be recovered, under PLE conditions corresponding to the central point of the 1st design, from samples extracted the same day of addition, but not when aged for several days at room temperature. THIOM was found to be partially degraded to CAR during extraction, and mainly lost during evaporative concentration of the PLE extract to a final volume of 5 mL. To sum up, METHI, CYM and THIOM were identified as very labile species, prone to degradation during sample preparation. The metabolite of MET, CGA 108906, was not recovered except when adding FA to the extraction solution. This behavior agrees with the existence of two carboxylic acids in the structure of the compound. In fact, the use of a 5% of FA, combined with the mixture of MeOH:ACN (80º C, extraction in 2 cycles of 5 min), improved also the responses observed for CYM, METHI and CGA 62826; it worsened that of THIAB (Fig. S2), and it did not affect those of remaining compounds.

Considering above comments, it was decided to extract PLE cells twice. First extraction was carried out under mild conditions (80 ºC, 1 cycle of 5 min, MeOH:ACN (70:30)). Thereafter, cells were re-extracted at 120 ºC in 2 cycles of 5 min using a ternary mixture of MeOH:ACN:FA (65:30:5). In both cases, pressure was set at 1500 psi. For most compounds, responses in the 2nd extraction represented less than 5% of those observed in the 1st one. However, CAR, PYR and FEN showed relative responses above 10% in the 2nd fraction, THIAB and CGA 62826 were equally distributed between both fractions, and CGA 108906 was only noticed in the 2nd fraction, Fig. S3.

3.2. Performance of the method

EEs and MEs were investigated in two soils with OC contents of 1.5% and 8.3%, respectively. The 1st corresponds to the same matrix employed during optimization of PLE extraction conditions, the 2nd was chosen as representative of a high organic matter content vineyard soil. The addition level used to assess EEs was 125 ng g^{-1}. Spiked samples were aged overnight to limit the degradation risks of labile compounds (CYM); moreover, THIOM was removed from the spiking solution in order to prevent an overestimation in the EEs of CAR.

As illustrated in the supplementary section (Fig. S4), EEs were scarcely affected by the organic carbon content of soil samples. Within the set of selected compounds, only the fungicides THIAB and METHI showed non-quantitative yields. In the first case, the average extraction yield for both samples stayed around 50%, whereas that of METHI remained below 40%, Fig. S4. From data provided in the previous section, very likely THIAB is not completely extracted from soil (a possible explanation is formation of complexes with metals existing in all vineyard soils, such as copper (Gamba et al., 2017), whilst METHI is partially degraded (Sun and Lee, 2002).

Detailed values of MEs are provided as supplementary information, Table S3. Values close to 100% correspond to non-significant changes in the efficiency of compounds ionization between soil extracts and solvent-based standards. Data below and above 100% point out to suppression or enhancement of the ESI ionization efficiency, respectively. Fig. 2 displays the number of compounds displaying MEs distributed in five segments: < 60%, 60-80%, 80-100%, 100-120% and >120%. For the lower OC soil, the neonicotinoid insecticides showed MEs below 80%. Thus, the efficiency of their ESI ionization was significantly (CLO, IMI and THM, with MEs below 60%), or moderately attenuated (THC and ACE, MEs between 60 and 80%) in soil extracts versus solvent-based standards. For the rest of compounds, MEs remained between 80 and 120%. When increasing the OC of the soil, the number of compounds showing significant or moderate signal attenuation (MEs < 60% and 60-80%, respectively) were 5 and 17, respectively. Focusing on the group of the 5 neonicotinoid insecticides, the magnitude of signal attenuation rose with the increase in the OC content of soil, Table S2.

Table 1 compiles the overall recoveries of the method corresponding to two different vineyard soils, spiked with selected compounds at two different concentration levels: 20 and 100 ng g^{-1}. These levels were fixed considering the previously reported residues of organic fungicides in vineyard soils (Bermúdez-Couso et al. 2007; Pose-Juan et al. 2015; Vallejo et al. 2019). Extractions of non-spiked and spiked samples were carried out in triplicate. Recoveries were estimated against solvent-based calibration standards using a limited number of ISs. Selection of ISs was made considering compounds behavior during extraction and ionization (EEs and MEs, values) and availability. When a specific isotopic labelled analogue was not available for a given compound, its response was corrected with that obtained for the IS showing the closest retention time and/or belonging to the same chemical family. For species not affected by significant MEs (see Table S2 and Fig. 2), MET-$^{13}C_6$ was used as IS. Data for METHI are not included in this list given the non-effective extraction of this compound from soil.

Considering average values of the overall method recoveries (Table 1): THIAB, CYM, THC and CGA 108906 showed recoveries below 70%; whereas, values above 130% were noticed for the fungicide mandipropamid (MAN). Thus, the use of solvent-based calibration standards does not provide accurate concentration data for these 5 compounds. Accuracy values obtained for the remaining species (44 compounds) were deemed suitable to investigate their occurrence in soil samples. Procedural blanks, corresponding to extraction of diatomaceous earth spiked only with the mixture of ISs, did not reflect existence of contamination problems. Thus, the LOQs of the method were calculated from ILOQs divided by the EEs and MEs observed for each compound (normalized values between 0 and 1) and multiplied by the ratio between the volume of the final extract (5 mL) and the extracted sample amount (2 g). Calculated LOQs varied from 0.2 ng g^{-1} to 13 ng g^{-1} (referred to freeze-dried soil), Table 1. The linear response range of the method extends up to 500 ng g^{-1}, referred to freeze-dried soil samples.

The number of pesticides covered by the developed method (44 compounds) is significantly higher than those included in previous applications of GC-MS (Bermúdez-Couso et al., 2007; Schreck et al., 2008b) and LC-ESI-MS/MS to the same matrix (Pose-Juan et al., 2015; Vallejo et al., 2019). These previous methodologies permitted to quantify a maximum of 17 species. PLE extractions are carried out in an automated way, they are faster and they require less volume of organic solvents than conventional solid-liquid extraction approaches proposed in earlier studies (Schreck et al., 2008b; Pose-Juan et al., 2015; Vallejo et al., 2019).

3.3. Pesticide residues in vineyard soils

Fig. 3 summarizes data corresponding to residues in soil samples, from 9 different vineyards, taken before starting the vegetative cycle of vines. In some points, soil was sampled in consecutive years. Depicted data reflect the total residues level, the identity and concentration of the pesticide found at the highest concentration in each vineyard and the number of compounds with concentrations above 10 ng g^{-1}. Individual concentrations for all compounds above method LOQ are provided in Table S4. For some soils, dilution of the PLE extract was required to accommodate certain compounds within the linear response range of the method. Total pesticide residues ranged from 60 ng g^{-1} (vineyard code 7) to more than 2800 ng g^{-1} (vineyard code 6, year 2019). Within the set of processed samples, species found at highest concentrations were DIM, CAR, MYC, PYRA and FLUO. Except CAR, the rest of compounds are employed as fungicides in the treatment of *mildew* (DIM and FLUO) and *oidium* (MYC and PYRA) infections in vineyards. CAR is not authorized for vines treatment, but is recognized as a transformation product of THIOM, which is included in commercial formulations for the treatment of *mildew*. As explained in the previous section, the latter compound was not stable during the employed sample preparation process; so, additional formation of CAR from THIOM residues, remaining in soil, cannot be excluded.

When compared to residues in soils from other wine production areas, values depicted in Fig. 3 are much higher than those reported in Rioja (Pose-Juan et al., 2015), the Basque country (Vallejo et al., 2019) and France (Schreck et al. 2008b), and in the same range as the concentrations of MET in soil samples, from the same geographic area, taken at the end of summer (Bermúdez-Couso et al. 2007). Furthermore, as far as we could trace, residues of the degradation species CAR, the fungicide FLUO, and the insecticide IMI are reported in vineyard soils for the first time. Total residues summarized in Fig. 3 are also higher than those reported for the sum of herbicides, insecticides and fungicides in a collection of 300 arable soil samples from several EU countries (Silva et al., 2019).

Fig. 4A shows the normalized values for the sum of pesticide residues for samples obtained in March-April versus those measured, at same sampling points, at the beginning of October from the previous year. With a single exception (vineyard code 1, 2017-18 campaign) total residues in March were lower than those measured in October from the previous year. On average (excluding that value), a 40% reduction of the total soil residues was found. Hydrolysis reactions, microbiological degradation, run-off during raining events and uptake by herbaceous vegetation growing in vineyard soils, during autumn and the beginning of spring, might explain the above reduction. Climate parameters (temperature and accumulated rain) in each of the sampling sites compiled in Fig. 4A, during the non-vegetative period of vines, are provided as supplementary information (Table S5). Data were obtained from the closer station from the regional meteorological net.

Fig. 4B displays the individual removal percentages for pesticides found at significant concentrations (above 10 ng g^{-1}) in samples compiled in Fig. 4A. Error bars are included for compounds quantified in at least three pairs of samples, Table S6. The average dissipation factors varied from 12% for DIM to 75% in case of BEN. When compounds are grouped in chemical families, or attending to the kind of pest they are designed to treat, some additional conclusions are observed. The acylalanine fungicides MET and BEN, as well as the by-product CGA 62826, are removed with relatively high efficiencies from soil samples during winter time. The azolic compounds MYC and PEN, employed in the treatment of *oidium* infections, present removal efficiencies lower than 40%. Within the group of anti-*botrytis* fungicides, PYR was more persistent than CYP and FLUD. The latter two species are normally combined in commercial formulations for the treatment of *botrytis* infections. The fungicide PYRA and the insecticide IMI also showed relatively low dissipation efficiencies; however, the first compound seems to have a low frequency detection and the open uses of IMI have been forbidden since the end of 2018. So, further applications of this species are not expected. Some highly efficient fungicides employed in the prevention and treatment of *mildew* infections in vines were scarcely removed from vineyard soils during winter time. Particularly, CAR (arising from THIOM degradation) was removed with an average efficiency of 36%, which varied significantly among the investigated vineyards. In case of DIM, the estimated dissipation efficiency was 12%, the lowest percentage within the set of compounds included in Fig. 4B.

