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Abstract

Knowledge of the degradation extent and pathways of fungicides in the environment is scarce. Fungicides may have isomers with distinct fungal-control efficiency, toxicity and fate in the environment, requiring specific approaches to follow up the degradation of individual isomers. Here we examined the degradation of the widely used fungicide dimethomorph (DIM) in a vineyard catchment using the signatures of carbon stable isotopes ($\delta^{13}C$) and E/Z isomer fractionation (IF(Z)). In a microcosm laboratory experiment, DIM degradation half-life in soil was 20 ± 3 days, and was associated with significant isomeric ($\Delta IF(Z)$ = +30%) and isotopic ($\Delta\delta^{13}C$ up to 7 ‰) fractionation. This corresponds to an isomer enrichment factor of ε_{IR} = -54 ± 6%, suggesting isomer selectivity and similar carbon stable isotopic fractionation values of $\varepsilon_{DIM-(Z)}$ = -1.6 ± 0.2 ‰ and $\varepsilon_{DIM-(E)}$ = -1.5 ± 0.2 ‰. Isomeric and isotopic fractionation values were used to estimate DIM degradation in topsoil and transport in a vineyard catchment over two wine-growing seasons. DIM concentrations following DIM application were up to 3 $\mu g.g^{-1}$ in topsoil and 29 $\mu g.L^{-1}$ in runoff water at the catchment outlet. Accordingly, the IF(Z) and $\delta^{13}C$ values of DIM in soil were similar to those observed in DIM commercial formulations. The gradual enrichments in DIM-(Z) and ^{13}C of the residual DIM in soil indicated DIM biodegradation over time. DIM biodegradation estimated based on E/Z isomer and carbon stable isotope ratios in topsoil and runoff water ranged from 0% after DIM application up to 100% at the end of the wine-growing season. DIM biodegradation was overestimated compared to conventional approaches relying on DIM mass balance, field concentrations and half-lives. Altogether, our study highlights the usefulness of combining carbon isotopes, E/Z isomers and classical approaches to estimate fungicide degradation at the catchment scale, and uncovers difficulties in using laboratory-derived values in field studies.

KEYWORDS: Dimethomorph, isotopes, isomers, CSIA, ISIA, vineyard, pesticides

HIGHLIGHTS:

- Degradation estimates of dimethomorph (DIM), a widely used fungicide in vineyards
- DIM half-life of 20 ± 3 days in vineyard topsoil
- DIM isomer selectivity (ε_{IR} = -54 ± 6%) during biodegradation
- Carbon isotopic fractionation (εc) similar for DIM-(E) and DIM-(Z) biodegradation
- Stable isotopes and isomers to estimate DIM degradation at the catchment scale

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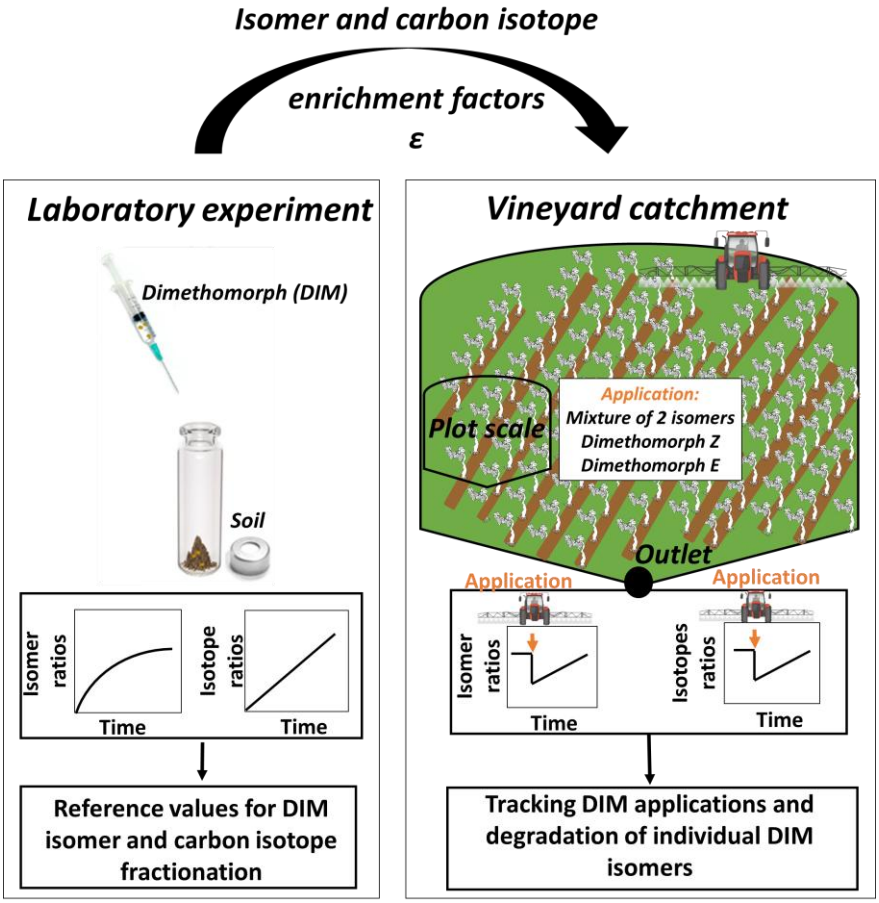

1. Introduction

The proportion of modern pesticide formumations with active ingredients containing several isomers has gradually increased over the last decade (Jeschke, 2018). Stereoisomers are molecules with the same molecular formula and constitution, but differing in the three-dimensional orientations of their atoms in space. The great majority of manufactured stereoisomeric pesticides are mixture of stereoisomers, although the bioactivity is primarily associated with only one stereoisomer (Kurihara et al., 1997). Individual stereoisomers, however, may differ in pest-control efficiency (Garrison, 2006), toxicity (Huang et al., 2012; Xie et al., 2018; Zhai et al., 2019), and fate (Buser et al., 2000; Gámiz et al., 2016; Hegeman and Laane, 2002). Within stereoisomers, diastereisomers (e.g., (E)/(Z)) are molecules which are neither superimposable nor the image of each other in a mirror. In environmental studies, stereochemistry is often neglected, while isomers are considered as a unique molecular entity. Stereoisomer-specific studies are thus crucial to assess the risks associated with stereoisomeric pesticides (Basheer, 2018; de Albuquerque et al., 2018; Liu et al., 2005; Petrie et al., 2015).

