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Dissipation of Fungicides in a Vineyard Soil Amended with Different Spent Mushroom Substrates

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ABSTRACT: The degradation kinetics and formation of metabolites for fungicides of different chemical classes (iprovalicarb, metalaxyl, penconazole, and pyrimethanil) and determination of bound residues for metalaxyl and penconazole were studied in both an unamended vineyard soil and in the same soil amended with two spent mushroom substrates (composted (C-SMS1) and fresh (F-SMS2)). The degradation kinetics was fitted to single first-order or first-order multicompartment patterns. Degradation rates decreased in C-SMS1-amended soils for all fungicides as compared to unamended soil, but in F-SMS2-amended soils, they decreased only for iprovalicarb and penconazole. The DT_{50} values were higher by up to 1.8 (metalaxyl), 3.8 (pyrimethanil), 4.1 (iprovalicarb), and >1000 (penconazole) times in the soil plus C-SMS1 compared to those for soil plus F-SMS2 or unamended soil. The dissipation mechanism recorded the highest mineralization in the unamended soil for ^{14}C-metalaxyl and ^{14}C-penconazole, with the highest formation of nonextractable residues in the F-SMS2-amended soil for ^{14}C-metalaxyl. The results are consistent with (1) the chemical characteristics of each SMS (total and soluble organic carbon) controlling sorption and the bioavailability of fungicides and (2) the microbial activity of SMS-amended soils, which affects fungicide biodegradation. The findings of this work highlight the potential of SMS amendments with different characteristics to decrease or increase the degradation rate of a fungicide in a vineyard soil.

KEYWORDS: *fungicide, soil, spent mushroom substrate, degradation, mass balance, dehydrogenase activity*

INTRODUCTION

The need to ensure the production of crops of sufficient quality and quantity and an abundant food supply makes the use of pesticides essential to combat all kinds of pests. In Spain, the use of pesticides increased by 4.7% from 2009 to 2010, representing >90000 tons of products and a market value of >600 million euro (www.aepla.es). The use of these chemical compounds potentially involves the diffuse contamination of the environment and has a particular impact on food, water, and biodiversity. Indeed, the presence of these compounds in the soil and in surface and groundwaters has increased in recent years.[1−3] Accordingly, knowledge of the behavior of pesticides and their dissipation in the environment is essential when the environmental contamination risk derived from the use of these compounds is assessed.

The dissipation of pesticides in soils can be affected by changes in soil management, such as the addition of organic amendments. The organic matter (OM) and nutrients added to the soil can heavily affect the structure and activity of bacterial and fungal populations due to the increased metabolism of the readily available nutrients. Some organic amendments may favor degradation by stimulating microbial activity.[4,5] However, other research has indicated that pesticides are less available for dissipation because the addition of organic amendments can enhance the sorption of these compounds.[6−8]

In the La Rioja region (northern Spain), the use of pesticides is high, amounting to 13.79 kg ha^{-1} in 2008. Fungicides are applied in higher amounts (7.19 kg ha^{-1}) than herbicides, insecticides, and other compounds (www.marm.es). They are applied mainly in vineyards, which represent 34% of the total cultivated area in this region (www.larioja.org). Vineyard soils have naturally low OM levels, and organic amendments are usually applied. In recent years, spent mushroom substrates (SMS) have been applied due to increased mushroom production in the region and the high amounts of this organic residue generated (250000 tons per year). SMS has a high content of OM and nutrients[9] and could be exploited as a soil fertilizer and amendment to increase the OM content of vineyard soils, as this crop is of huge economic importance in the region. However, SMS could affect the behavior of fungicides in amended vineyard soils, with implications for their persistence in soil and/or transport to groundwater.

The effectiveness of these residues as sorbents has been reported for the removal of pesticides and organic contaminants from water.[10,11] Marín-Benito et al.[12,13] have also reported the enhanced sorption and decreased leaching of certain fungicides in SMS-amended soils. The results from these studies reported changes in the environmental behavior of these fungicides in the amended soils related to soil characteristics and fungicide properties. However, to date only a few studies have evaluated the influence of SMS on the degradation and persistence of pesticides and organic compounds in soils amended with this residue.[5,14,15]

Accordingly, the objective of this research was to study the effect of SMS as a soil amendment on the dissipation of four

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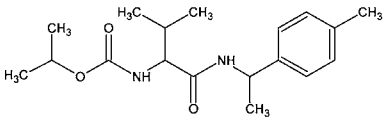
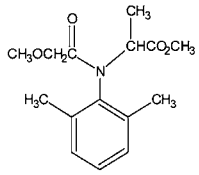
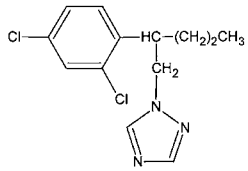
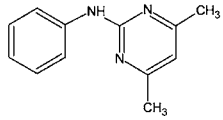
Table 1. Fungicide Characteristics[a]

common name / chemical name	chemical structure	water solubility (mg L^{-1})	log Kow	molecular mass (g mol^{-1})	pKa	sorption coefficients (Kd)[a] Al	Al+C-SMS1	Al+F-SMS2
Iprovalicarb 1-methylethyl [(1*S*)-2-methyl-1-[[[1-(4-methylphenyl)ethyl]amino]carbonyl]propyl]carbamate		11.0 (R, 20°C) 6.0 (S, 20°C)	3.20	320.43	-	0.69	3.12	2.23
Metalaxyl methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-DL-alaninate		8400 (22°C)	1.75	279.33	0	0.20	1.30	0.70
Penconazole 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole		73 (20°C)	3.72	284.18	1.51	3.95	26.5	15.2
Pyrimethanil 4,6-dimethyl-*N*-phenyl-2-pyrimidinamine		121 (25°C)	2.84	199.11	3.52	1.12	9.88	6.89

[a]Data for sorption coefficients taken from Marín-Benito.[17]

Table 2. Characteristics of Spent Mushroom Substrate (SMS) and Unamended and Amended Soils

	sample	pH	ash (%)	OM[a] (%)	C/N	OC[b] (%)	DOC[c] (%)	sand (%)	silt (%)	clay (%)
SMS										
	C-SMS1	7.5	48.3	51.7	11.9	26.7	1.22			
	F-SMS2	4.5	32.5	67.5	17.9	31.2	10.8			
soil										
	Al	7.87				0.59	0.005	64.4	14.2	21.4
	Al+C-SMS1	7.44				3.81	0.082			
	Al+F-SMS2	6.04				3.96	1.047			