Analysis of soil samples taken at shorter time intervals reflected some additional findings. As example, CYM, widely used in combination with other fungicides, was degraded very fast in soil, being detected only in samples collected at the end of summer, Fig. 5A. Such fast dissipation rate is in agreement with a soil half-life of 5 days obtained in laboratory experiments (PPDB: Pesticide properties database, 2020). Fig. 5B shows the levels of CYP and MYC in samples collected at different times from vineyard code 4. These species are classified as moderately (CYP) and highly persistent (MYC) in soil (PPDB: Pesticide properties database, 2020). Reduction of their concentrations was noticed in winter and beginning of spring; however, their levels remained stable during autumn. Incorporation of vine leaves to soil and wash-off from leaves by rain water might explain such behavior. Although this latter contribution was not quantified in the current research, the presence of significant fungicide residues in vine leaves has been recognized in previous studies (Turkoz-Bakirci, 2018). Considering data in Fig. 5B, CYP and MYC have a potential to be concentrated in soil if applied every campaign.

The removal of a given pesticide from vineyard soils does not necessary mean mineralization. Sometimes formation of a relatively stable by-product happens. Fig 5C shows the time-course of MET in samples taken from 31st august 2018 (time zero in the plot) at vineyard code 5. The residues of the commercial fungicide decreased steady with time from the 1st sampling; however, the levels of its soil metabolite (CGA 62826) remained more or less stable until the last sampling campaign. In soils with a higher pH, as those corresponding to Rioja Designation of Origin, formation of CGA 62826 was not detected in samples taken at the beginning of autumn, but just in those corresponding to winter and spring sampling (Pose-Juan et al., 2015). So, acidic soils might speed up the hydrolysis of the ester moiety of MET to produce CGA 62826.

Profiles of the E/Z isomers of the fungicide DIM were different among calibration standards, spiked soil samples (not polluted with this fungicide) and soils from vineyards treated with this fungicide. In the first two cases, responses measured for the earlier eluting isomer represent around 40% of that corresponding to sum of E/Z forms, (Fig. S5). However, in vineyard soils processed in this study 95% of DIM residues corresponded to later retention time isomer. Fig. 5D shows the evolution of total DIM concentration at vineyard code 6 for a two-year period. Data obtained from the 1st sampling until March 2018 point out to the following facts: (1) this fungicide was applied in larger doses in the 2018 campaign than in the 2017 one, (2) DIM concentrations increased in autumn of year 2018, likely from rain wash-off and incorporation of vine leaves to soil, and (3) the equilibrium between E/Z isomers of DIM in soils is shifted to the longer retention time species.

Fig. 6 summarizes the levels of additional fungicides, in heavy polluted vineyard soils, at different sampling times. For some compounds, such as CAR, PYR and PEN soil residues did not follow a clear trend, remaining above 100 ng g^{-1} in all samples. In other cases, MET, BEN, and BOS, residues increased after 2018 campaign, which clearly points out to their application at vineyards during the spring-summer period. Thereafter, their removal was compound dependent. MET and BEN were relatively labile, whereas the concentrations of BOS increased from the end of August 2018 to January 2019, Fig. 6, following the same trend as those depicted for DIM (Fig. 5D) and MYC (Fig. 5B), both classified as slow dissipation species in soil. Steady decrease, or initial increase, in soil concentrations of certain pesticides during autumn (when not sprayed in vine) likely depends on a balance between their soil degradability and extra inputs from leaves through fall and/or rain wash out. Thus, for relatively stable fungicides, soil concentrations might increase during autumn months due to the contribution of secondary inputs from aerial vine parts.

4. Conclusions

PLE extraction followed by UPLC-ESI-QqQ-MS/MS permitted the determination of a broad group of fungicides and insecticides in vineyard soils. The use of two sequential extractions of the same sample allowed recovering compounds with different stability during the PLE process. Despite using mild extraction conditions, certain compounds (THIOM and METHI) are totally or partially degraded during extraction. On the other side, overall recoveries in the range from 70 to 130% were achieved for 44 out of 51 compounds. Significant residues for 12 fungicides, from different chemical families, and the insecticide IMI were found in vineyard soil samples obtained at the beginning of spring. Half of them showed average dissipation rates below 50% from the end of each viticulture campaign to the beginning of the next year one; thus, they have a potential to be accumulated in the soils of the evaluated vineyards if same active ingredients are sprayed on consecutive campaigns. Global residues found in the current study point out to concerning pollution levels of vineyard soils in the investigated area, with several compounds lasting for more than one year, with average soil concentrations above 100 ng g^{-1}.

Acknowledgements

L.P.M acknowledges a FPU grant to the Spanish Ministry of Science. This study was supported by Xunta de Galicia and Spanish Government through grants GRC-ED431C 2017/36, PGC2018-094613-B-I00, both co-funded by the EU FEDER program.

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Table 1. Overall recoveries of the analytical procedure in two different vineyard soils spiked at two concentration levels and method LOQs (ng g^{-1}) referred to freeze-dried soil.

	Vineyard code 5 (OC, 5.3%)				Vineyard code 3 (OC, 6.2 %)				Global	
Compound	Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	recovery (%) with SD	LOQs (ng g^{-1})
ACE	68%	6%	67%	4%	99%	11%	89%	2%	81 (16)	1.0
AME	86%	6%	89%	5%	94%	5%	93%	3%	91 (3)	1.0
AZO	144%	7%	140%	1%	116%	7%	116%	5%	129 (15)	0.3
BEN	120%	4%	123%	4%	112%	5%	113%	2%	117 (6)	0.3
BIT	104%	5%	105%	3%	105%	3%	114%	4%	107 (5)	1.4
BOS	101%	3%	98%	2%	119%	10%	105%	10%	106 (9)	0.5
CAR	156%	12%	179%	8%	196%	7%	191%	7%	90 (18)	0.2
CGA 108906	72%	17%	69%	8%	57%	22%	45%	7%	**57 (12)**	2.6
CGA 62826	124%	12%	113%	8%	142%	4%	127%	5%	127 (12)	1.4
CHLOR	76%	8%	74%	3%	86%	8%	85%	2%	80 (6)	2.8
CHLORA	87%	3%	89%	6%	119%	18%	105%	2%	100 (15)	5.7
CHLORM	88%	36%	82%	8%	109%	23%	91%	2%	92 (12)	8.0
CLO	94%	28%	91%	18%	99%	13%	87%	9%	93 (5)	8.5
CLOF	85%	8%	76%	2%	95%	20%	91%	3%	87 (8)	5.1
CYF	86%	9%	82%	6%	106%	2%	106%	4%	95 (13)	1.4
CYM	81%	3%	88%	7%	56%	15%	47%	20%	**68 (19)**	2.1
CYP	121%	4%	114%	3%	104%	2%	110%	3%	112 (7)	1.3
DIF	104%	2%	101%	3%	116%	5%	115%	3%	109 (7)	0.8

Compound	Vineyard code 5 (OC, 5.3%) Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	Vineyard code 3 (OC, 6.2 %) Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	Global recovery (%) with SD	LOQs (ng g^{-1})
DIM	98.5%	4%	110%	7%	n.e.	-	144%	6%	127 (24)	2.2
DIN	98%	3%	99%	4%	104%	4%	110%	2%	103 (6)	2.0
FEN	85%	8%	86%	3%	95%	10%	91%	4%	89 (4)	0.8
FENA	82%	10%	81%	2%	100%	10%	96%	8%	90 (10)	0.8
FENH	114%	10%	103%	9%	96%	12%	103%	3%	104 (7)	1.4
FLUD	107%	18%	84%	33%	88%	33%	107%	26%	97 (12)	1.4
FLUF	74%	3%	79%	2%	102%	3%	105%	1%	90 (16)	1.4
FLUO	112%	5%	106%	2%	119%	5%	114%	3%	113 (5)	1.4
FLUS	98%	4%	98%	3%	110%	3%	105%	8%	103 (6)	0.5
IMA	79%	4%	77%	4%	88%	6%	89%	4%	83 (6)	0.8
IMI	109%	25%	115%	18%	133%	6%	117%	12%	119 (10)	13
IPR	117%	4%	113%	5%	120%	5%	111%	4%	115 (4)	0.5
MAN	152%	11%	147%	10%	122%	7%	123%	5%	**136 (16)**	1.4
MET	n.e.	-	115%	3%	108%	3%	107%	1%	110 (4)	0.5
METR	112%	10%	100%	6%	128%	5%	128%	1%	117 (14)	0.8
MYC	102%	5%	98%	3%	96%	5%	101%	2%	99 (3)	1.4
PEN	103%	6%	99%	5%	115%	7%	112%	3%	107 (8)	0.3
PROC	85%	4%	87%	5%	95%	7%	96%	11%	91 (6)	2.5
PROP	101%	2%	101%	3%	120%	4%	116%	2%	110 (10)	1.3
PYR	105%	5%	122%	15%	109%	7%	110%	5%	111 (7)	0.6
PYRA	n.e.	-	116%	2%	111%	1%	114%	3%	114 (3)	0.5

	Vineyard code 5 (OC, 5.3%)				Vineyard code 3 (OC, 6.2 %)				Global	
Compound	Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	Recovery (20 ng g^{-1})	SD	Recovery (100 ng g^{-1})	SD	recovery (%) with SD	LOQs (ng g^{-1})
QUIN	77%	4%	77%	2%	85%	4%	86%	1%	81 (5)	0.9
TEBF	121%	6%	120%	4%	110%	4%	117%	4%	117 (5)	2.5
TEBU	104%	9%	101%	5%	118%	9%	112%	2%	108 (8)	1.4
THC	56%	9%	53%	7%	69%	10%	68%	3%	**61 (8)**	1.9
THIAB	57%	5%	60%	4%	81%	20%	76%	5%	**69 (12)**	1.2
THM	99%	6%	97%	14%	81%	12%	86%	9%	91 (9)	4.3
TRIAF	101%	9%	99%	4%	107%	5%	106%	4%	104 (4)	1.9
TRIAL	98%	10%	96%	3%	121%	4%	113%	4%	107 (12)	0.9
TRIF	98%	5%	95%	4%	108%	5%	105%	3%	101 (6)	2.1
ZOX	101%	6%	93%	4%	107%	4%	107%	5%	102 (7)	0.3

n.e., not evaluated values since soil levels were much higher than the addition level.