The widely used fungicide dimethomorph (4-[3-(4-chlorophenyl)-3-(3-4-dimethox-yphenyl) acryloyl] morpholine) (DIM) contains a double bond and consists of the stereoisomers DIM-(E) and DIM-(Z). The frequent detection of DIM in vineyard soils, waters and grapes mirrors regular DIM applications and moderate DIM degradation in soil (half-life $T_{1/2}$ range: 41-96 days in aerobic soils (EFSA, 2006)), posing a risk for surrounding living organisms (Imfeld et al., 2020; Liu et al., 2012; Shabeer et al., 2015). DIM was selected in this study as a model stereoisomeric pesticide because (i) it is used worldwide and in several crops to control plant diseases, such as mildew and root rot, (ii) the fungicidal activity stems

from the (Z)-isomer of DIM (PPDBwebsite), although commercial formulations are not enriched in the (Z)-isomer, and (iii) knowledge of the degradation of individual DIM stereoisomers and the direction of stereoselective degradation of DIM is scarce.

Among degradative and nondegradative processes contributing to pesticide dissipation in the environment, DIM biodegradation may be isomer-selective (Huang et al., 2012; Li et al., 2012; Masbou et al., 2018b). DIM biodegradation with preferential use of the E-stereoisomer has been reported (Jia and Zhu, 2015; Suryawanshi et al., 2018; Zhang et al., 2020), supporting the use of isomeric ratios as a DIM biodegradation proxy. In stereoisomeric pollutants, one isomer may degrade faster than the other due to preferential microbial uptake or enzyme activity, stemming from different geometrical recognitions of the isomer molecules (Jammer et al., 2014). Hence, gradual changes in the isomeric fractions (IF) or ratios (IR) from the initial isomeric signature may help evaluate degradation in the field (Jin and Rolle, 2016; Williams et al., 2003). However, this approach may be limited for DIM due to its sensitivity to isomerization mechanisms (i.e., interconversion of (E) and (Z) isomers, (Avetta et al., 2014)) and the isomer-selective dependence of DIM degradation on specific soil properties, such as organic matter and pH (Li et al., 2013; Yang and Ji, 2015).

In this context, combining isomer analysis and compound-specific stable isotope analysis (CSIA) in the so-called isomer-selective isotope analysis (ISIA or ESIA for enantiomer-specific cases) may help to identify sources and transformations of pesticide isomers in the environment (Badea and Danet, 2015; Maier et al., 2013). Similar to isomers, pesticide molecules with light and heavy stable isotopes are degraded at slightly different rates, which results in a kinetic isotope effect (Hoefs, 1997). Pesticide CSIA can be used to evaluate the *in situ* biodegradation of pesticides and has been applied in various experimental settings (Cui et al., 2021; Drouin et al., 2021; Droz et al., 2021; Masbou et al., 2018a; Melsbach et al., 2020; Meyer et al., 2009; Pérez-Rodríguez et al., 2021; Torrentó et al., 2021)

and in modelling studies (Lutz et al., 2017). However, field studies involving the pesticide CSIA (Alvarez-Zaldívar et al., 2018; Wu et al., 2018) remain limited thus far, mainly due to conceptual and analytical limits. ESIA has been developed for several chiral compounds (e.g., polar herbicides, HHCB, phenoxyalkanoic methyl herbicides, anilide fungicides and PCBs) (Jammer et al., 2014; Maier et al., 2013; Masbou et al., 2018b; Milosevic et al., 2013; Tang et al., 2017; Wang et al., 2013). In contrast, ISIA is currently limited to hexachlorocyclohexane (HCH) isomers (Badea et al., 2011; Bashir et al., 2013; Zhang et al., 2014). Here we hypothesised that the application of DIM-ISIA may help to evaluate the contribution of DIM biodegradation to the overall DIM dissipation in the field, where both degradative and nondegradative processes co-occur.

The purpose of this study was thus to examine the degradation and transport of DIM isomers at the scale of a small (42.7 ha) vineyard catchment (Rouffach, France) using isotopic and isomeric signatures. ISIA and isomeric ratios measurements were developed for DIM isomers and applied to both laboratory microcosm experiments and field samples to evaluate (i) the degradation of DIM isomers in vineyard topsoil and runoff water, and (ii) the applicability of isomeric and isotopic approaches to estimate the contribution of degradation to the overall DIM dissipation at the catchment scale.

2. Materials and methods

2.1. Chemicals and solution preparation

DIM standard (PESTANAL, purity: >97%), metolachlor-d11 (analytical grade purity, >97%), solvents (dichloromethane DCM, acetonitrile ACN, ethyl acetate EtOAc; HPLC grade purity, >99.9%) and sulfate magnesium ($MgSO_4$, ReagentPlus® ≥99.5%) were purchased from Sigma–Aldrich. Primary secondary amide (PSA)-bonded silica was

purchased from Supelco. For further calibration standard preparation and reproducibility check, a stock solution of DIM at 1 $g.L^{-1}$ was prepared in dichloromethane (DCM) and stored at -18 °C until analysis. In the absence of DIM(E) or DIM(Z) standards, all measurements and experiments were carried out using available DIM standard from Sigma-Aldrich (IF(Z) = 0.61 ± 0.01, n=10).

2.2. Study site

The 42.7 ha Hohrain vineyard catchment is located in the Alsatian foothills (47°57′9 N, 07°17′3 E) and is representative of northern vineyards (Duplay et al., 2014; Imfeld et al., 2020) (Figure S1). The catchment has a mean slope of 15% and is mainly occupied by vineyards (59%, 25.3 ha), forests and pasture (29%), grass strips and ditches (7%), and roads and paths (5%). The soil is a calcareous clay loam developed on a loess basement with a bulk density of 1.4 g cm^{-3}. The main soil types are Cambisol (Hypereutric Clayic) and Haplic Cambisol (Calcaric Siltic) (Duplay et al., 2014). Vineyard plots are permanently covered by grass every second inter row to limit soil erosion, and grass-free interrows are ploughed down to a 5 cm depth to enhance water infiltration.

Rainfall-runoff events generate intermittent discharge at the outlet of the catchment, driven by Hortonian overland flow. Vineyard plots contribute to discharge when rainfall intensity overcomes saturated hydraulic conductivity (Tournebize et al., 2012). Grass strips with a 2 to 3 m width at the plot edges limit overland flow on plots (Lefrancq et al., 2014).

2.3. Pesticide applications and sampling

The dose and frequency of pesticide applications were obtained from surveys addressed to wine producers of the catchment in 2015 and 2016. Answers from producers covered 75% and 74% of the total vineyard area in 2015 and 2016, respectively. The dose and timing of applications were extrapolated for the non-surveyed area to calculate the seasonal

pesticide applications. The amount of DIM applications in the vineyard catchment estimated by surveys of the farmers was 119 g in 2015 and 5406 g in 2016 (Dose: 2 to 6 $kg.ha^{-1}$) (Imfeld et al., 2020). The high mildew pressure in 2016 associated with unfavourable wet and warm climatic conditions in early summer explained the significant difference in applied amounts between the two years. DIM was applied on June 19th in 2015 and between June 20th and 23rd in 2016.