[a]Organic matter. [b]Organic carbon. [c]Dissolved organic carbon.

fungicides, which represent different chemical classes and are widely used in viticulture: iprovalicarb, metalaxyl, penconazole, and pyrimethanil. Degradation kinetics, metabolite formation, and dissipation mechanism (mineralization and nonextractable residue formation) were evaluated under laboratory conditions in a vineyard soil amended with two different SMS (fresh or composted). Soil dehydrogenase activity was also determined for all SMS−soil−fungicide combinations to analyze the effect of the amendment and the fungicide on soil microbial activity. No published data are currently available on the extent to which these compounds dissipate in SMS-amended soils or the rate at which they do so, and the results could be used (1) to evaluate the potential environmental impacts of this soil management strategy and (2) to improve the knowledge on sustainable use and management of soil, which can have an indirect effect on the agricultural production process.

MATERIALS AND METHODS

Chemicals. The characteristics of the fungicides iprovalicarb, metalaxyl, penconazole, and pyrimethanil are included in Table 1.[16,17] Penconazole and metalaxyl were kindly supplied by Novartis Crop Protection AG (Basel, Switzerland). Unlabeled (>97.2% purity) and labeled [*triazole*-U-^{14}C]-penconazole and [*phenyl*-U-^{14}C]-metalaxyl (specific activities of 1.02 and 1.37 MBq mg^{-1} and 98.1 and 97.2% purities, respectively) were used. Pyrimethanil and iprovalicarb (>97.5% purity) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Metalaxyl metabolites CGA 62826 (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine) and CGA 67868 (*N*-methoxyacetyl-2,6-dimethylaniline) were kindly supplied by Syngenta Crop Protection (Basel, Switzerland). HPLC grade methanol was supplied by Merck (Darmstadt, Germany). 2,3,5-Triphenyltetrazolium chloride (TTC) and 2,3,5-triphenylformazan (TPF) were supplied by Sigma-Aldrich Química SA (Spain).

Spent Mushroom Substrates and Soil. A composted SMS (commercial name, Intracompost SPCH-SPS) from *Agaricus bisporus* (75%) and *Pleurotus* spp. (25%) cultivation (C-SMS1) and a fresh SMS from *Lentinula edodes*, or shiitake, cultivation (F-SMS2) were supplied by INTRAVAL (La Rioja, Spain). The F-SMS2 was obtained immediately after its removal from mushroom houses. The C-SMS1 was used after composting under aerobic conditions to increase the homogeneity of the SMS. The SMS samples were sieved, and the <2 mm fraction was selected. The characteristics of the SMS (on a dry weight basis) are included in Table 2. The pH was determined in a SMS/water suspension (1:2 w/v ratio), and ash percentage was determined by weight difference after ignition at 540 °C for 24 h. Organic carbon (OC) content was determined by oxidation (Walkley−Black method). Dissolved organic carbon (DOC) was determined in a suspension of residue in Milli-Q ultrapure water (1:100 w/v ratio) after 24 h of shaking at 20 °C, centrifuging (20 min at 12857*g*), and filtering (Minisart NY25 filter 0.45 μm, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 organic carbon

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Table 3. Parameters of Fungicide Analysis

fungicide	RT (min)	m/z	cone voltage (V)	λ (nm)	calibration range (μg mL^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})
iprovalicarb[a]	5.0	321.30	10	193.0	0.125−2.0	0.689	2.274
metalaxyl[a]	5.4	280.33	20	194.0	0.05−5.0	0.570	1.881
penconazole[a]	6.8	284.18	20	201.7	0.05−2.5	0.780	2.574
pyrimethanil[b]	6.2	198.00			0.03−1.5	0.540	1.782

[a]Determined by HPLC-DAD-MS. [b]Determined by GC-MS.

analyzer (Shimadzu, Columbia, MD, USA). Total N content was determined according to the Kjeldahl method.

A soil sample (Al) was collected from the surface layer (0−15 cm) of a vineyard in Aldeanueva de Ebro (La Rioja, Spain). The sandy clay loam soil was sieved (<2 mm) and stored at 4 °C until further use. The soil characteristics determined by the usual analytical methods[18] are included in Table 2. The pH was determined in a soil/water suspension (1:2 w/v ratio), and particle size distribution was determined using the pipet method. The OC content and DOC content in soil extracts (1:2 w/v ratio) in Milli-Q ultrapure water were determined as previously indicated for SMS.

Amended soils were prepared by uniformly mixing Al soil with C-SMS1 or F-SMS2 at a rate of 10% w/w. Subsamples were analyzed to assess both OC and DOC contents and the pH as previously indicated (Table 2).

Degradation Studies. Initially, fungicides were individually dissolved in methanol to give a concentration of 1000 mg L^{-1}. Solutions of each fungicide were then prepared in Milli-Q ultrapure water, and a volume of 10 mL of appropriate concentration was added to 300 g of fresh weight of unamended or amended soils to give a concentration of 2 mg kg^{-1} dry soil. Soil samples were incubated at 20 °C in the dark. The moisture content of soil samples was previously adjusted to 40% of the maximum soil water-holding capacity, and it was maintained by adding sterile Milli-Q ultrapure water when necessary. Each soil treatment was prepared in duplicate. A sterilized soil sample was also prepared by autoclaving soil at 120 °C for 1 h on three consecutive days. Sterilized unamended soil was treated with each fungicide and incubated as indicated above, and these samples were used as controls to check the chemical degradation of fungicides. Finally, soils for microbiological control were prepared by adding only sterile Milli-Q ultrapure water. All soils were thoroughly stirred with a sterilized spatula, and all of the steps were performed in a sterile cabinet. Soil samples were taken at day 0 for fungicide analysis and thereafter repeatedly at different time intervals (up to 258 days) depending on the degradation rate of each fungicide.