In bold compounds whose average overall recoveries are outside the 70-130% range.

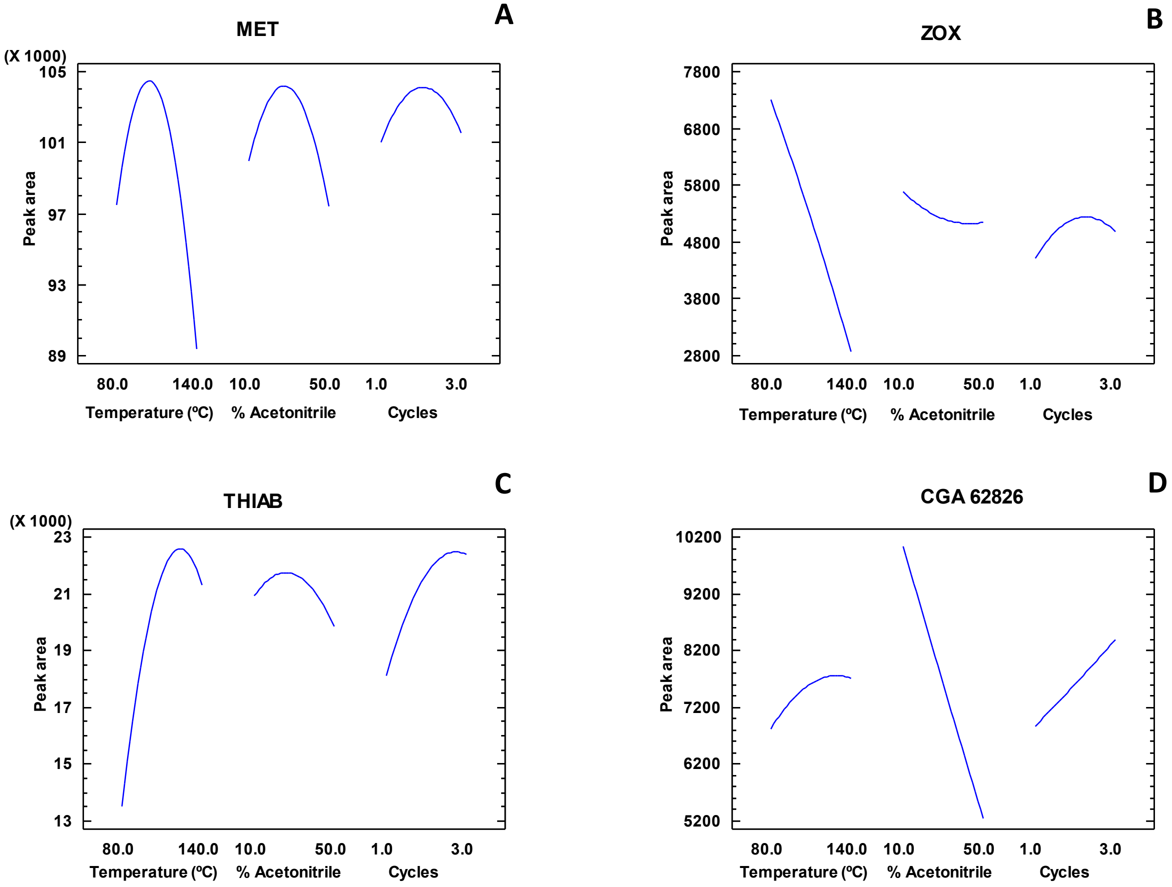

Fig. 1. Main effect plots showing the different trends in the responses of selected compounds as function of values for factors involved in the Box-Behnken experimental factorial design.

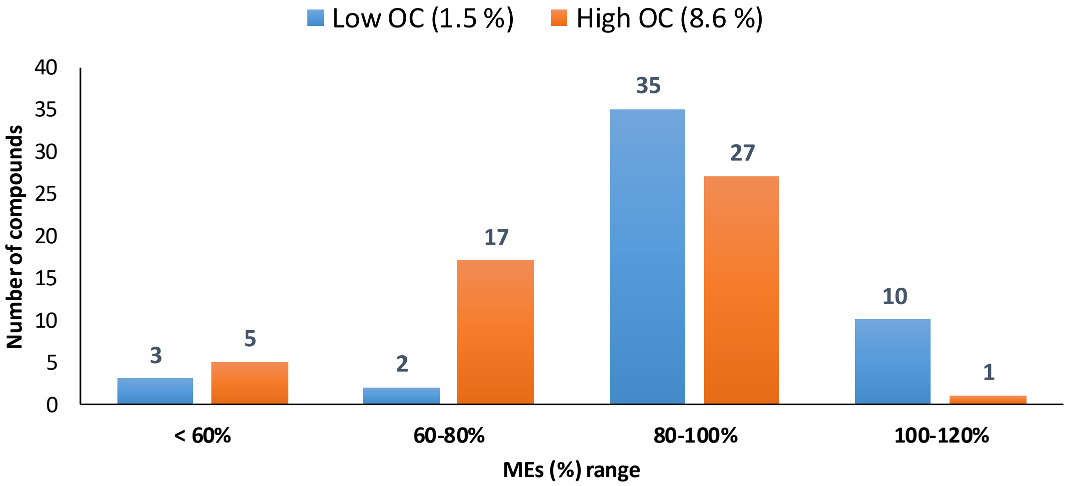

Fig. 2. Distribution of matrix effects (MEs, %), defined as ratio of slopes for spiked sample extracts and solvent-based standards, as function of the organic carbon content of soil samples.