The sampling procedure is detailed in Table S1. Briefly, topsoil samples (0-1 cm) were collected weekly from March to October 2015 and 2016 across four transects across the catchment (Figure S1) (Imfeld et al., 2020). Topsoil samples were also collected every 7 to 30 days in two adjacent and representative vineyard plots, 1486 m^2 each, namely, P1 and P2 (locations on Figure S1). Composite topsoil samples (1 kg) consisted of mixed topsoil subsamples collected every 10 m across the transects in the catchment and every 2 m across 5 interrows for each plot. Topsoil samples were transported in coolers and stored at -18 °C until DIM extraction.

Runoff samples were collected at the catchment and plot outlets (Imfeld et al., 2020). Briefly, automatic runoff measurement and water sampling were carried out from March 26th to October 6th, 2015, and from March 30th to October 10th, 2016. Water depth was measured at the catchment outlet using a bubbler flow module (Hydrologic) combined with a Venturi channel to monitor discharge runoff, with an uncertainty of ±8%. Flow proportional samples (100 ml every 6 L) of runoff water were collected during each runoff event using a 4010 Hydrologic automatic refrigerated sampler. Runoff water was collected in a 24-bottle sampler with precleaned glass bottles.

Runoff water from the plots was collected in a gutter located downstream of each plot. The gutters were covered to limit photolysis and contamination during the passage of agricultural machinery. Water flow at the outlet of the plots was monitored continuously

using an ultrasonic height sensor (ISMA) connected to a flowmeter and combined with an exponential section Venturi channel (ISMA). Runoff water samples were collected in proportion to the flow rate at each flowing event (100 mL every 3 L) using a flow-controlled refrigerated automatic sampler (ISCO). Samples were combined weekly into a composite sample. Water samples were refrigerated during sampling and placed on ice during transport to the laboratory to limit pesticide degradation. Water samples were immediately filtered using a 0.45 µm glass filter membrane and frozen (-18 °C) until extraction and DIM analysis.

2.4. DIM biodegradation experiment in microcosms

Topsoil (0-5 cm) from the vineyard plots (Table S3 for soil physicochemical characteristics) was collected and pooled on August 6th, 2015, to evaluate isomer and isotope fractionation during aerobic DIM biodegradation. The DIM concentration in topsoil before DIM spiking was below the LOD. The soil microcosms consisted of 30 mL glass vials. To maintain aerobic conditions while limiting water loss and avoiding contamination, a 0.2 µm syringe filter (Rotilabo®, Carl Roth®, France) was mounted on a syringe tip stuck through the vial cap (see experimental setup in the SI, Figure S5). Approximately 15 g of soil was incubated, and deionized water was added to reach a volumetric water content of 20% of soil. The soil was spiked with an environmentally relevant concentration of 10 µg.g^{-1} DIM. Abiotic control experiments were prepared similarly to sterilized soils (autoclaved three times at 12-hour intervals). Ten separate experiments and 10 abiotic controls were incubated at 20 ± 1 °C and collected using a sacrificial approach at Days 1, 10, 25, 50 and 100 (duplicate measurements at each sampling date).

2.5. Dimethomorph extraction and analysis

DIM was extracted from water samples using solid-phase extraction (SPE) and from soil samples using QUECHERS modified extraction procedures, as previously described

(Gilevska et al., 2022). DIM isomers were quantified by gas chromatography (Trace 1300, Thermo Fisher Scientific) coupled with a mass spectrometer (ISQTM, Thermo Fisher Scientific) using a Metolachlor-d11 isotope-labelled internal standard. As the DIM standard was not certified in DIM(E) and DIM(Z) isomers, the ratios of peak areas for DIM(E) and DIM(Z) isomers obtained from GC-MS in SIM/Fullscan mode and GC-IRMS were used to characterized the DIM standard. Both GC-MS and GC-IRMS measurements gave similar and reproducible (IF(Z) = 0.61 ± 0.1) isomer ratios. Distinct calibrations curves were then obtained to evaluate separately DIM(E) and DIM(Z) concentrations. Extraction recoveries, DL and QL from water and soil samples are provided in Table S2.

DIM isomer separation was sufficient for accurate isotope analysis (isomer resolution, R>2) (Hengel and Shibamoto, 2000). Typical chromatograms (pure standard, water and topsoil samples) are provided in SI (Figure S2). The soil extraction procedure had no significant effect on the Z/E isomer ratios and the carbon isotope composition (SI, Section S-3).

2.6. Carbon stable isotope analysis of dimethomorph

The carbon isotope compositions of DIM isomers ($\delta^{13}C_{DIM\text{-}(Z)}$ and $\delta^{13}C_{DIM\text{-}(E)}$) were determined using a GC-C-IRMS system consisting of a gas chromatograph (Trace GC Ultra, Thermo Fisher Scientific) coupled *via* a GC IsoLink/Conflo IV interface (Thermo Fisher Scientific) to an isotope ratio mass spectrometer (DeltaV Plus, Thermo Fisher Scientific). The $\delta^{13}C$ values were calibrated using a three-point calibration using the international reference materials AIEA-600, USGS-40, and USGS-41 (σ <0.05 ‰) and reported in parts per thousand (‰) against the Vienna Pee Dee Belemnite (VPDB) reference standard, according to Equation 1:

$$\delta^{13}C_{DIM-(X)} = \left(\frac{(^{13}C_{DIM-(X)}/^{12}C_{DIM-(X)})_{sample}}{(^{13}C_{CO2}/^{12}C_{CO2})_{ref}} - 1\right) \times 1000 \quad (1)$$

with X = bulk, (Z) or (E)

Details of the DIM-ISIA validation protocol are provided in the SI (Section S-3). Briefly, linearity tests were performed on DIM standards, indicating a minimum injection of 6 ngC for both DIM-(E) and DIM-(Z) for reliable DIM CSIA. The measurement precision of $\delta^{13}C_{DIM\text{-}(Z)}$ = -33.6 ± 0.7 ‰ (± σ, n = 20) was similar to that of $\delta^{13}C_{DIM\text{-}(E)}$ = -33.5 ± 0.7 ‰ and $\delta^{13}C_{Bulk\text{-}DIM}$ = -33.5 ± 0.5 ‰. The total analytical uncertainty of σ < 0.7 ‰ for amplitude >100 mV (m/z 44) on each isomer (>6 ngC) was consistent with typical CSIA measurements (Schürner et al., 2016). Long-term $\delta^{13}C$ values obtained during 3 different GC-C-IRMS sessions within 3 months (Figure S4 and Table S2) did not significantly differ from the bulk value ($\delta^{13}C_{Bulk\text{-}DIM}$ = -33.0 ± 0.5 ‰; ± σ, n = 3) obtained with an elemental analyser-isotopic ratio mass spectrometer (Flash EA IsoLinkTM CN IRMS, Thermo Fisher Scientific). Cross-calibration of the DIM Sigma–Aldrich standard confirmed insignificant isotopic fractionation associated with the extraction procedure and DIM-ISIA.