Mineralization and Mass Balance of ^{14}C-Metalaxyl and ^{14}C-Penconazole. For metalaxyl and penconazole, simultaneous incubations were carried out with ^{14}C-labeled fungicides to study the dissipation mechanism (mineralization kinetics and the formation of nonextractable residues over time). Aqueous solutions of unlabeled fungicide of an appropriate concentration were labeled with ^{14}C-fungicides, and a volume of 10 mL of these solutions was added to 300 g fresh weight of unamended or amended soils to give a concentration of 2 mg kg^{-1} dry soil and an activity of approximately 100 Bq g^{-1}. In these soil samples, a $^{14}CO_2$ trap, consisting of a scintillation vial containing 1 M NaOH (1 mL), was attached to the lid via a stainless steel clip as described by Reid et al.[19]

Fungicide Extraction. At each sampling time, 2 × 5 g of each duplicate treatment (300 g sample of unamended or amended soil treated with different fungicides) was taken and shaken at 20 °C for 24 h with methanol (10 mL) in glass tubes. The samples were then centrifuged at 5045*g* for 15 min, and the fungicide extracts were filtered in a GHP Acrodisc filter (Waters Corp.) to remove particles >0.45 μm. For the determination of the fungicides and their metabolites, a volume of the extract (6 mL) was transferred to a clean glass tube and evaporated at 25 °C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany) until dryness. The residue was dissolved in 0.75 mL of methanol and transferred to a glass vial for analysis. The recoveries of the extraction method were determined by spiking three unamended and amended soil samples with analytical grade fungicide to a final concentration of 2 mg kg^{-1} and performing the extraction procedure as described above. The mean recovery values varied between 87 and 111% for all of the fungicides studied.

Determination of Unlabeled Fungicides. Iprovalicarb, metalaxyl, and penconazole were quantified by HPLC with diode array (DAD) and mass spectrometer (MS) detectors (Waters Associates, Milford, MA), using a Waters Symmetry C18 column (75 × 4.6 mm i.d., 3.5 μm) at ambient temperature. The mobile phase was 70:30 (v/v) methanol/water (0.1% formic acid) for metalaxyl and 80:20 methanol/water (0.1% formic acid) for iprovalicarb and penconazole. The flow rate of the mobile phase was 0.3 mL min^{-1}, and the sample injection volume was 20 μL. The retention time (RT), ranges of calibration curve concentrations, and limits of detection (LOD) and quantification (LOQ) are included in Table 3. Quantitative analysis was performed using the peak area of each compound obtained from the total ion chromatogram (TIC) in SIM mode. The positive molecular ions (m/z) [M]$^+$ corresponding to each fungicide in the positive ionization mode are included in Table 3. Monitoring also involved positive molecular ions (m/z) 136.2, 137.2, and 167.1 for iprovalicarb metabolites[20] (2-(4-methylphenyl)ethylamine, iprovalicarbcarboxylic acid, and terephthalic acid, respectively), 266.2 and 194.2 for metalaxyl metabolites[21] (CGA 62826 and CGA 67868), and 128.1 and 287.1 for penconazole metabolites[22] (1,2,4-triazole-1-ylacetic acid and 2-(2,4-dichlorophenyl)-3-[1,2,4]-triazole-1-ylpropionic acid).

Pyrimethanil was quantified using a 7890A Agilent gas chromatograph coupled to a 5975C Agilent mass spectrometer (Agilent Technologies, Avondale, AZ, USA) using a 30 m × 0.25 mm i.d., 0.25 μm film thickness, HP-5MS capillary column (J&W, Folsom, CA, USA). A volume of 1.0 μL was injected in splitless mode at 250 °C. The carrier gas was ultrapure helium at a flow of 1.5 mL min^{-1}. Measurements were performed in SIM mode. The more abundant ion was chosen for pyrimethanil quantification (m/z 198.0). The major pyrimethanil metabolite[23] (2-amino-4,6-dimethylpyrimidine) formed in soil was monitored during the experiment (m/z 124.0). Calibration range, LOD, and LOQ are included in Table 3. The iprovalicarb, penconazole, and pyrimethanil metabolites were only qualitatively monitored.

Determination of ^{14}C-Fungicides. The quantitative determination of ^{14}C-penconazole and ^{14}C-metalaxyl after extraction was performed by liquid scintillation using a Beckman LS6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA). The radioactivity of the solution was measured in disintegrations per minute (dpm), being determined in duplicate in 1 mL of methanol extract to which 4 mL of scintillation cocktail was added (Ecoscint TMA, National Diagnostics, Atlanta, GA, USA).

Residues of ^{14}C-fungicides remaining in the soil after extraction were determined by the combustion of triplicate 1 g dried soil samples, using a Biological Oxidizer (R. J. Harvey OX-500 Instrument Corp., NJ) under O_2 excess at 900 °C. The $^{14}CO_2$ generated was trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxisolve C-400, Zinsser Analytic, Berkshire, U.K.; 15 mL) and determined as indicated above. $^{14}CO_2$ from mineralized ^{14}C-fungicides in the scintillation vial containing 1 M NaOH (1 mL) was determined at the different sampling times by mixing with 4 mL of scintillation cocktail and determined as previously indicated.

Soil Dehydrogenase Activity. Soil dehydrogenase activity (DHA) was determined following the Tabatabai method[24] at different times after fungicide application. The method is based on the