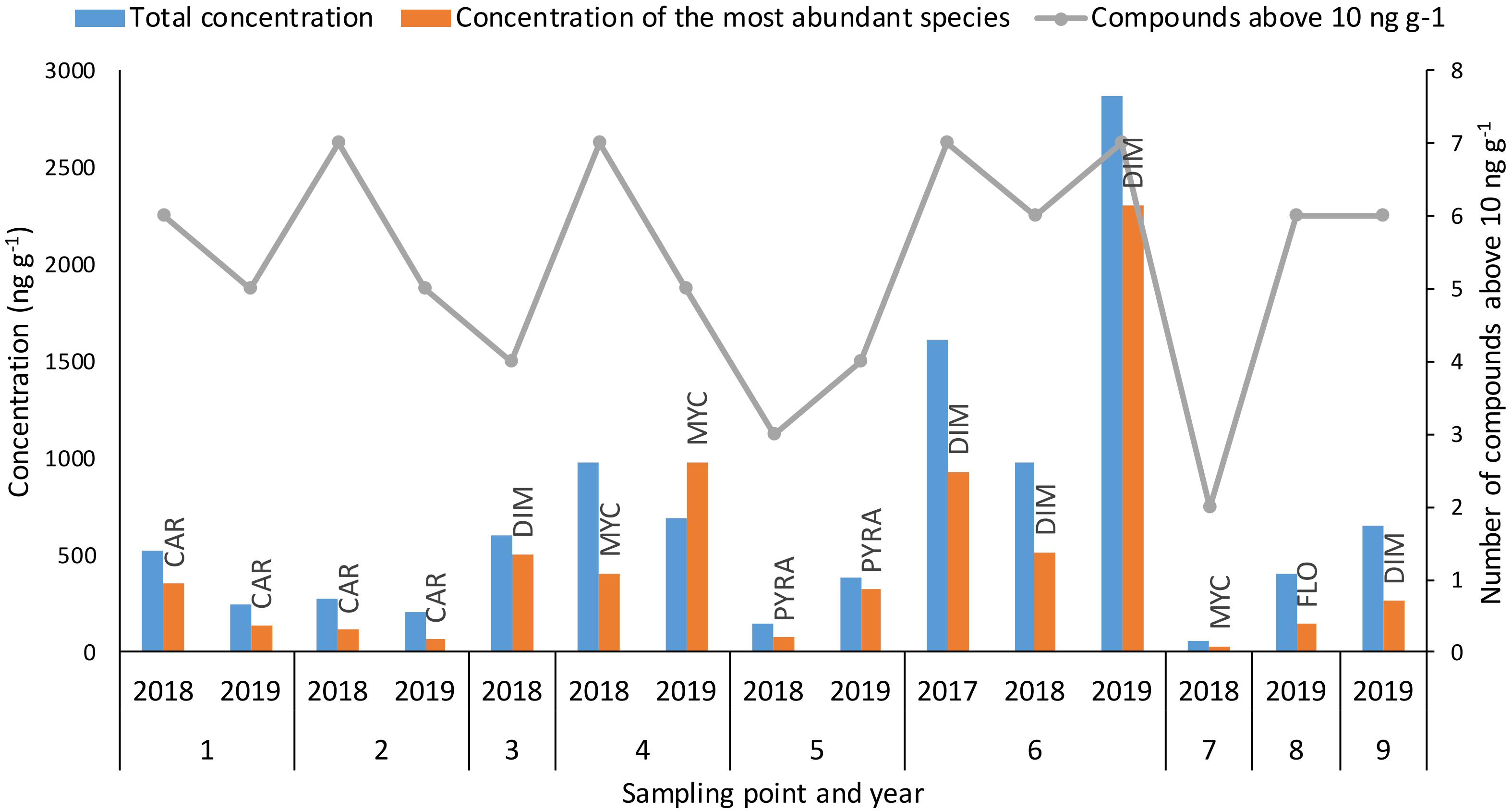

Fig. 3. Plot of total pesticide residues (ng g^{-1}), concentration of the most abundant compound (ng g^{-1}), and number of pesticides above 10 ng g^{-1} in vineyard soils taken at spring time, before starting the seasonal application campaign.

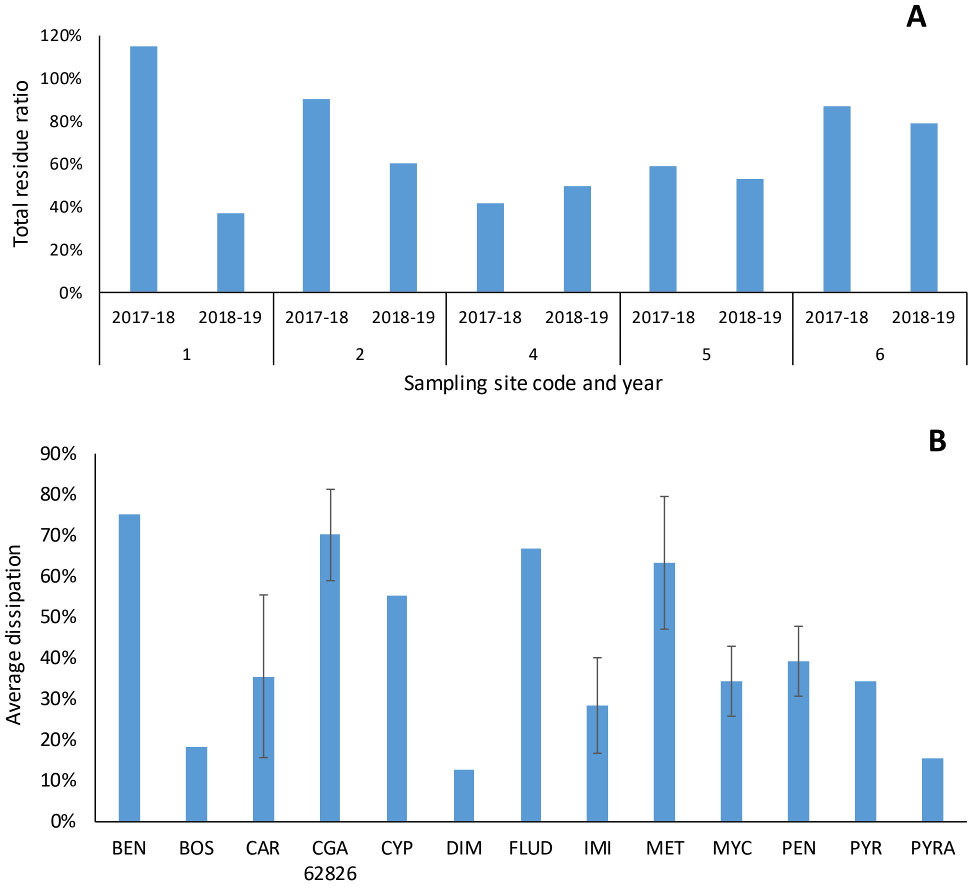

Fig. 4. A, Normalized ratios for total pesticide residues in vineyard soils in March versus October of the previous year. B, Average dissipation percentages observed for different fungicides.

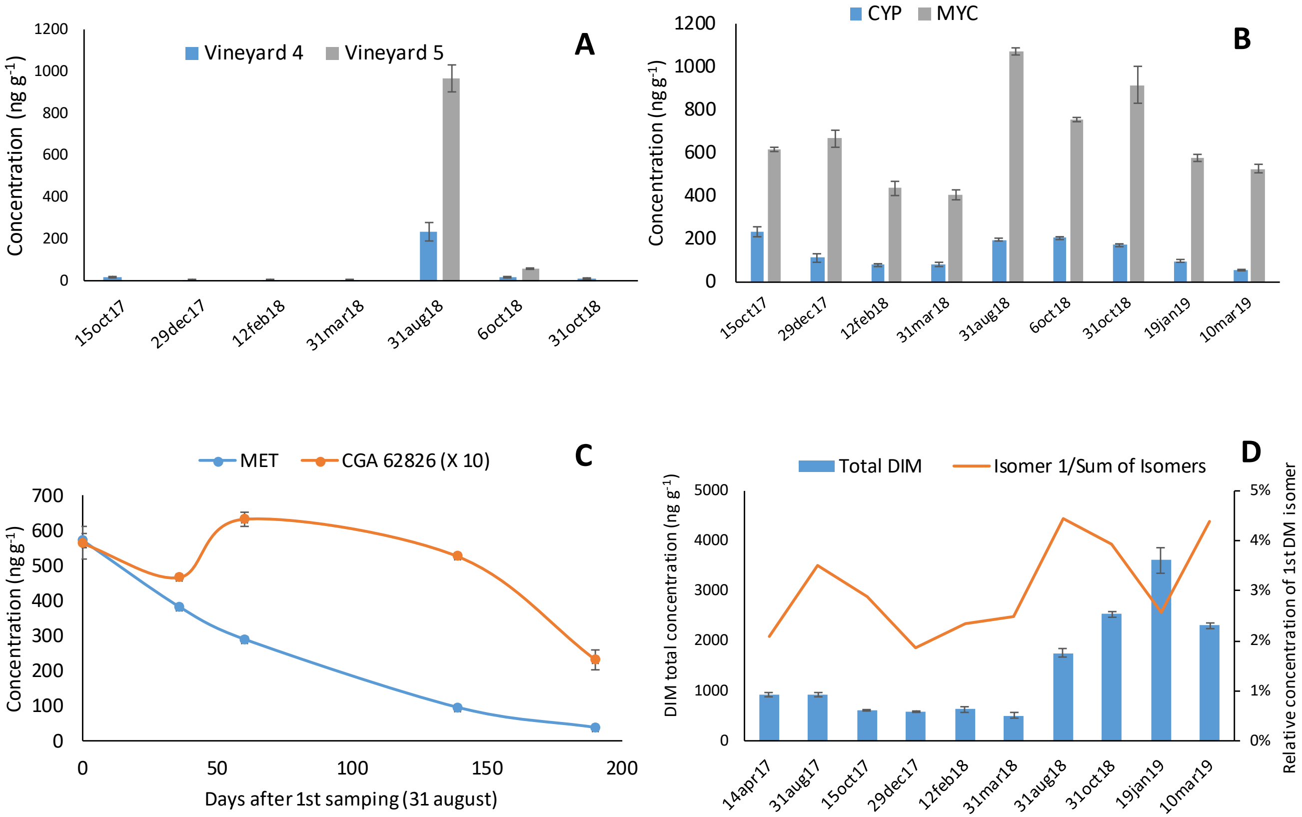

Fig. 5. Concentration values obtained at different sampling dates. A, CYM concentrations in two different vineyards. B, Levels of CYP and MYC at vineyard code 4. C, evolution of MET and its hydrolysis transformation product (CG 62826) from August to March. D, Total concentration of DIM and ratios between isomers during a 2-years period in the same vineyard.