Despite the robustness of DIM-ISIA analytical method, several field samples (n = 50) failed the quality check due to either low concentrations, yielding peak amplitude <100 mV, or co-elution. The validated DIM-ISIA measurements (n = 29) were thus limited, leading to a discrepancy between soil and water samples in 2015 and 2016.

2.7. Isomeric fractionation and carbon isotope calculations

Change in the proportion of each isomer was calculated by isomer fractionation IR(Z) and IF(Z) isomeric ratio and fractionation respectively, and according to:

$$IR(Z) = \frac{[DIM-(Z)]}{[DIM-(E)]} \qquad (2)$$

$$IF(Z) = \frac{[DIM-(Z)]}{[DIM-(Z)]+[DIM-(E)]} \qquad (3)$$

Rac-DIM mixture thus displays IR(Z) = 1 and IF(Z) = 0.5, while an enrichment in DIM-(Z) features an IR(Z) > 1 and IF(Z) > 0.5.

An isotopic balance calculation enabled determining the isotopic composition of bulk-DIM ($\delta^{13}C_{bulk\text{-}DIM}$) from the stable isotope ratios of the individual DIM isomers determined by GC-C-IRMS (Badea et al., 2011):

$$\delta^{13}C_{Bulk-DIM} = IF_{DIM-(Z)} \times \delta^{13}C_{DIM-(Z)} + IF_{DIM-(E)} \times \delta^{13}C_{DIM-(E)} \quad (4)$$

The bulk-DIM $\delta^{13}C_{bulk\text{-}DIM}$ of the analytical standard was also determined by GC-IRMS analysis as a double-peak integration method and by EA-IRMS, confirming the isotopic balance approach (Table S2). Isotopic fractionation values (ε) for DIM-(E) and DIM-(Z) were calculated from the logarithmic linearization using the Rayleigh equation:

$$\ln\left(\frac{\delta^{13}C_x+1}{\delta^{13}C_0+1}\right) = \varepsilon \times \ln\left(\frac{C_x}{C_0}\right) \quad (5)$$

where $\delta^{13}C_0$ and $\delta^{13}C_x$ are the measured carbon isotope ratios at the beginning (t = 1 day) and at t = *x* days from the beginning of the experiment, and C_0 and C_x are the corresponding concentrations.

The carbon isotopic fractionation value (ε), derived from closed soil degradation experiments under mixed aerobic conditions, were used to quantify field degradation B(%) (Alvarez-Zaldívar et al., 2018; Höhener et al., 2022; Hunkeler et al., 2008):

$$B(t,\%) = \left(1 - \left(\frac{\delta^{13}C_t + 1000}{\delta^{13}C_0 + 1000}\right)^{\frac{1000}{\varepsilon}}\right) \times 100 \quad (6)$$

Similarly, the Rayleigh equation allows deriving isomeric enrichment factors ε_{IR} from linear regression of:

$$\ln\left(\frac{IR(Z)x}{IR(Z)0}\right) = \varepsilon_{IR} \times \ln\left(\frac{C_x}{C_0}\right) \quad (7)$$

IR is generally prefered to IF for ε_{IR} calculations (Jammer et al., 2014). The carbon isotopic fractionation value (ε_{IR}), derived from closed soil degradation experiments under mixed aerobic conditions, were used to quantify field degradation B(%):

$$B(t,\%) = \left(1 - \left(\frac{IR\,(Z)_t}{IR\,(Z)_t}\right)^{\frac{1}{\varepsilon}}\right) \times 100 \qquad (8)$$

The error of the isotopic fractionation values and the isomeric enrichment factors was given as the 95% confidence interval (CI) and determined using regression analysis, as described elsewhere (Elsner et al., 2007).

2.9. Statistical analysis

Statistical analyses were carried out using JMP software (SAS Institute, USA) and the function "Data Analysis" from the Add-In "Analysis Tool Pack" of Microsoft Excel®. Isotopic and isomeric fractionation values were calculated from linear regressions of Equation (5) and (7) respectively, and rate constants (or half-lives) were determined using a single first-order rate model (SFO) from plots of ln (C/C_0) versus time (see SI for details). Measurements were performed in triplicate and all data are displayed as the mean ±95% CI unless otherwise specified.

3. Results and discussion

3.1. Isomer-selective biodegradation of DIM in soil microcosms

In abiotic microcosm experiments, DIM_{tot} concentrations in soils did not vary over time (Figure 1), except on Day 100 ($[DIM]_{t=100}/[DIM]_{t=1}$ = 0.65) in a single replicate. High DIM half-lives ($T_{1/2}$ >100 days) in abiotic experiments coincided with insignificant isomer selectivity (IF(Z) = 0.61 ± 0.01, n = 10). In contrast, $T_{1/2}$ = 19.6 ± 3.2 days for DIM in biotic

microcosms (Table 1) emphasised the prevailing contribution of DIM biodegradation to its overall dissipation in soil. Degradation of the (E)-isomer of DIM ($T_{1/2\ (DIM\text{-}(E)}$ = 13.7 ± 2.7 days) was faster than that of the (Z)-isomer ($T_{1/2\ (DIM\text{-}(Z)}$ = 22.2 ± 5.2 days). These half-lives are in the range of those reported in previous field dissipation studies ($T_{1/2\ (DIM\text{-}(Z)}$ = 9-86 days and $T_{1/2\ (DIM\text{-}(E)}$ = 11-30 days) (FAO, 2014). Here, the vineyard soil used to derive $T_{1/2}$ was frequently treated with GripTop® (commercial formulation containing DIM), which may favour DIM-adapted microbial communities and higher DIM dissipation rates.

DIM-(Z) isomer fraction (IF(Z)) increased from 0.61 ± 0.01 up to 0.91 ± 0.01 (n = 2) after 100 days, indicating an enrichment in the DIM-(Z) isomer during biodegradation. The relationship between IF(Z) (or IR(Z)) and the degradation extent was significant (r^2 >0.97, p* <0.001, n = 8), yielding an IR enrichment factor (ε_{IR}) = -54 ± 6% (Figure S6, equation 7). Although no ε_{IR} for DIM was previously retrieved, ε_{IR} for other organic molecules, including enantiomers, typically ranged from -4 to -237% (Esslinger et al., 2011; Jammer et al., 2015; Qiu et al., 2014; Souchier et al., 2016). Altogether, these results highlight significant isomer-selectivity during DIM biodegradation in soil.