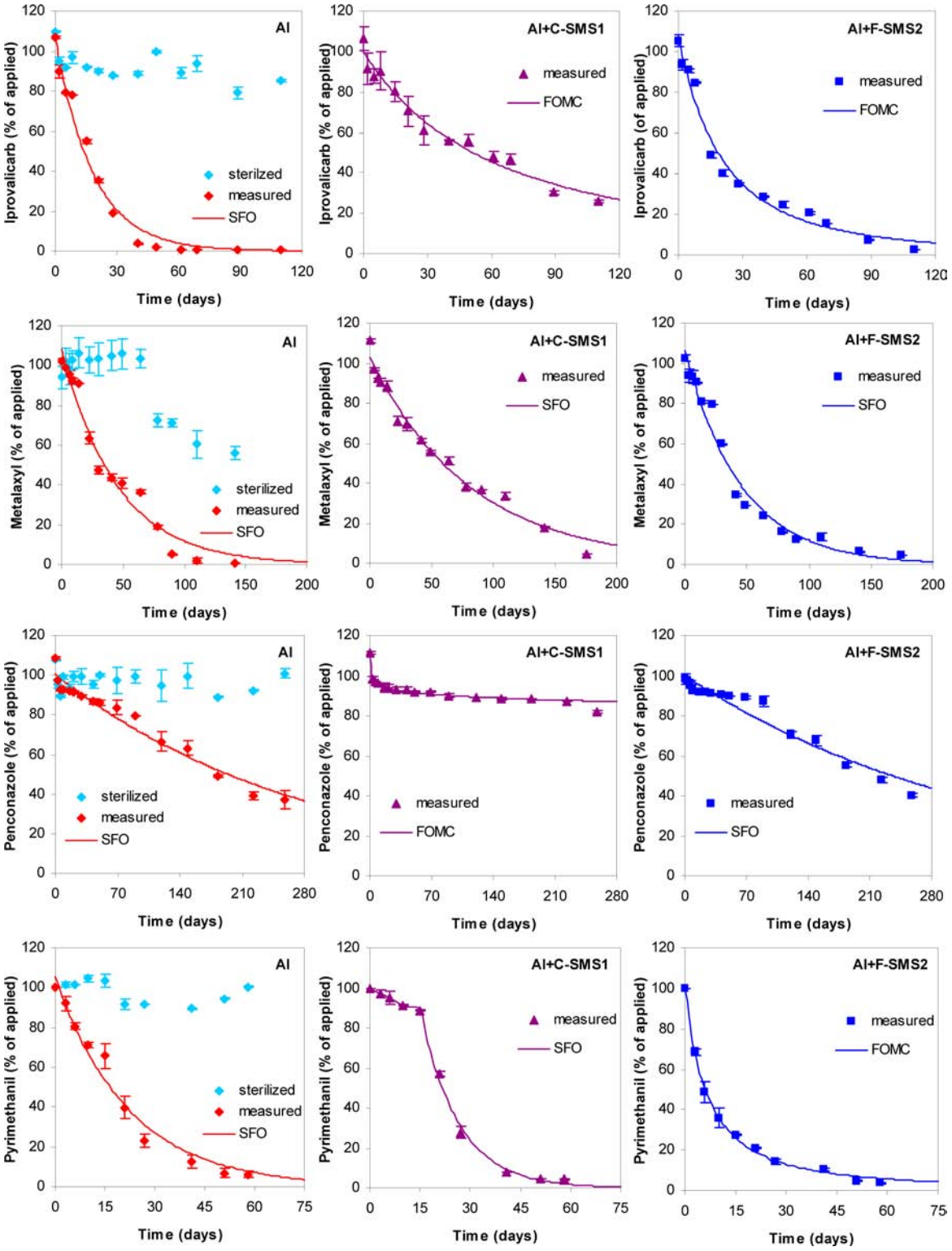

Figure 1. Degradation kinetics of iprovalicarb, metalaxyl, penconazole, and pyrimethanil in unamended sterilized and nonsterilized soil (Al) and in SMS-amended soils (Al+C-SMS1 and Al+F-SMS2). Bars indicate the standard deviation of the mean.

extraction and colorimetric determination of the intensely colored TPF produced from the reduction of colorless TTC in soils.

Data Analysis. The degradation kinetics for the fungicide was fitted to a single first-order (SFO) kinetic model ($C = C_0\, e^{-kt}$) or first-order multicompartment (FOMC) model ($C = C_0/((t/\beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. C is the fungicide concentration at time t, C_0 is the initial fungicide concentration, k (day^{-1}) is the degradation rate, α is a shape parameter determined by the coefficient of variation of k values, and β is a location parameter. For the selection of the kinetic model that best describes the degradation results, FOCUS work group guidance recommendations were followed.[25] The coefficient of determination (r^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. The χ^2 test considers the deviations between observed and calculated values relative to the uncertainty of the measurements for a specific fit and was used to compare the goodness of fit of the two models tested.

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Table 4. Kinetics Parameters for the Degradation of Fungicides in Soils Unamended and Amended with SMS (10% w/w) Obtained from Fitting Kinetics to a Single First-Order (SFO) and Gustafson and Holden (FOMC) Models

	single first order (SFO)				Gustafson and Holden (FOMC)				
	k (day^{-1})	DT_{50} ± SD[a] (days)	r^2	χ^2	α	β	DT_{50} ± SD[a] (days)	r^2	χ^2
Iprovalicarb									
Al	0.055	12.6 ± 0.1a	0.984	11.0	5.69×10^4	10.3×10^5	12.6 ± 0.2a	0.984	11.4
Al+C-SMS1	0.012	55.9 ± 1.7c	0.943	5.4	1.67	99.7	51.4 ± 1.4c*	0.949	5.2
Al+F-SMS2	0.035	20.1 ± 0.5b	0.968	10.9	2.28	47.2	16.7 ± 1.0b*	0.978	9.1
LSD ($p < 0.05$)	1.72								
Metalaxyl									
Al	0.022	31.1 ± 0.4a	0.970	9.8	2.51×10^4	10.6×10^5	29.4 ± 0.4a	0.968	10.4
Al+C-SMS1	0.012	56.7 ± 0.1b	0.978	5.8	1.99×10^2	1.63×10^4	56.9 ± 0.7b	0.978	6.0
Al+F-SMS2	0.022	31.4 ± 0.2a	0.974	8.6	1.41×10^4	6.38×10^5	31.4 ± 0.2a	0.974	8.9
LSD ($p < 0.05$)	1.12								
Penconazole									
Al	0.004	192 ± 4.9a	0.951	4.5	3.30×10^4	9.12×10^6	172 ± 7.1a*	0.951	4.6
Al+C-SMS1	0.007	>1000[b]	0.599	3.5	0.029	0.10	≫1000[b]	0.937	1.4
Al+F-SMS2	0.003	235 ± 3.8b	0.939	4.4	1.14×10^4	3.66×10^6	222 ± 4.2b	0.937	4.6
LSD ($p < 0.05$)	18.9								
Pyrimethanil									
Al	0.045	15.3 ± 0.3b	0.972	8.7	4.82×10^4	1.07×10^6	15.3 ± 0.4b	0.972	9.1
Al+C-SMS1	0.088	22.9[c] ± 0.5c	0.994	6.1	9.26×10^3	1.05×10^5	22.9[c] ± 1.0c	0.994	6.7
Al+F-SMS2	0.085	8.2 ± 0.7a	0.974	12.3	1.50	10.4	6.1 ± 0.8a*	0.994	3.6
LSD ($p < 0.05$)	1.88								

[a]Average DT_{50} values ± standard deviation. [b]DT_{50} values are extrapolated and not considered in the ANOVA. [c]DT_{50} value includes a lag phase (slow dissipation) of 15 days before degradation started. The same letter in DT_{50} values within a column indicates mean values that are not significantly different, and an asterisk in DT_{50} values in a row indicates mean values that are significantly different according to LSD between soil groups and kinetic models.