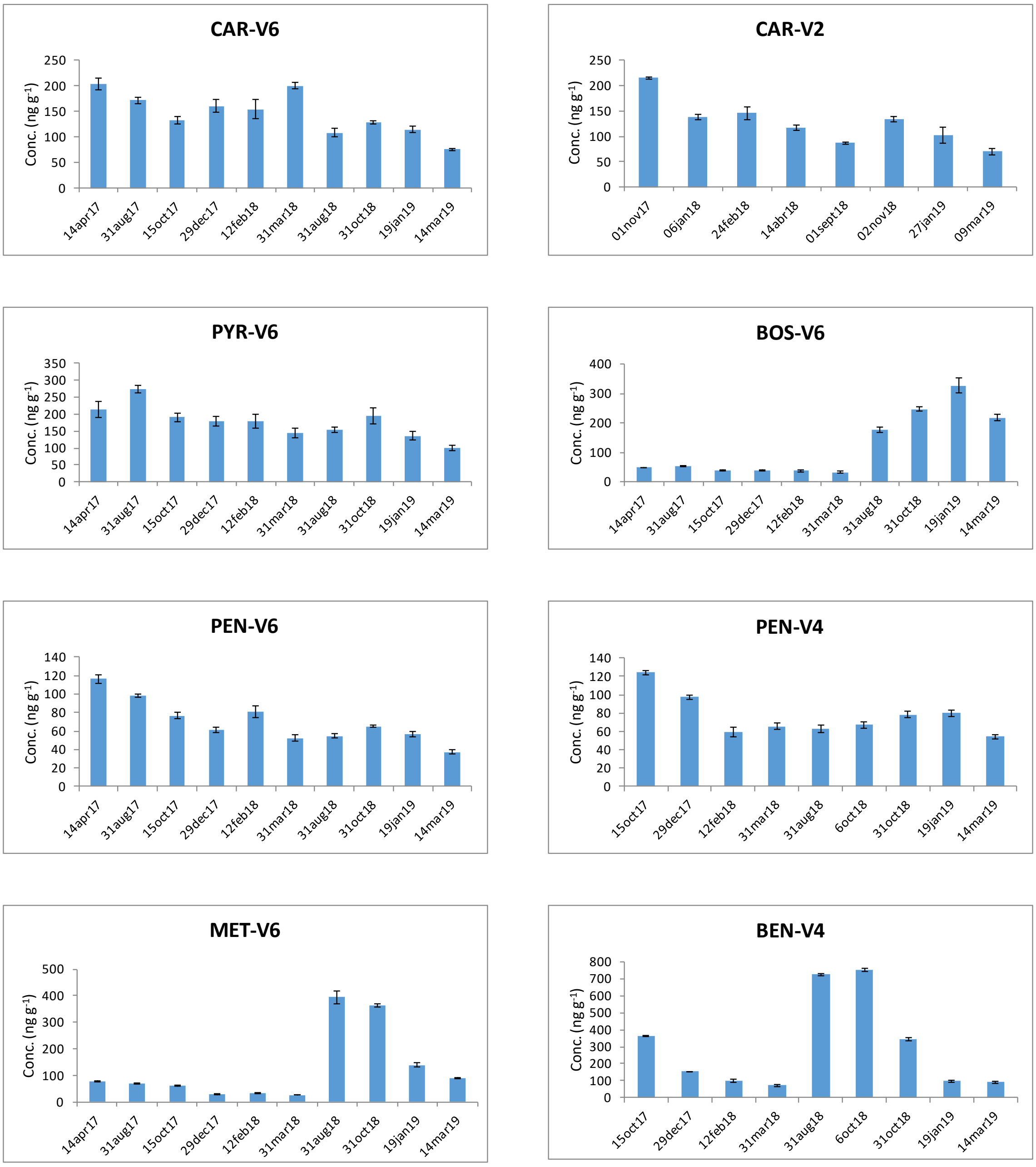

Fig 6. Concentration of selected pesticides found in different sampling sites through a 2-year period.

Supplementary information to manuscript:

Multiresidue procedure to assess the occurrence and dissipation of fungicides and insecticides in vineyard soils from Northwest Spain

L. Pérez-Mayán, M. Ramil, R. Cela, I. Rodríguez*

Department of Analytical Chemistry, Nutrition and Food Sciences. Research Institute on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, 15782-Santiago de Compostela, Spain.

*corresponding author
e-mail: isaac.rodriguez@usc.es

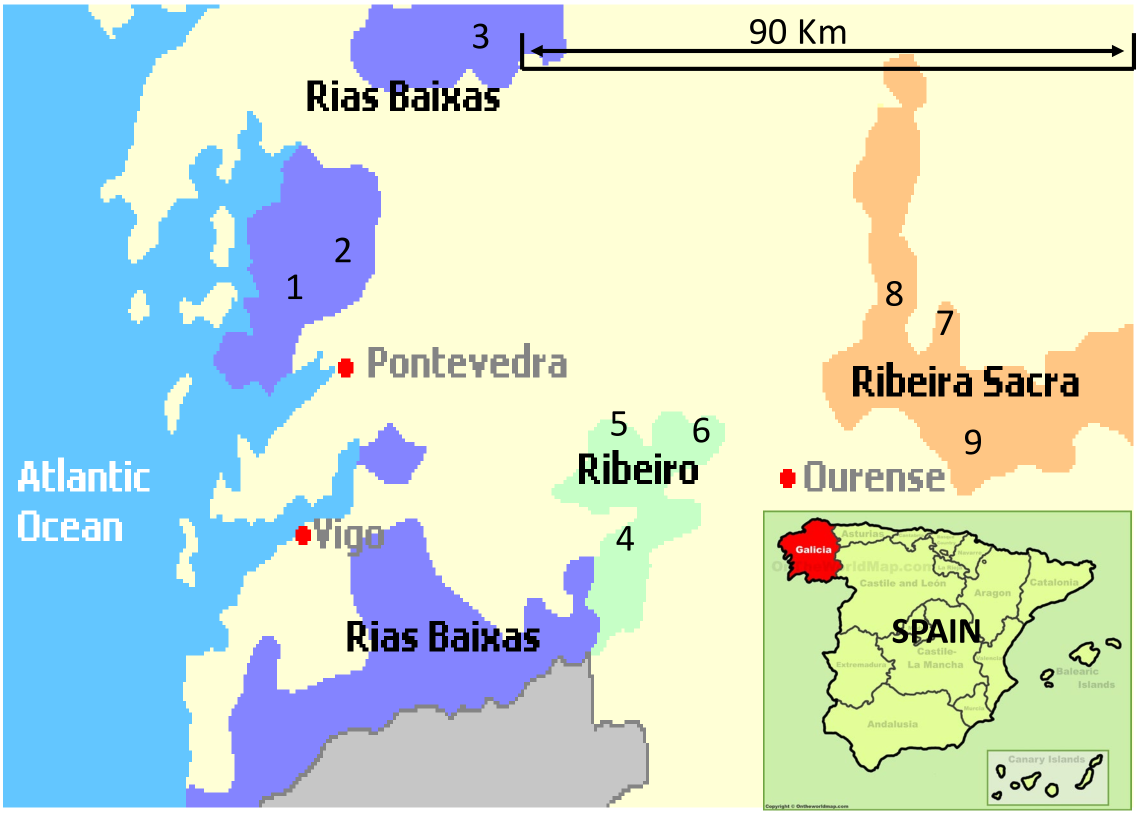

Fig. S1. Sampling points (1 to 9) corresponding to vineyards in the Designation of Origin Rias Baixas, Ribeiro and Ribeira Sacra in Galicia, Northwest Spain.

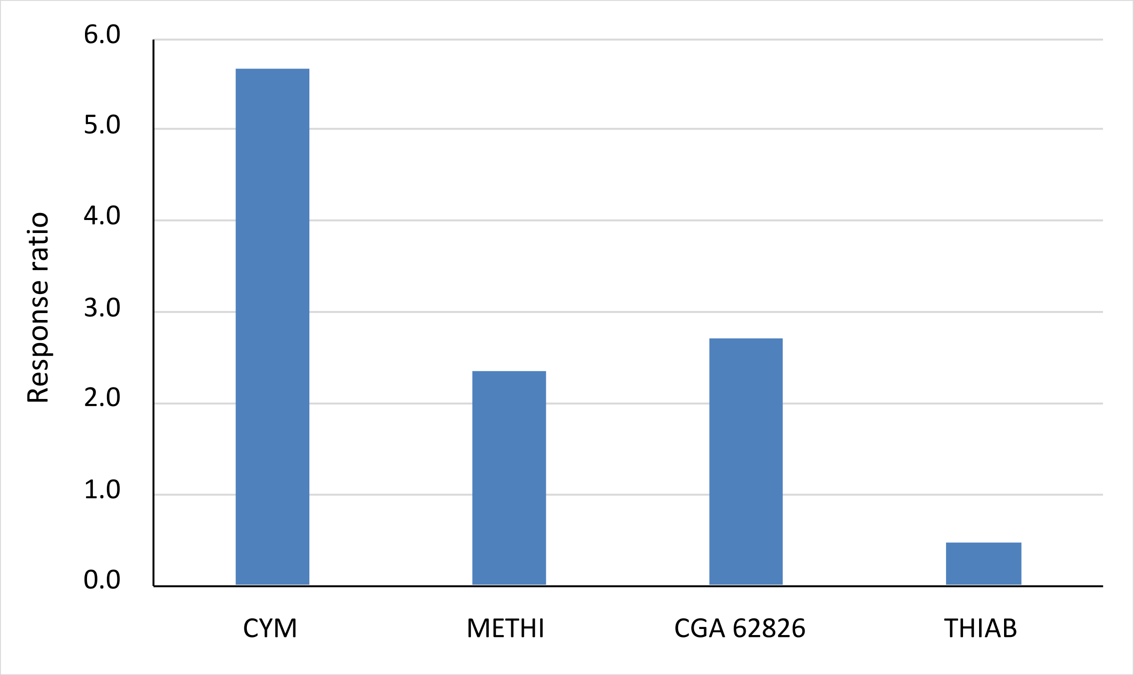

Fig. S2. Effect of formic acid (FA) in the relative extraction efficiencies of selected compounds from a spiked soil sample. PLE extraction at 80 ºC, 2 cycles of 5 min, MeOH:ACN (70:30), flush volume 100%. Ratio of peak areas with and without FA (5%) in the extraction solution.