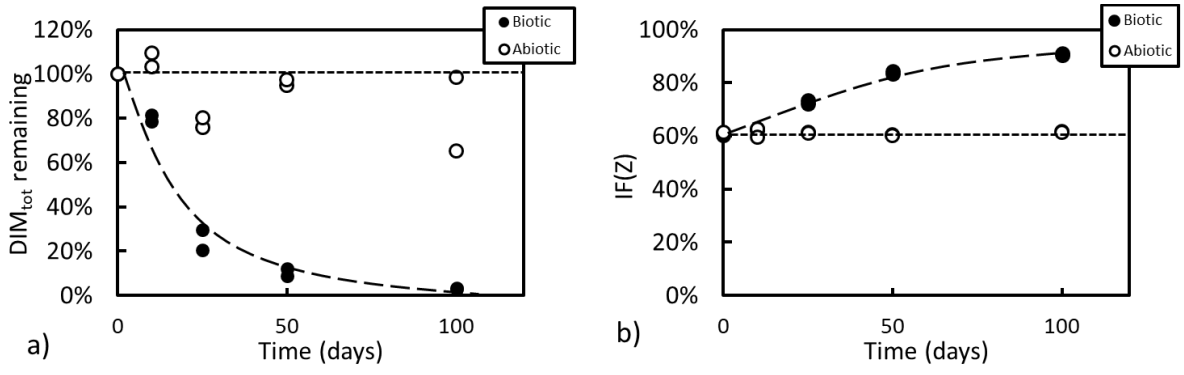

Figure 1: DIM concentrations (a) and IF(Z) (b) in biotic and abiotic soil experiments.

3.2. Isomer-selective isotope analysis of DIM in soil microcosms

The carbon isotope composition ($\delta^{13}C$) of DIM in biotic soil experiments varied from $\delta^{13}C_{DIM-(Z)}$ = -33.7 ± 0.3 ‰ and $\delta^{13}C_{DIM-(E)}$ = -33.5 ± 0.1 ‰ (t = 1 day) to $\delta^{13}C_{DIM-(Z)}$ = -29.1 ± 0.1 ‰ and $\delta^{13}C_{DIM-(E)}$ = -26.5 ± 0.5 ‰ (t = 100 days). In contrast, no significant change of the $\delta^{13}C$ of both DIM isomers after 100 days in the abiotic experiment confirmed no or limited DIM biodegradation. Isotopic fractionation values for DIM-(Z) ($\varepsilon_{DIM-(Z)}$ = -1.6 ± 0.2 ‰, equation 5) and DIM-(E) ($\varepsilon_{DIM-(E)}$ = -1.5 ± 0.2 ‰) in the biotic soil experiments were similar (Table 1 and Figure S6). Since this is the first report for DIM bulk or isomer-selective isotopic fractionation, ε_c values cannot be compared. The low isotopic fractionation values for DIM reflect isotope dilution since the DIM molecule contains 21 carbon atoms. The two main metabolites expected from a demethylation pathway are M550F006 (Z67, meta desmethyl dimethomorph) and M550F007 (Z69, para desmethyl dimethomorph) (Avetta et al., 2014; Zhang et al., 2020). However, the DIM degradation pathways in soil could not be confirmed based on DIM degradation products as analytical standards are currently lacking.

Table 1: DIM biodegradation kinetics and isomeric (ε_{IR}) and isotopic (ε_c) fractionation observed in the laboratory biodegradation experiment with the vineyard soil.

	$T_{1/2}$ ± 95% CI [days]	**ε_{IR} ± 95% CI [%]**	**ε_c ± 95% CI [‰]**
DIM-(E)	13.7 ± 2.7		- 1.6 ± 0.2
DIM-(Z)	22.2 ± 5.2	- 54 ± 6	- 1.5 ± 0.2
DIMtot	19.6 ± 3.2		- 1.5 ± 0.2

3.3. DIM degradation and runoff transport in the vineyard plots

Overall, the DIM concentrations in the soil significantly ($p < 0.001$) differed in 2015 (0.25 ± 0.12 $\mu g.g^{-1}$; n = 114, plots and catchment samples) and 2016 (1.61 ± 0.14 $\mu g.g^{-1}$; n = 93) and followed a seasonal application pattern. At the plot scale, DIM concentrations ranged

329 from below the DL up to 1.8 μg.g^{-1} and 10.1 μg.g^{-1} in the topsoil of the vineyard plots in 2015
330 and 2016, respectively (Figure 2). The DIM concentrations in the P1 and P2 plots were
331 similar ($p > 0.05$), confirming the reproducibility of the sampling strategy.

332 DIM_{tot} concentrations in runoff water from the plots ranged from <LD up to 12 μg.L^{-1}
333 and were above the French drinking water limit of 0.1 μg. L^{-1} for 31 of the 39 samples.
334 Although EFSA estimates that DIM acute toxicity towards aquatic organisms is low (EFSA,
335 2006), several studies underscored an ecotoxic risk for several living organisms, including
336 soil and water microflora (Avetta et al., 2014; Lunn, 2007) or in mixtures with other
337 substances, such as difenoconazole (Fan et al., 2021) or copper (Megateli et al., 2013).

338 Altogether, isomeric fractionation values of the IF(Z) of DIM in the plot topsoil and
339 runoff water indicated significant DIM degradation. Indeed, the IF(Z) values from 0.5 to 0.8
340 in the vineyard plots in 2015 and 2016 were generally larger than the IF(Z) value of the DIM
341 commercial formulation (GripTop®; 0.50 ± 0.02). This highlights that DIM-(Z) was
342 systematically enriched in topsoil and runoff, while DIM-(E) degraded preferentially, in
343 agreement with the results of the soil microcosm experiments. Similar IF(Z) values in topsoil
344 and in runoff water at the plot scale suggest that DIM in runoff stems from a unique DIM pool
345 in soil.