The error value at which the χ^2 test is fulfilled at a given degree of freedom should be below 15% (at 5% significance level). Values for the time to 50% degradation, or DT_{50} values, were used to characterize the decay curves and compare variations in degradation rates. The parameters of the kinetic models were estimated using the Excel Solver add-in package.[25]

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments on the dissipation of fungicides. Standard deviation (SD) was used to indicate variability among replicates, and the least significant difference (LSD), at a confidence level of 95%, was determined to evaluate the effects of different soil treatments on DT_{50} values and dehydrogenase activity. Statgraphics Plus version 5.1 statistical software (Statgraphics Plus Corp., Princeton, NJ, USA) was used.

RESULTS AND DISCUSSION

Degradation Kinetics of Fungicides and Metabolite Formation. The decrease in the concentrations of unlabeled fungicides (expressed as a percentage of the fungicide initially applied) in unamended soil, sterile unamended soil, and C-SMS1- and F-SMS2-amended soils is shown in Figure 1. The study was carried on for 58 (pyrimethanil), 110 (iprovalicarb), 175 (metalaxyl), and 258 (penconazole) days of incubation of the fungicide in the soils. At the end of incubation, the degradation of pyrimethanil and metalaxyl was practically total (93−98% range for pyrimethanil and 95−99% range for metalaxyl), and the degradation of iprovalicarb and penconazole was in the range of 74−100 and 18−63%, respectively.

Data for residual concentrations of fungicides as a function of time were fitted to SFO and FOMC models, and kinetic parameters were calculated for each fungicide and soil treatment (Table 4). Most of the degradation kinetics fitted the SFO model better than the FOMC model. Only the degradation kinetics of iprovalicarb in both SMS-amended soils, penconazole in C-SMS1-amended soil, and pyrimethanil in F-SMS2-amended soil fitted the FOMC model better (χ^2 error values were lower than those corresponding to the SFO model). Previous studies have also reported the degradation curves of metalaxyl and penconazole in unamended and/or amended soils fitted to a SFO model.[8,26,27] However, Karanasios et al.[28] fitted degradation curves of metalaxyl in unamended soil and biomixtures of soil with organic residues to multicompartment kinetic models. The degradation kinetics of pyrimethanil in C-SMS1-amended soil recorded a lag phase of 15 days with a very slow degradation rate, and this was followed by a rapid degradation phase, which fitted well to a SFO model. The existence of a lag phase has been observed for some pesticides,[29] and it reflects the adaptation time needed for the microbial community to degrade the pesticide.

In general, the degradation rate decreased in the order pyrimethanil > iprovalicarb > metalaxyl > penconazole. The DT_{50} value for the degradation of pyrimethanil (15.3 days) in unamended soil was lower than that reported in the literature (27.9−71.8 days) for unamended soils.[23] However, DT_{50} values obtained in this study for iprovalicarb (12.6 days), for metalaxyl (31.1 days), and for penconazole (192 days) in unamended soil are in the range of the DT_{50} values found in the literature, which varied in the ranges of 2−30 days for iprovalicarb,[20] 14−42 days for metalaxyl,[21] and 55.3−488 days for penconazole[22] in unamended soils.

For iprovalicarb and penconazole, degradation was more rapid in unamended soil than in C-SMS1- and F-SMS2-amended soils. For metalaxyl and pyrimethanil, degradation in C-SMS1-amended soil was slower than in F-SMS2-amended soil and DT_{50} values followed the order Al+C-SMS1 > Al ≥ Al +F-SMS2. The DT_{50} values calculated in the Al+C-SMS1 soil increased 1.8−1.8 (metalaxyl), 3.8−1.5 (pyrimethanil), 3.1−4.1

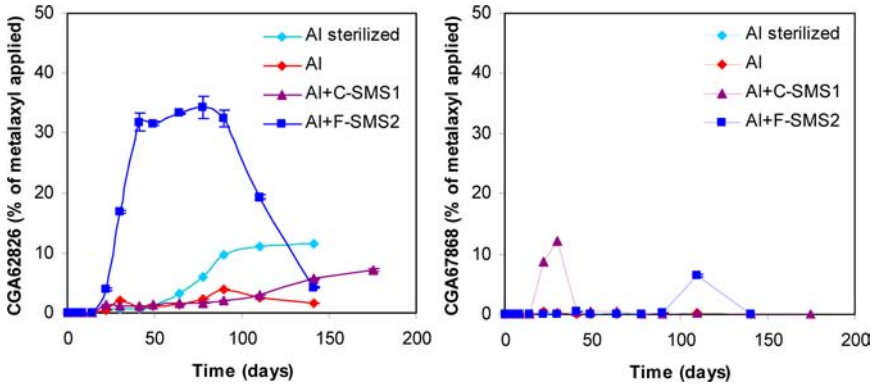

Figure 2. Metalaxyl metabolite formation over time. Bars indicate the standard deviation of the mean.

(iprovalicarb), and >1000 (penconazole) times compared to those in the Al+F-SMS2 or unamended soil, respectively. Higher sorption coefficients of fungicides by Al+C-SMS1 than by Al+F-SMS2[17] (Table 1) could explain the slower degradation rate in C-SMS1-amended soil for all of the fungicides studied. The influence of sorption on the degradation kinetics of pesticides in soils has been observed in many studies,[30] due to a decrease in the bioavailability and biodegradation of these compounds sorbed by soil.[31] For pyrimethanil and metalaxyl, the degradation rate in Al+F-SMS2 was faster than or similar to that in unamended soil, despite their higher sorption. This could be explained by the higher DOC content of Al+F-SMS2. Pyrimethanil and metalaxyl could be sorbed by the DOC in solution, as indicated for other pesticides,[32] increasing their bioavailability to be degraded.