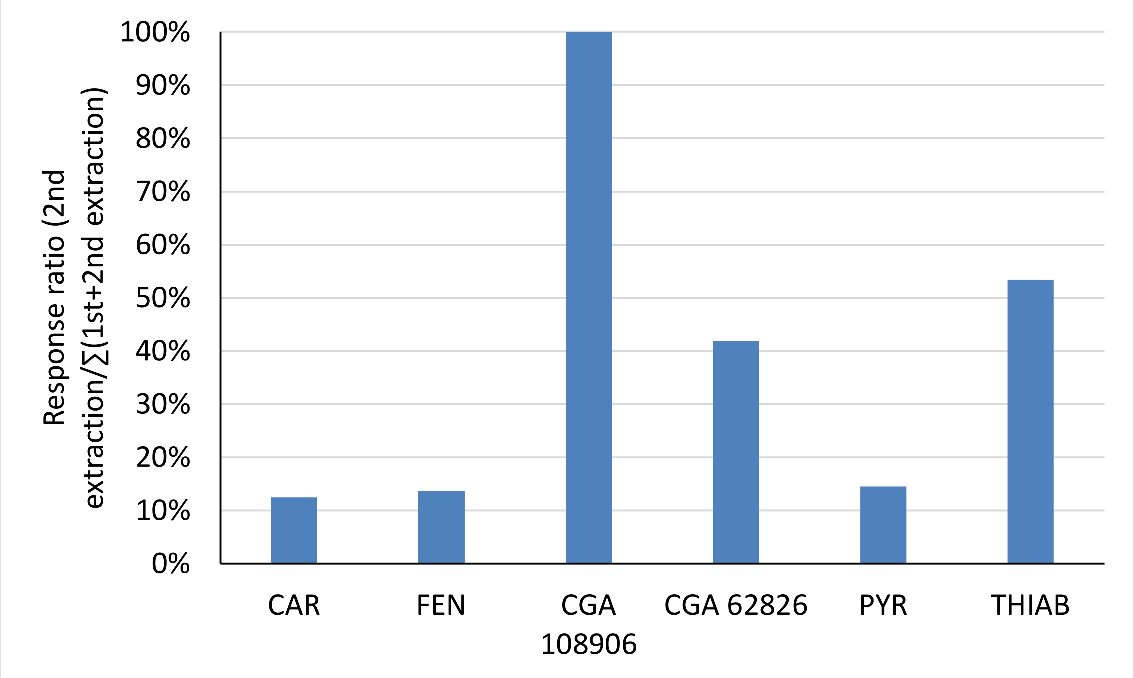

Fig. S3. Relative responses in 2nd extraction of a packed PLE cell containing 2 g of spiked vineyard soil. Extraction conditions: 1st: 80 ºC, 1 cycle 5 min, MeOH: ACN (70:30); 2nd: 120 ºC, 2 cycles, 5 min, flush volume 70%, MeOH:ACN:FA (65:30:5).

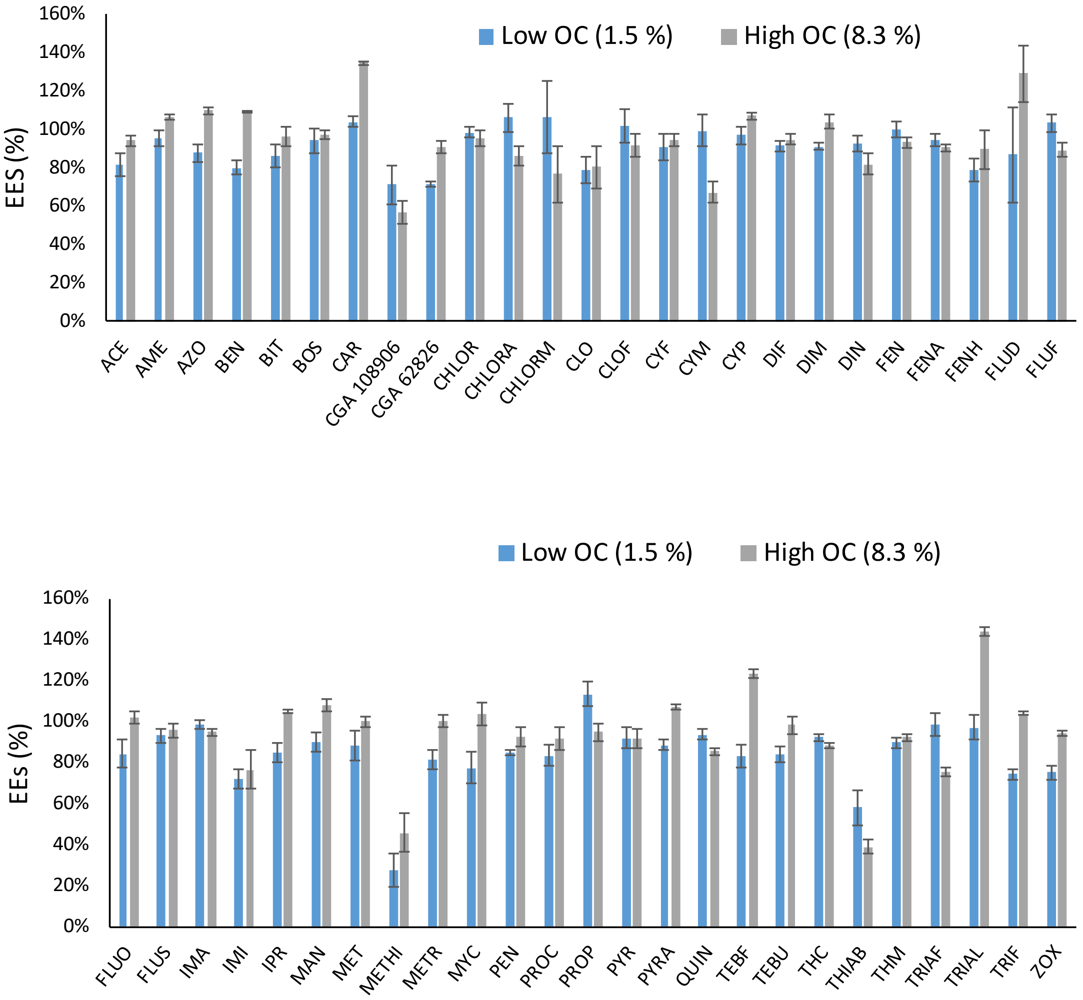

Fig. S4. Extraction efficiency (EEs, %) of the PLE procedure for spiked soil samples (added concentration 125 ng g^{-1}) with different organic carbon (OC) contents, n=3 replicates.

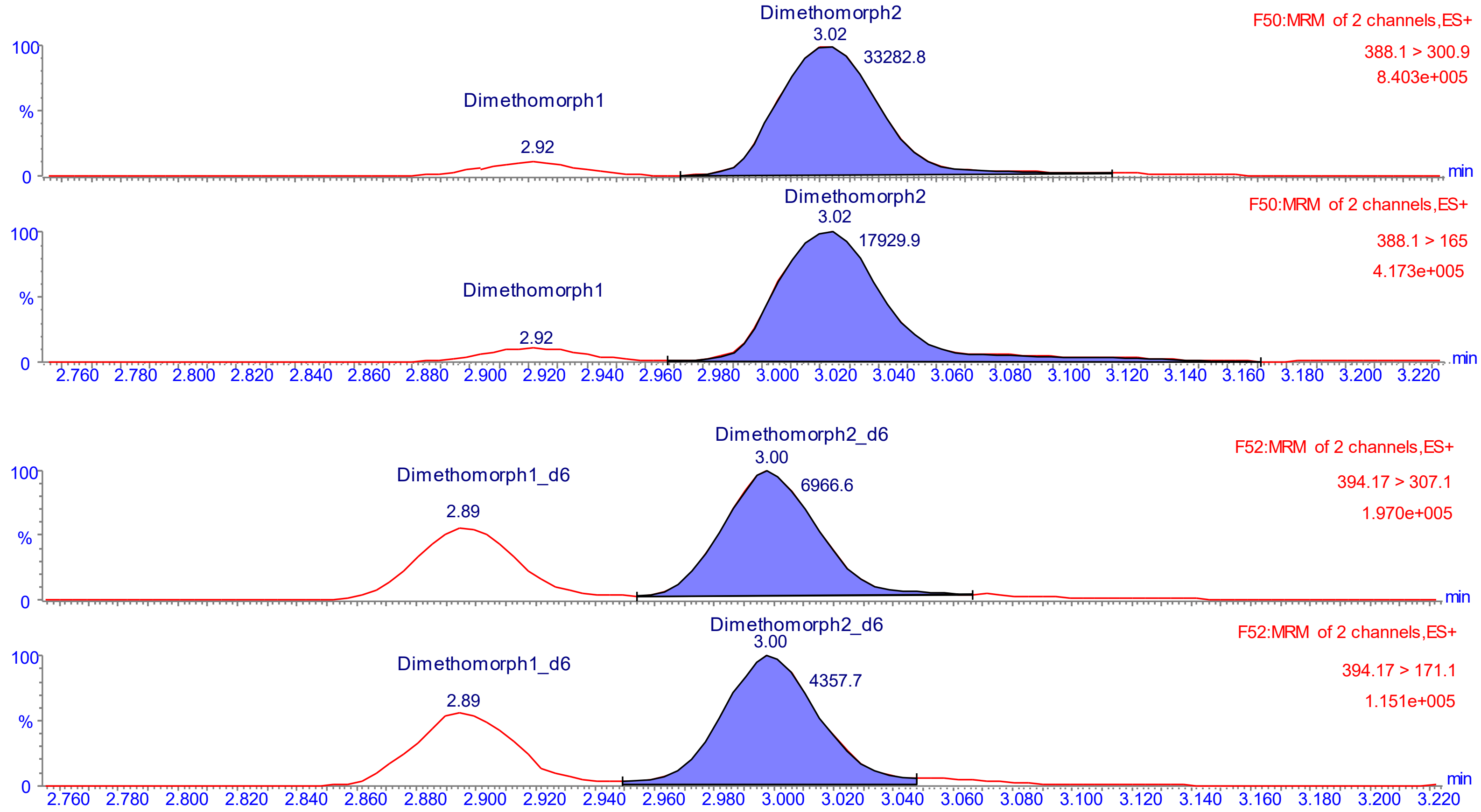

Fig. S5. LC-ESI-MS/MS chromatograms for Q1 and Q2 transitions of DIM and DIM-d_6 in a non-spiked vineyard soil.