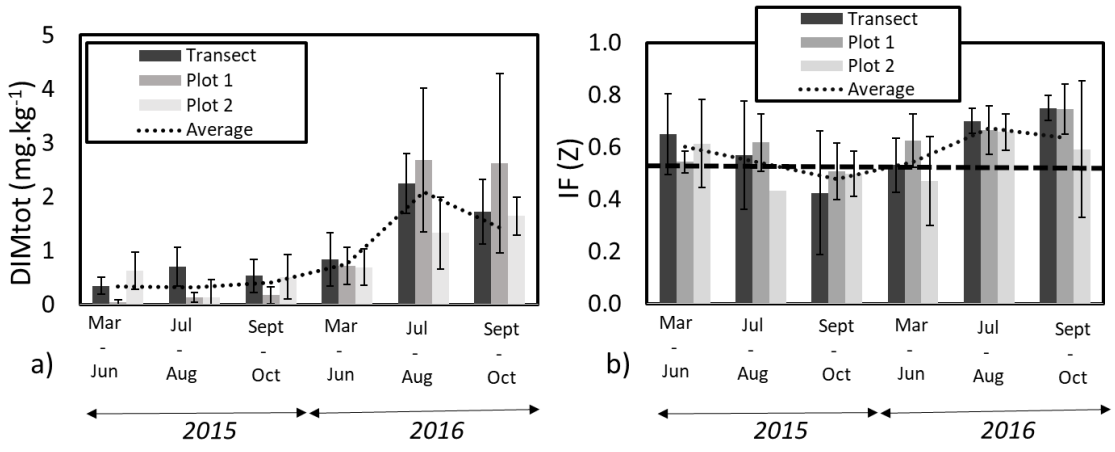

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Figure 2. DIM topsoil concentrations (a) and IF(Z) (b) at the plot and catchment scales. The periods correspond to vine budding and initial growth before DIM application (March–June), vine growth following DIM application (July–August) and the end of the season after harvesting (September–October). Dashed line in Figure 2b) represent the IF(Z) of the applied formulation.

3.4.DIM degradation and runoff transport in the vineyard catchment

At the catchment scale, the DIM concentrations and IF(Z) in the topsoil were similar to those obtained at the plot scale (Figure 2), confirming the homogeneity of DIM applications. DIM concentrations in runoff collected at the catchment outlet were similar in 2015 (3.0 ± 6.2 µg. L^{-1}; n = 13) and 2016 (5.0 ± 9.5 µg. L^{-1}; n = 15) (Table 2). The DIM mass export at the catchment outlet reached 2.3 g in 2015 and 4.7 g in 2016 (Imfeld et al., 2020). The DIM concentrations increased in both topsoil and runoff water following DIM application, with DIM export coefficients reaching 0.7% (Figure 3a and (Imfeld et al., 2020)). The DIM concentrations in topsoil in 2016 reached 2.9 µg.g^{-1} (June 22nd), during the main application (June 20th – 23th 2016), and gradually decreased to 1.2 µg.g^{-1} at the end of the sampling campaign (October 5th). Considering a layer of 1 cm of topsoil, this corresponds to DIM masses from 4.3 to 10 kg in the overall catchment topsoil. The DIM masses were similar to the applied masses (5.4 kg in 2016), supporting the idea of DIM storage in topsoil with limited yearly DIM degradation and export by surface runoff.

Table 2: DIM concentrations, IF(Z) and $\delta^{13}C$ values in the vineyard catchment.

Year	Rainfall depth (mm)	Outlet runoff volume (m^3)	Number of runoff events	Matrix	[DIM_{tot}] (µg.g^{-1} or µg.L^{-1})	IF(Z)	$\delta^{13}C_{DIM-(Z)}$ (‰)	$\delta^{13}C_{DIM-(E)}$ (‰)
Commercial formulation Grip®Top (BASF)					90 g.kg^{-1} *	0.50 ± 0.02 (n = 6)	-33.2 ± 0.7 (n = 3)	-33.7 ± 0.3 (n = 3)
2015	239	1577	32	Topsoil	0.11 ± 0.09 (n = 15)	0.63 ± 0.08 (n = 15)	n.d.	n.d.

				Runoff	3.0 ± 6.2 (n = 13)	0.81 ± 0.15 (n = 12)	-32.1 ± 0.9 (n = 7)	-29.2 ± 1.4 (n = 6)
2016	310	2027	47	Topsoil	1.5 ± 1.1 (n = 20)	0.65 ± 0.10 (n = 20)	-30.7 ± 1.7 (n = 8)	-28.6 ± 2.7 (n = 8)
				Runoff	5.0 ± 9.5 (n = 15)	0.72 ± 0.09 (n = 15)	n.d.	n.d.

n.d.: not determined

** DIM concentration in the commercial formulation Grip®Top (BASF)*

The apparent DIM field dissipation of 128 ± 60 days ($T_{1/2\ field}$ ± 95% CI, n = 13) was higher than the half-life of DIM ($T_{1/2\ lab}$ = 19.6 ± 3.2 d) in laboratory experiments (Figure S7). This indicates that DIM biodegradation was slower in the field than under laboratory-controlled conditions. Overall, IF(Z) values reflected DIM applications, with IF(Z) in runoff decreasing after DIM applications. IF(Z) >0.9 in runoff in early 2015 indicated degradation in soil of the residual DIM from the previous DIM application in 2014. IF(Z) decreased to □0.5 following the application of the Griptop® formulation (IF(Z) = 0.50 ± 0.02, Table 2), and gradually increased up to 0.8-1 over time. In contrast, IF(Z) did not change much (IF(Z) = 0.63 ± 0.08) in topsoil across the 2015 season and was globally lower than in runoff water (IF(Z) = 0.81 ± 0.15 Table 2).

IF(Z) variations in topsoil and runoff water were consistent with DIM biodegradation estimates (Figure 3). IF(Z) in 2016 ranged from 0.6 to 0.8 in runoff, whereas it decreased after DIM applications (June 20th to 23rd) and gradually increased from 0.5 to 0.8 in topsoil (Figure 3b). Accordingly, estimates of biodegradation extent (B(%)) derived from ε_{IR} values obtained in laboratory experiments (equation 8) were significantly higher in the runoff water (B(%) = 82 ± 8%) than in the topsoil (B(%) = 58 ± 10%) in 2015 and 2016 (Figure 3b). Overall, this suggests a preferential export of the degraded fraction of DIM from the topsoil into the runoff water. This supports the idea that DIM sorption (Koc = 300 – 600 L.kg^{-1}, (Boesten and Matser, 2017)) may reduce the DIM bioavailability, and thus DIM degradation, in topsoil.