A decrease in the dissipation rate of metalaxyl in soil amended with different organic residues was observed[6,8] and attributed to a higher sorption of this fungicide by the amended soil. Komárek et al.[33] reported a relationship between the high affinity of pyrimethanil by the soil solid phase and its difficulty to be degraded. However, the amendment of soil with a biocompost enhanced the degradation of penconazole compared to unamended soil due to an increase in the OC content and soil microbial activity after biocompost amendment.[27]

The degradation of fungicides in sterilized soils indicated that the soil microbial community had an active role in fungicide degradation (Figure 1). No degradation was observed for pyrimethanil and penconazole in sterilized soils, or degradation was much slower for iprovalicarb (85.4% remained after 110 days) and metalaxyl (56.1% remained after 141 days) than for nonsterilized soils. The degradation of iprovalicarb and metalaxyl in sterilized soils may be influenced by other abiotic factors, although photodegradation was not considered, as soils were kept in the dark during incubation. Several authors have indicated that the dissipation of metalaxyl and pyrimethanil under aerobic conditions is due mainly to biodegradation.[26,34]

During the incubation experiment, some metabolites of iprovalicarb described in the literature (2-(4-methylphenyl)-ethylamine; iprovalicarbcarboxylic acid, and terephthalic acid)[20] and pyrimethanil (2-amino-4,6-dimethylpyrimidine)[23] were qualitatively monitored in the Al+F-SMS2 and the unamended soils, respectively (data not shown). However, penconazole metabolites monitored (2-(2,4-dichlorophenyl)-3-[1,2,4]-triazole-1-ylpropionic acid; 1,2,4-triazole-1-ylacetic acid; and 1*H*-1,2,4-triazole)[22] were not detected in the soil extracts.

More evident was the presence of metalaxyl metabolites, CGA 62826 and CGA 67868, in the soil extracts from unamended and amended soils during the degradation experiment (Figure 2). CGA 62826 is the main acid metabolite of metalaxyl obtained in the soil by cleavage of the methyl ester group or by benzylic hydroxylation of the methyl chain or aromatic hydroxylation.[35] The higher amount of CGA 62826 was detected in Al+F-SMS2 between 30 and 110 days (34.3% at 78 days). In Al and Al+C-SMS1, the maximum amounts of CGA 62826 were 3.96 and 7.15% at 90 and 175 days, respectively. CGA 67868 is formed either directly from metalaxyl or from the metabolite CGA 62826 by N dealkylation. It was detected at lower amounts than the metabolite CGA 62826, and the maximum amounts detected were 0.42% (Al), 12.3% (Al+C-SMS1), and 6.54% (Al+F-SMS2) $\mu g\ g^{-1}$ at 22, 30, and 110 days, respectively (Figure 2). The formation of metalaxyl metabolites in unamended soils and soils amended with different organic residues has frequently been detected.[8,26,35]

Mineralization and Mass Balance of ^{14}C-Metalaxyl and ^{14}C-Penconazole. Figure 3 shows the total ^{14}C balance corresponding to mineralized, extracted (as parent or metabolites), and nonextractable (bound residues) ^{14}C-metalaxyl and ^{14}C-penconazole in unamended and SMS-amended soils over time. The total mass balance (expressed as percentage of the ^{14}C initially applied) was, in general, >80% (71−112% range) for ^{14}C-metalaxyl and >90% (88−110% range) for ^{14}C-penconazole.

The mineralization of metalaxyl was very low up to 30 days, and then there was an increase in $^{14}CO_2$ evolution, which reached a plateau after 175 days. The low mineralization phase corresponds to a previous period of adaptation of the soil microbial community. Subsequently, the degradation of the phenyl ring, where ^{14}C is labeled, occurred more quickly. For penconazole, mineralization increased slowly over the incubation period, especially in SMS-amended soils.

^{14}C-Metalaxyl and ^{14}C-penconazole mineralization was significantly higher in unamended soils relative to amended soils due to the higher fungicide sorption by amended soils, as indicated above. A decrease has been reported in the amount of pesticide mineralized in amended soils because of the higher sorption,[36] although other authors attributed this decrease to the use of the OM added with the amendment by the microorganisms instead of the pesticide.[6] The amounts of ^{14}C-metalaxyl mineralized to $^{14}CO_2$ after 175 days were 19.6, 3.42, and 1.00% in Al, Al+C-SMS1, and Al+F-SMS2 soils, respectively. There is lower mineralization in Al+F-SMS2,

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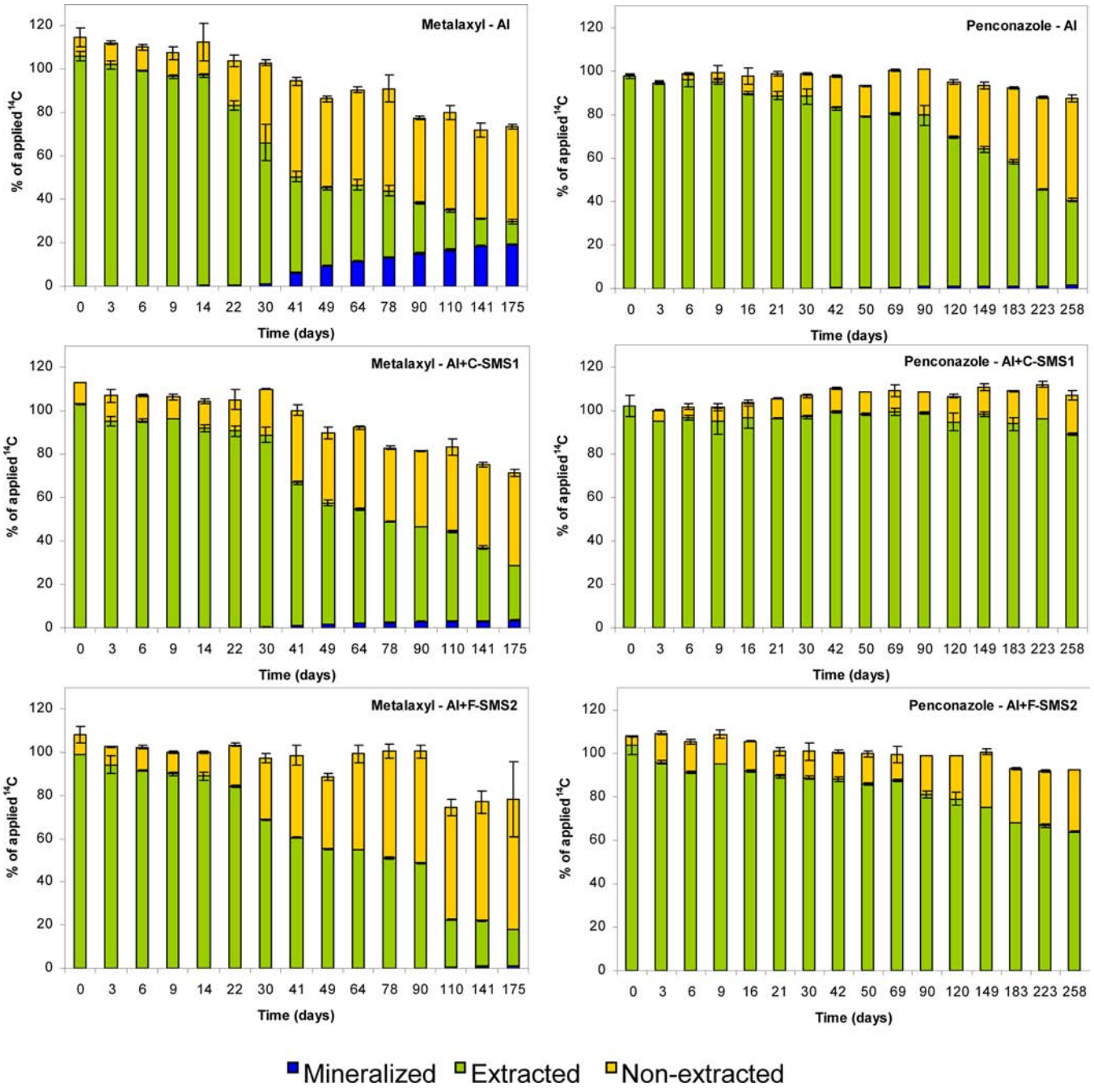