Table S1. Summary of UPLC-ESI-MS/MS determination conditions, linearity and instrument limits of quantification (ILOQs) in ng mL^{-1}.

Compound	Abbreviation	Retention time (min)	[M+H]$^+$ ion (*m/z*. Da)	Cone voltage (V)	Q1 (CE. V)	Q2 (CE. V)	Q2/Q1 ratio	R^2 (1-200 ng mL^{-1})	ILOQ (ng mL^{-1})
Acetamiprid	^{g}ACE	2.24	223.0	34	126.0 (20)	56.1 (15)	0.6	0.9998	0.3
Ametoctradin	^{g}AME	3.25	276.0	60	176.0 (35)	70.0 (50)	0.16	0.9994	0.4
Azoxystrobin	^{g}AZO	3.75	404.0	28	372.0 (15)	329.0 (30)	0.19	0.9956	0.1
Benalaxyl	^{g}BEN	4.85	326.1	26	148.0 (20)	91.0 (34)	0.67	0.9971	0.1
Bitertanol	^{g}BIT	4.27	338.1	21	99.1 (16)	70.1 (8)	0.91	0.9968	0.5
Boscalid	^{g}BOS	3.89	342.9	41	139.9 (20)	307.0 (20)	0.11	0.9928	0.2
Carbendazim	^{a}CAR	1.77	192.1	33	160.1 (18)	132.1 (28)	0.14	0.9998	0.1
Chlorantraniliprole	^{g}CHLORA	3.18	484.0	18	286.0 (12)	453.0 (17)	1.01	0.9975	2
Chlorpyrifos	^{g}CHLOR	6.13	349.9	36	97.0 (32)	198.0 (20)	0.64	0.9995	1
Chlorpyrifos methyl	^{g}CHLORM	4.95	321.8	34	125 (20)	289.9 (16)	0.35	0.9947	3
Clofentezine	^{g}CLOF	5.24	303.0	28	138.0 (22)	102.0 (35)	0.72	0.9974	2
Clothianidin	^{b}CLO	2.14	250.0	24	169.0 (22)	132.0 (18)	0.4	0.9654	2
Cyflufenamid	^{g}CYF	5.56	413.2	36	295.1 (15)	203.0 (35)	0.47	0.9980	0.5
Cymoxanil	^{g}CYM	2.33	199.0	23	128.0 (8)	111.0 (18)	0.51	0.9993	0.7
Cyprodinil	^{c}CYP	3.11	226.0	56	93.0 (33)	108.0 (25)	0.61	0.9921	0.5
Difenoconazole	^{g}DIF	4.87	406.0	46	251.1 (25)	111.1 (60)	0.34	0.9994	0.3

Compound	**Abbreviation**	**Retention time (min)**	**$[M+H]^+$ ion (*m/z*. Da)**	**Cone voltage (V)**	**Q1 (CE. V)**	**Q2 (CE. V)**	**Q2/Q1 ratio**	**R^2 (1-200 ng mL^{-1})**	**ILOQ (ng mL^{-1})**
Dimethomorph	[d]DIM	3.01; 3.11	388.1	40	300.9 (20)	165.0 (30)	0.57	0.9985	0.7
Diniconazole	[g]DIN	4.43	326.1	46	70.2 (25)	159.0 (34)	0.05	0.9991	0.5
Fenamidone	[g]FENA	3.77	312.1	31	92.0 (25)	236.1 (14)	0.57	0.9979	0.3
Fenhexamide	[e]FENH	3.86	302.1	41	97.2 (22)	55.3 (38)	0.44	0.9982	0.5
Fenpropidin	[g]FEN	2.57	274.2	55	147.1 (28)	86.1 (28)	0.93	0.9992	0.1
Fludioxonil	[g]FLUD *	3.47	247.0	50	180.0 (28)	126.0 (35)	0.81	0.9989	5
Flufenoxuron	[g]FLUF	6.12	489.1	40	158.0 (22)	141.0 (46)	0.58	0.9991	0.5
Fluopicolid	[g]FLUO	4.1	383.0	40	172.9 (20)	365.0 (15)	0.1	0.9977	0.5
Flusilazol	[g]FLUS	4.01	316.0	36	247.0 (18)	165.0 (28)	0.78	0.9996	0.2
Imazalil	[g]IMA	2.38	296.9	41	68.9 (18)	158.9 (34)	0.5	0.9960	0.3
Imidacloprid	[f]IMI	2.19	256.1	34	209.1 (15)	175.1 (20)	0.91	0.9935	3
Iprovalicarb	[g]IPR	3.61	321.1	28	119.1 (16)	203.1 (10)	0.42	0.9990	0.2
Mandipropamid	[g]MAN	3.92	412.0	25	328.0 (16)	125.0 (35)	0.63	0.9999	0.5
Metalaxyl	[g]MET	2.83	280.1	26	220.1 (13)	192.1 (17)	0.57	0.9986	0.2
Metalaxyl metabolite 108906	[g]CGA 108906	2.13	296.1	25	160.0 (25)	250.1 (10)	0.46	0.9988	0.8
Metalaxyl metabolite 62826	[g]CGA 62826	2.44	266.1	25	220.0 (12)	192.0 (15)	0.53	0.9994	0.5
Methiocarb	[g]METHI	3.36	226.0	28	169.0 (10)	121.0 (22)	0.58	0.9969	1
Metrafenone	[g]METR	5.55	409.0	28	209.1 (14)	226.9 (16)	0.43	0.9999	0.3
Myclobutanil	[g]MYC	3.65	289.1	34	70.2 (18)	125.1 (32)	0.29	0.9987	0.5
Penconazole	[g]PEN	4.18	284.0	34	70.1 (16)	159.0 (34)	0.31	0.9999	0.1
Prochloraz	[g]PROC	3.21	376.0	22	70.1 (34)	307.1 (16)	0.001	0.9957	0.9
Propiconazole	[g]PROP	4.46	342.0	46	69.0 (22)	159.0 (34)	0.59	0.9997	0.5
Pyraclostrobin	[g]PYRA	5.23	388.1	31	193.9 (12)	163.0 (25)	0.66	0.9970	0.2
Pyrimethanil	[h]PYR	2.72	200.0	51	107.0 (24)	82.0 (24)	0.71	0.9993	0.2

Compound	Abbreviation	Retention time (min)	[M+H]$^+$ ion (*m/z*. Da)	Cone voltage (V)	Q1 (CE. V)	Q2 (CE. V)	Q2/Q1 ratio	R^2 (1-200 ng mL^{-1})	ILOQ (ng mL^{-1})
Quinoxyfen	[g]QUIN	5.47	308.0	61	197.0 (32)	161.9 (44)	0.62	0.9997	0.3
Tebuconazole	[g]TEBU	3.97	308.0	40	70.1 (22)	125.0 (40)	0.07	0.9996	0.5
Tebufenozide	[g]TEBF	4.73	353.1	19	133.0 (20)	297.1 (8)	0.72	0.9998	1
Thiabendazole	[a]THIAB	1.85	202.0	51	175.0 (25)	131.0 (30)	0.62	0.9990	0.3
Thiacloprid	[g]THC	2.37	253.0	41	126.0 (20)	90.1 (40)	0.17	0.9988	0.5
Thiamethoxam	[i]THM	2.03	292.0	28	211.2 (12)	132.0 (22)	0.47	0.9907	1
Thiophanate methyl	THIOM	2.57	343.0	28	151.0 (46)	93.0 (19)	0.01	0.9956	10
Triadimefon	[g]TRIAF	3.8	294.1	31	69.3 (20)	197.2 (15)	0.69	0.9985	0.7
Triadimenol	[g]TRIAL	3.2	296.1	21	70.2 (10)	99.1 (15)	0.13	0.9968	0.9
Trifloxystrobin	[g]TRIF	5.62	409.0	34	186.0 (16)	145.0 (40)	0.44	0.9984	0.1
Zoxamide	[g]ZOX	5.13	336.0	38	187.1 (25)	159.0 (38)	0.45	0.9994	0.3
Carbendazim-d_3	[a]CAR-d_3	1.76	195.1	33	160.0 (18)	132.0 (33)	0.14		
Clothianidin-d_3	[b]CLOT-d_3	1.95	253.0	20	172.0 (10)	132.0 (15)	0.62		
Cyprodinil-d_5	[c]CYP-d_5	3.08	231.0	56	93.0 (33)	108.0 (25)	0.5		
Dimethomorph-d_6	[d]DIM-d_6	2.88	394.2	40	307.1 (20)	171.1 (30)	0.59		
Fenhexamide-d_3	[e]FENH-d_3	3.42	305.0	50	100.0 (20)	55.0 (30)	0.20		
Imidacloprid-d_4	[f]IMID-d_4	2.18	260.1	34	213.1(15)	179.1 (20)	0.98		
Metalaxyl $^{13}C_6$	[g]MET-$^{13}C_6$	2.83	286.1	26	226.1 (13)	198.1 (17)	0.63		
Pyrimethanil-d_5	[h]PYR-d_5	2.44	205.0	51	82.0 (24)	107.0 (24)	0.83		
Thiamethoxam-d_4	[i]THM-d_4	1.84	296.0	20	215.0 (10)	132.0 (20)	0.44		

*FLUD: ionization mode ESI$^-$

[a] to [i] identify the IS associated to each compound.