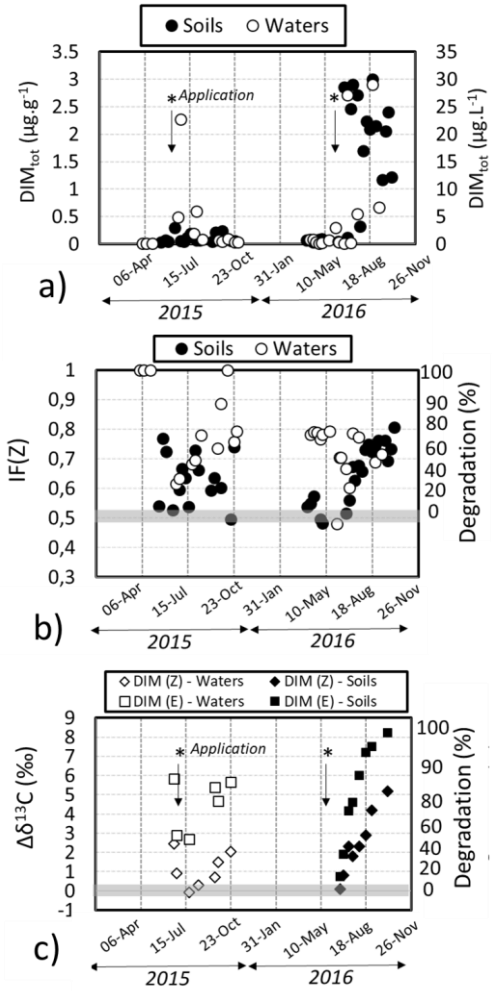


Figure 3: Total DIM concentration (DIM_{tot}) (a), DIM isomeric fractionation (IF(Z)) (b), and DIM isomer specific isotope composition for carbon ($\delta^{13}C$) compared to $\delta^{13}C$ of the commercial formulation (c) in transect topsoils (filled symbols) and outlet runoff waters (empty symbols). DIM biodegradation (B(%)) were estimated as described in the Materials and Methods section (equation 5 and 6).

3.5. *DIM isomer-specific isotopic fractionation in vineyard catchment topsoil and runoff*

DIM ISIA was carried out in runoff (2015) and topsoil (2016) samples with sufficient DIM concentrations (Figure 3c). In 2015, the initial $\delta^{13}C$ values of DIM in runoff ($\delta^{13}C_{DIM\text{-}(Z)}$ = -30.7 ± 1.2 ‰ and $\delta^{13}C_{DIM\text{-}(E)}$ = -27.9 ± 1.2 ‰, june 18th) decreased ($\delta^{13}C_{DIM\text{-}(Z)}$ = -33.3 ± 1.9 ‰ $\delta^{13}C_{DIM\text{-}(E)}$ = -31.0 ± 2.2 ‰, july 23rd) after DIM application, and were close to $\delta^{13}C_{DIM}$ value of the DIM commercial formulation (GripTop®; $\delta^{13}C_{DIM\text{-}(Z)}$ = -33.2 ± 0.7; $\delta^{13}C_{DIM\text{-}(E)}$ = -33.7 ± 0.3, Table 2). However, the $\delta^{13}C$ values gradually increased ($\delta^{13}C_{DIM\text{-}(Z)}$ = -31.2 ± 0.5 ‰; $\delta^{13}C_{DIM\text{-}(E)}$ = - 28.03 ± 0.5 ‰) at the end of the season (Figure 3c). This gradual enrichment in ^{13}C of the remaining DIM fraction exported during runoff indicates DIM biodegradation in the vineyard soil, as previously observed with *S*-metolachlor in a crop catchment (Alvarez-Zaldívar et al., 2018).

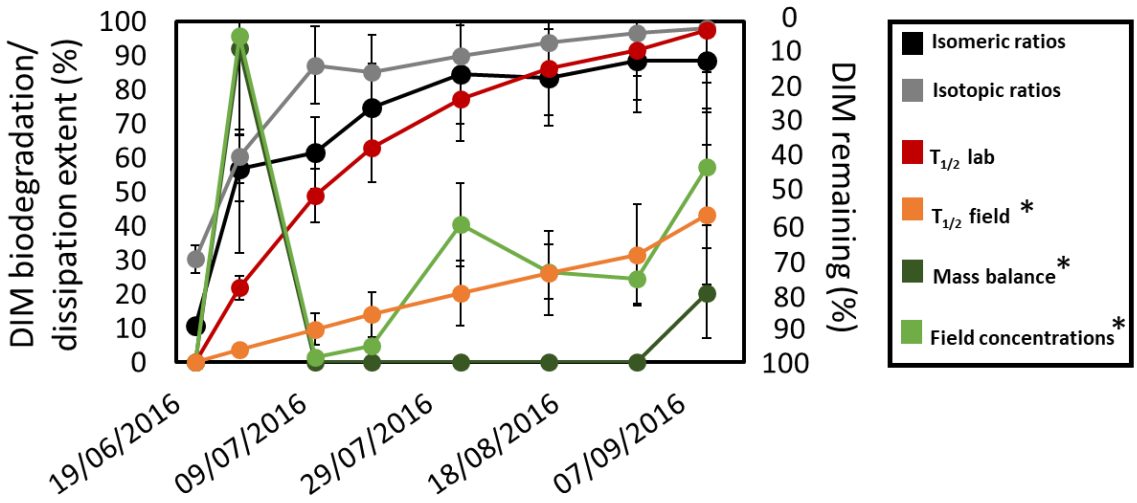
The isotopic fractionation values ($\varepsilon_{DIM\text{-}(Z)}$ = -1.6 ± 0.2 ‰ and $\varepsilon_{DIM\text{-}(E)}$ = -1.5 ± 0.2 ‰, Table 1) derived from the soil microcosm experiments were used to estimate DIM biodegradation, following the isotopic approach. Biodegradation estimates using the isotopic approach (equation 6) ranged from 38 to 99% for DIM-(E) and from 0 to 97% for DIM-(Z) in topsoil and runoff water (Figures 3c). Changes in DIM isotope signatures ($\Delta\delta^{13}C$ up to 6 ‰) corresponded to an average biodegradation of 67% for DIM-(E) and 85% for DIM-(Z) in topsoil, whereas biodegradation estimations in runoff average 44% for DIM-(E) and 92% for DIM-(Z) (Figure 3c). Overall, DIM ISIA confirmed the gradual DIM degradation in topsoil following DIM application.

3.6. Comparison of DIM degradation estimates in the vineyard catchment

Overall, estimates of biodegradation extent B(%) obtained using the isomeric and isotopic approaches in runoff significantly differed ($p<0.05$), whereas B(%) in topsoil was similar (Figure S8b). This suggests that distinct processes may affect DIM isomeric fractionation in topsoil and runoff. For instance, DIM photolysis in water may lead to the fast (i.e., a few minutes) interconversion of DIM isomers from (E) to (Z) (Avetta et al., 2014;

425 Lunn, 2007). In contrast, DIM sorbed in topsoil would be less prone to photolysis and
426 interconversion of DIM isomers. The interconversion during photolysis of DIM isomers in
427 runoff water may thus affect isomeric ratios since the water residence time in the vineyard
428 catchment ranges from 10 to 30 minutes. However, no isotope fractionation occurred ($\Delta\delta^{13}C$
429 <1 ‰ after 90% degradation, data not shown) during DIM photolysis in water under solar
430 irradiation (QSUN XE-1 test chamber, Xe arc lamp, and daylight-Q filter with a nominal cut-
431 on of 295 nm). Hence, DIM ISIA may lead to more accurate estimates of DIM biodegradation
432 than estimation relying on isomeric ratios since solar irradiation apparently has limited effect
433 on DIM $\delta^{13}C$ but may affect IF(Z) values.