Figure 3. Mass balances of mineralized, extracted, and nonextracted ^{14}C (expressed as percentage of applied ^{14}C) for the dissipation studies of ^{14}C-metalaxyl and ^{14}C-penconazole in unamended and SMS-amended soils. Bars indicate the standard deviation of the mean.

despite the greater degradation rate than in Al+C-SMS1. This is consistent with the formation of a higher amount of metabolite CGA 62826 (Figure 2), which was not finally degraded to $^{14}CO_2$ as mineralization kinetics indicated.

For penconazole, the percentages mineralized were 1.45, 0.10, and 0.22% in Al, Al+C-SMS1, and Al+F-SMS2 soils, respectively, after incubation for 258 days. The low mineralization of this fungicide would indicate the difficulty microorganisms face in degrading the triazole ring, where ^{14}C is labeled, and this is consistent with the degradation rates of fungicide in unamended and amended soils.

In Al and Al+C-SMS1 the total amount of $^{14}CO_2$ evolved from metalaxyl exceeded the level of radiochemical impurities in the ^{14}C-labeled compound. However, for metalaxyl in Al+F-SMS2 and penconazole in all soil samples, the amount of $^{14}CO_2$ evolved was not above the impurity level, so actual mineralization of the compounds may be lower than indicated or may even not have occurred.

For both fungicides, the extracted amounts decreased as incubation time increased. The decrease was higher in the unamended soil with respect to the amended soils as indicated for nonlabeled fungicides. The extracted ^{14}C amounts were 10.7, 25.3, and 17.0% of the ^{14}C-metalaxyl initially added in unamended and C-SMS1- and F-SMS2-amended soils after 175 days of incubation. For penconazole, these amounts were 38.8, 89.4, and 63.6% of the ^{14}C-fungicide added in the soils after 258 days. The extracted amounts of ^{14}C corresponded to the parent compound and its metabolites formed during degradation and

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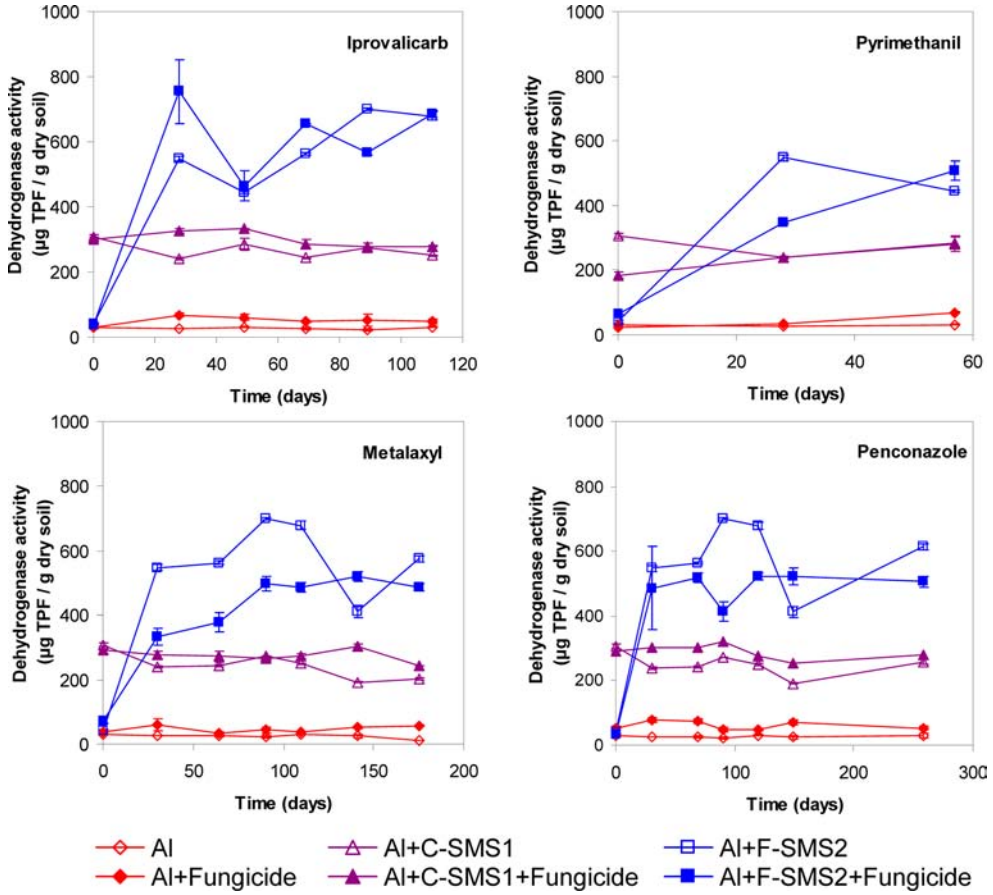