Table S2. Codes and physical properties of soils involved in the study.

Designation of Origin	Vineyard code	[a]Soil pH	Organic carbon (%)	Nitrogen (%)	Sand	Silt	Clay	Soil texture
Rías Baixas	1	4.3	7.5	0.55	59.1	23.6	17.3	Sandy loam
Rías Baixas	2	4.4	8.1	0.60	57.4	22.7	19.9	Sandy loam
Rías Baixas	3	4.7	6.2	0.47	61.5	24.4	14.1	Sandy loam
Ribeiro	4	5.7	3.9	0.19	59.4	27.8	12.8	Sandy loam
Ribeiro	5	4.7	5.4	0.37	32.8	45.8	21.4	Loam
Ribeiro	6	3.8	3.4	0.24	36.8	45.3	17.9	Loam
Ribeira Sacra	7	4.7	8.3	0.73	69.5	16.8	13.7	Sandy loam
Ribeira Sacra	8	5.7	5.1	0.40	65.6	16.7	17.7	Sandy loam
Ribeira Sacra	9	4.4	5.1	0.27	59.6	26.6	13.8	Sandy loam

[a]Values measured in $CaCl_2$ 0.01M.

Table S3. Matrix effects (MEs, %) in two different vineyard soils with low and high organic matter content.

Compound	Matrix Effects (%)			
	OC 1.5%		OC 8.3%	
	Average	SD	Average	SD
ACE	68.0%	1.2%	52.6%	2.1%
AME	101.7%	1.6%	101.8%	3.3%
AZO	103.0%	1.4%	97.2%	4.3%
BEN	99.0%	1.3%	84.3%	3.5%
BIT	95.7%	1.6%	78.8%	1.9%
BOS	90.9%	2.9%	92.7%	2.8%
CAR	117.4%	1.2%	90.3%	5.3%
CGA 108906	108.5%	1.8%	89.8%	2.5%
CGA 62826	95.9%	0.9%	85.8%	4.0%
CHLOR	97.6%	2.6%	72.2%	3.1%
CHLORA	93.6%	1.0%	66.1%	3.9%
CHLORM	103.5%	3.8%	88.7%	0.8%
CLO	49.3%	3.5%	44.2%	9.3%
CLOF	102.5%	2.1%	97.7%	4.6%
CYF	94.8%	1.7%	69.3%	2.9%
CYM	83.1%	2.4%	88.4%	5.7%
CYP	99.5%	2.0%	93.6%	4.3%
DIF	95.2%	1.7%	74.3%	2.0%
DIM	85.3%	1.55	61.8%	4.1%
DIN	93.4%	1.7%	79.3%	2.9%
FEN	105.6%	1.5%	99.3%	3.4%
FENA	91.3%	1.3%	94.2%	3.4%
FENH	97.1%	1.5%	86.9%	6.0%
FLUD	116.5%	4.7%	75.3%	4.1%
FLUF	93.0%	2.4%	75.2%	3.1%
FLUO	97.3%	1.8%	83.7%	3.8%
FLUS	97.6%	1.6%	86.0%	5.7%
IMA	100.0%	1.4%	98.9%	2.9%
IMI	53.3%	4.6%	39.7%	3.1%
IPR	102.7%	2.3%	90.5%	3.4%
MAN	91.5%	2.0%	82.4%	3.4%
MET	95.9%	0.9%	85.8%	4.0%
METHI	97.4%	2.7%	77.1%	2.4%
METR	96.0%	1.3%	75.9%	4.3%
MYC	96.1%	2.2%	86.9%	5.5%
PEN	96.6%	1.8%	88.9%	3.3%

Compound	**Matrix Effects (%)**			
	OC 1.5%		**OC 8.3%**	
	Average	**SD**	**Average**	**SD**
PROC	98.8%	1.9%	91.5%	6.0%
PROP	93.4%	1.5%	78.4%	2.4%
PYR	92.7%	1.6%	85.7%	3.8%
PYRA	98.0%	0.7%	75.6%	4.6%
QUIN	90.0%	1.5%	71.9%	3.2%
TEBF	98.6%	1.6%	88.5%	3.4%
TEBU	98.3%	2.1%	84.7%	2.7%
THC	60.4%	1.9%	40.8%	4.4%
THIAB	88.4%	2.2%	74.0%	3.0%
THM	52.4%	5.0%	33.5%	1.2%
TRIAF	98.7%	2.3%	91.2%	2.2%
TRIAL	98.9%	2.9%	91.4%	5.1%
TRIF	92.7%	1.8%	69.9%	2.9%
ZOX	96.1%	2.0%	69.0%	4.6%

Table S4. Summary of concentrations in soil samples before each annual application campaign. Values in ng g^{-1}, n=3 replicate extractions.

Vineyard code	**1**		**2**		**3**	**4**		**5**		**6**			**7**	**8**	**9**
Year	**2018**	**2019**	**2018**	**2019**	**2018**	**2018**	**2019**	**2018**	**2019**	**2017**	**2018**	**2019**	**2018**	**2019**	**2019**
BEN	8.6	4.2	10.3	2.8		72.7	90.5							7.3	
BOS										47.6	32.3	216.9			
CAR	356.6	142.1	117.9	69.9	29.2	15.8	11.5	8.0	11.8	203.6	200.6	75.9		31.2	18.7
CGA 108906	8.3														
CGA 62826	23.2	12.2	6.2	5.4				13.7	23.3	22.8	4.4	45.6			
CHLOR	5.2		11.1												
CYP					2.9	78.4									
DIF		3.0		3.8											
DIM	34.7	2.4	6.2	4.5	502.4	2.7				929.4	514.8	2302.3			262.7
DIN														12.0	
FENH	13.0	15.6	21.7	23.4											
FLUD						316.4									
FLUO	9.0	4.2	24.1	20.8										149.0	
IMI	42.2	45.6	46.1	49.8											
IPROV	4.7	3.0	8.9	8.3	28.2		7.4								
MET	15.8	11.4	18.6	15.0	8.4	9.3	6.8	35.9	9.0	78.1	27.1	90.1		10.6	14.1
MYC			2.3			405.5	525.1	9.4	10.4				30.8	93.9	230.3
PEN					7.0	65.5	54.3			116.4	52.4	37.3			
PROP						12.7									
PYR							53.1			213.8	145.5	100.1			25.0
PYRA								81.0	329.8						7.3
TEB														101.4	93.0
TRIAL					26.5								30.7		
SUM	521.2	243.7	273.5	203.5	604.5	979.0	748.8	147.9	384.3	1611.7	977.0	2868.2	61.5	405.4	651.1
Compounds above method LOQs	11	10	11	10	7	9	7	5	5	7	7	7	2	7	7

Table S5. Rain (mm) and average temperature (ºC) at different sampling points during the non-vegetative period of vines.

Vineyard code	October 2017	November 2017	December 2017	January 2018	February 2018	March 2018	Total rain (mm)
	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	
1,2	48.6, 16.7	60.2, 11.5	170.9, 9.8	161.7, 10.0	134.0, 9.4	271.5, 9.8	847
4	22.4, 15.1	78.8, 7.5	173.0, 6.2	91.0, 7.9	88.2, 6.2	314.6, 8.6	768
5,6	23.4, 16.2	69.6, 8.4	123.8, 6.9	75.4, 8.3	65.0, 6.8	265.4, 8.8	623

Table 5, cont.

Vineyard code	October 2018	November 2018	December 2018	January 2019	February 2019	March 2019	Total rain (mm)
	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	Rain (mm), Tª (ºC)	
1,2	128.0, 15.2	268.0, 12.3	182.0, 11.8	113.0, 8.6	42.0, 11.5	89.0, 12.2	822
4	101.0, 13.4	243.0, 10.4	87.4, 9.2	64.2, 4.3	41.2, 8.1	88.8, 10.9	626
5,6	67.8, 14.6	175.4, 10.8	84.0, 9.5	73.2, 5.1	30.8, 8.7	61.2, 11.4	492

Table S6. Dissipation percentages of different pesticides during the non-vegetative cycle of vines (October-March).

Compound	**Year**	**Vineyard code**					**Average (%)**	**SD**
		1	**2**	**4**	**5**	**6**		
BEN	2017-18			80			75	
	2018-19			71				
BOS	2017-18					18	18	
	2018-19					18		
CAR	2017-18			52		10	36	20
	2018-19	62	32	18		39		
CGA 62826	2017-18				75	68	70	11
	2018-19	81	82		58	56		
CYP	2017-18			66			55	
	2018-19			45				
DIM	2017-18					15	12	
	2018-19					10		
FLUD	2017-18			67			67	
	2018-19							
IMI	2017-18	36	11				28	12
	2018-19	31	35					
MET	2017-18		45	70	61	55	63	16
	2018-19	86	39	57	82	75		
MYC	2017-18			34	27		34	8
	2018-19			43				
PEN	2017-18			47		32	39	8
	2018-19			32		46		
PYR	2017-18					23	34	

Compound	Year	Vineyard code					Average (%)	SD
		1	2	4	5	6		
	2018-19					45		
PYRA	2017-18				8		15	
	2018-19				23			