434 Overall, estimates of B(%) or P(%) (proportion of remaining DIM) in topsoil at the
435 catchment scale largely differ depending on the estimation approach (Figure 4). The isotopic
436 and isomeric approaches yielded B(%) >88% in topsoil, which are close to B(%) estimates
437 obtained using laboratory half-life values ($T_{1/2}$ = 19.6 d). B(%) estimated with isotopic,
438 isomeric and $T_{1/2}$ approaches from laboratory experiments were correlated (R^2 >0.86, p <0.05)
439 (Figure S9). However, estimates with these lab-derived approaches differed from the observed
440 $T_{1/2\ (field)}$ of 128 days derived from DIM concentrations in the vineyard soil after the DIM
441 application during the 2016 campaign (SI, Section S-5 and Figure S7). Similarly to $T_{1/2\ (field)}$
442 approach, the so-called "field concentration" approach relies on comparing concentrations
443 shift in topsoils between t and t0 (first samples after application date; SI, Section S-5).



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445 *Figure 4: DIM dissipation in the vineyard soil following DIM application (2016) estimated*
446 *with different approaches. See Section S-5 in the SI for calculations. *Estimation of*
447 *dissipation extent (otherwise, biodegradation extent is estimated). Error bars represent the*
448 *standard deviation of the method estimated via error propagation.*

449 This dicrepency between approaches suggests that the isotopic and isomeric
450 approaches overestimated B(%) in the field and/or that using the field $T_{1/2}$ value
451 underestimated B(%). Estimates of DIM dissipation from DIM application onwards using the
452 mass balance approach indicated that B(%) was close to zero (i.e., no DIM biodegradation) to
453 balance DIM applications and stocks in the vineyard topsoil (SI, Section S-5). However, the
454 mass balance results must be considered with caution because of the large uncertainties in
455 such calculations. For instance, the low B(%) derived from this approach may be due to an
456 overestimation of the DIM mass budget in soil (6920 ± 1594 g, 95% CI, n = 15, from June
457 22nd to October 5th, 2016), which is based on the DIM concentrations in topsoil (i.e., top first
458 cm) from transect. In addition, the doses of DIM application (5406 g in 2016, (Imfeld et al.,
459 2020)) may have been underestimated when extrapolating the survey results to the entire
460 catchment or due to underreporting of DIM usage by winegrowers.

Overall, the discrepancy among complementary approaches to estimate DIM degradation highlights the interest and difficulties to quantify the contribution of pesticide biodegradation to the overall dissipation in the field. Although different approaches yielded similar estimates when examining S-metolachlor dissipation at the catchment scale (Alvarez-Zaldívar et al., 2018), different approaches led to distinct estimates of DIM dissipation. Several reasons may explain this difference. First, reference values derived from laboratory experiments (i.e., isotopes, isomers and $T_{1/2}$ (lab)) may overestimate the dissipation compared to field-derived parameters (i.e., mass balance, $T_{1/2}$ (field) and the field concentrations). This may reflect the high sensitivity of DIM to degradation conditions, including microbial communities, temperature, humidity, and DIM formulations, differing under laboratory and field conditions. Second, additional degradation/volatilization and unknown processes affecting DIM on the wine leaves may alter the isomeric and stable isotope composition of DIM before it enters into the soil and runoff water. Whil *S*-metolachlor is applied directly to soil, DIM is applied to leaves, where it is supposed to remain as long as possible to protect plants from fungi after rainfall events. Indeed, the GripTop manufacturer (BASF) states that DIM is safe from leaching once the spray has dried on the treated plant organs, approximately one hour after treatment. Hence, DIM may persist (approximately 12-14 days) on the leaves independent of the occurrence of rainfall. Overall, we hypothesise that the wine leaves may be an important intermediate compartment before DIM reaches the soil. This also advocates considering all compartments where pesticide degradation can occur once applied in the field and carefully comparing field and laboratory degradation estimates.

4. Conclusions

Knowledge of the contribution of fungicide degradation to the overall dissipation in agricultural catchments is scarce. In this study, we examined the potential of isomer and

485 stable isotope fractionation to evaluate DIM degradation at the vineyard plot and catchment
486 scales. Despite the large amount of carbon in the DIM molecule, significant carbon isotope
487 fractionation ($\Delta\delta^{13}C$ □5 ‰) occurred during biodegradation in microcosm soil experiments
488 and in the field ($\Delta\delta^{13}C$ up to 7 ‰). Enrichment in both the (Z)-isomer and in ^{13}C of DIM
489 following DIM application indicated significant DIM degradation in both topsoil and runoff
490 water. Altogether, our results underscored substantial DIM immobilization in the vineyard
491 soil, while a small pool of bioavailable DIM may be degraded and exported by surface runoff
492 waters. Indeed, the isomeric approach, highlighted the preferential export of a highly
493 degraded fraction of DIM in runoff water compared to the DIM stored in the topsoil, with
494 limited (<1%) DIM export by surface runoff.

495 The comparison of the DIM degradation estimates obtained from isomeric and
496 isotopic fractionation with those retrieved from classical approaches, including $T_{1/2\ (lab)}$ and
497 $T_{1/2\ (field)}$, and mass balances, underlined the difficulty to use values derived from laboratory
498 experiments to estimate fungicide degradation in the field. For instance, the use of
499 degradation kinetics and values retrieved from the laboratory experiments (i.e., isotopic,
500 isomeric and $T_{1/2\ (lab)}$) overestimated the DIM dissipation compared to field estimates relying
501 on $T_{1/2\ (field)}$, mass balance, and field concentrations. Since fungicides are primarily applied to
502 plant leaves, the foliar compartment cannot be neglected as a potential site of degradation
503 processes, including photolysis and biodegradation, which may result in significant isomer
504 and/or isotope fractionation. Laboratory experiments targeting fungicide formulation
505 behaviour on leaf surfaces may help in the future to improve estimations of fungicide
506 degradation in vineyards. Overall, this study also emphasised the interest of crossing
507 independent information sources, including application surveys, and applying isotopic and
508 isomeric approaches to estimate fungicide degradation in agricultural catchments.

509

Author contribution statement

Jérémy Masbou: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft. **Sylvain Payraudeau**: Formal analysis; Resources; Software; Validation; Writing – review & editing. **Benoit Guyot**: Data curation; Formal analysis; Methodology; Writing – review & editing**. Gwenaël Imfeld:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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