Figure 4. Soil dehydrogenase activity for unamended and SMS-amended soils, untreated (open symbols) and treated with fungicides (solid symbols) at different sampling times. Bars indicate the standard deviation of the mean.

were slightly higher than the extracted amounts of unlabeled fungicides where we determined the parent compound and metabolites (if formed) separately. The lower amount of extracted fungicides in the unamended soil with regard to amended soils was consistent with higher fungicide mineralization in this soil after 175 days for metalaxyl (Figure 3). However, for penconazole this was mainly supported by the formation of a higher percentage of nonextractable residues after 258 days in the unamended soil.

The amounts of nonextractable residues increased with the incubation time of fungicides in the soils, as reported for other pesticides and soils amended with different amendments.[37] The percentages of nonextractable residues formed at the end of the incubation time were 43.8, 42.7, and 60.3% for metalaxyl and 46.8, 17.7, and 28.1% for penconazole in the Al, Al+C-SMS1, and Al+F-SMS2 soils, respectively. The growing amounts of nonextractable residues built up over time also correspond to both the ^{14}C-fungicide and its ^{14}C-metabolites.

The formation of nonextractable residues in the soils was in general higher for metalaxyl than for penconazole. This could be explained because the sorption of metalaxyl by soils is low[17] (Table 1) and the degradation of fungicide was enhanced as previously indicated (Figure 2). Metabolite CGA 62826 might form bound residues in unamended and amended soils because its sorption was up to 8 times higher than the sorption of metalaxyl by the same soils.[17] The higher formation of bound residues of metalaxyl in Al+F-SMS2 in relation to Al+C-SMS1 is consistent with the higher degradation in this soil with higher DOC content. DOC could sorb the fungicide, enhancing its bioavailability for degradation as we have previously indicated.

In the case of penconazole, a lower amount of bound residues was detected in the SMS-amended soils, although it is generally indicated that the formation of nonextractable residues depends on the OC content of soils.[38] However, Barriuso et al.[39] observed a decrease in the formation of bound residues for dimefuron when the amount of compost added to the soil increased, indicating that the effect of the amendments in the formation of bound residues depends on pesticide characteristics. In our study, the formation of bound residues decreased in the order Al > Al+F-SMS2 > Al+C-SMS1, being inversely related to the sorption coefficients of penconazole by soils (Table 1). The increase in bound residues in unamended and Al+F-SMS2 soils after 50 and 90 days of incubation, respectively, could be related to the increase in penconazole degradation with the formation of more reactive metabolites to form bound residues than the parent compound.[40] In relation to this, a report drafted by the European Food Safety Authority[22] has indicated the possible degradation of this fungicide in soil, leading to different metabolites and nonextractable residues that could be slowly mineralized to CO_2.

Soil Dehydrogenase Activity. The DHA values for unamended and SMS-amended soils either untreated (controls) or treated with fungicides over the incubation period are presented in Figure 4. DHA mean values were higher in amended soils than in the unamended one, indicating the positive effect of the amendment on soil microbial

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activity.[15,41,42] The addition of organic residues to soil stimulated DHA due to the greater OC content available in the amended soil and the presence of new soil microbial populations introduced with the amendment. Furthermore, DHA was higher in the Al+F-SMS2 soil, due to its highest OC content and the degradation of more labile compounds provided by the amendment as DOC. The higher DHA in this soil is also consistent with the higher degradation rate of fungicides when compared with C-SMS1-amended soil.

DHA values were significantly higher at the beginning than at the end of the incubation time for the unamended soil (LSD = 5.36, $p < 0.01$) and C-SMS1-amended soil (LSD = 19.5, $p < 0.01$). DHA then remained practically constant in all subsequent measurements, indicating the stabilization of microbial activity. However, the F-SMS2-amended soil had initial DHA values similar to those of the unamended soil, and DHA values increased after 30 days (LSD = 17.8, $p < 0.01$) due to the adaptation period required for the microorganisms.

DHA values in the soils treated with the fungicides were, in general, significantly higher ($p < 0.05$) than in the control soils (without fungicides), indicating the soil microbial activity was stimulated by the addition of fungicides to the soil. This effect has also been reported for other fungicides or herbicide−amendment−soil combinations in the literature,[41,42] In the F-SMS2-amended soil treated with metalaxyl or penconazole, DHA decreased significantly with respect to the F-SMS2-amended soil without fungicide (control soil) (LSD = 33.5, $p < 0.05$; and LSD = 79.8, $p < 0.05$, respectively). The fungicide had a toxic effect on soil microorganisms and slightly inhibited soil DHA. This effect was more significant in the soil treated with metalaxyl and could be related to the lower mineralization kinetics of this fungicide in the F-SMS2-amended soil than in the C-SMS1-amended soil.

The study showed that the application of SMS as an amendment to a vineyard soil had an impact on the dissipation of fungicides representative of different chemical classes. A reduction in the degradation rate was seen for all fungicides in the soil amended with C-SMS1, but this reduction was observed only for iprovalicarb and penconazole when the soil was amended with F-SMS2. The results could be explained in terms of the sorption coefficients of fungicides by soils, which increased in the order Al < Al+F-SMS2 < Al+C-SMS1. The retention of fungicides by soil amendments reduced the bioavailability of fungicides to soil microorganisms and decreased the degradation rates of these compounds in the vineyard soil. However, the degradation rates of metalaxyl and pyrimethanil in the Al+F-SMS2 soil were similar or close to those in the unamended soil, and they were consistent with the high DOC content or the high DHA activity of Al+F-SMS2 soil. The influence of SMS amendments was also evident in the dissipation mechanism of ^{14}C-fungicides in relation to the formation and/or fate of degradation products formed during incubation. Therefore, the results obtained provide evidence that the influence of SMS amendments on the dissipation of fungicides in vineyard soils have to be evaluated to predict the persistence of these compounds in soil and/or possible transport to waters when SMS and fungicides are applied simultaneously.

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Notes

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