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Behavior of Metalaxyl and Its Pure *R*-Enantiomer in Sunflower Plants (*Helianthus annus*)

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A possible stereospecific and/or stereoselective mechanism of biodegradation for metalaxyl and metalaxyl-M was studied to elucidate their behavior in sunflower plants and to compare their biodegradation. Greenhouse experiments were carried out to confirm the same efficacy of the two fungicides against infections by *Plasmopara helianthi* in sunflower plants. The two fungicides appear to have the same behavior regarding both the protection against plant infections and the mode of translocation and the rate and pathway of biotransformation, but we have evidence that this biotransformation process is enantioselective. Furthermore, we propose procedures for a chromatographic separation of enantiomers and acid metabolites of the fungicides and for the determination of the *R*:*S* ratio by HPLC chiral analyses. This study emphasizes the importance of examining the fate of both stereoisomers of a chiral agrochemical in an environmental system for the correct use of enantiomerically pure agrochemical compounds.

KEYWORDS: Metalaxyl; chirality; Oomycetes; enantiomers; biodegradation

INTRODUCTION

Many agrochemicals, about 25% of the total (*1*), are chiral molecules and may exist in different stereoisomeric forms which may have different properties in asymmetrical environments such as living systems. At the present time, a significant number of these chiral agrochemicals are released into the environment as racemates or a mixture of stereoisomers, but in some cases the biological activity of a pesticide may be attributed to one stereoisomer, while the other stereoisomers have little or no activity (*2*). Indeed, the stereoisomers may have complementary biological activity, or one enantiomer may produce a completely different biological response than its antipode, and therefore the stereoisomers have different biological and physiological properties in terms of qualitative and/or quantitative effects. It is important to point out that stereochemistry is a factor influencing not only biological activity but also processes such as uptake, distribution, and metabolic behavior in organisms and in the environment. After their field application, chiral pesticides generally undergo a series of biologically mediated reactions and, if a racemic mixture of stereoisomers is applied, there may well be a predominance of an isomer in the residue, depending on the stereospecificity in the biological processes that may be responsible for the preferential biodegradation of one of the stereoisomers (*3*). Therefore, the stereochemical control in biodegradation process has important implications in the manufacture and use of chiral agrochemicals, and the separation, identification, and use of the biologically active isomer is essential to minimize contaminants in the environment. Indeed, regulatory actions have already limited the use of racemates in The Netherlands and in Switzerland for chiral herbicides such as dichlorprop and mecoprop, for which only one form is herbicidally active (*4*).

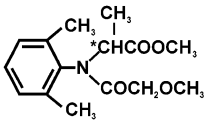

Figure 1. Chemical structure of metalaxyl. *Asymmetric carbon atom.

Metalaxyl [(*R*,*S*)-methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate] is an important acylanilide fungicide introduced in 1977, widely used in the control of plant diseases caused by pathogens of the *Oomycota* division. It is a systemic, apoplastically transported fungicide, highly active against fungi of the order *Peronosporales* by selectively interfering with the synthesis of ribosomal RNA (*5*, *6*). Metalaxyl is chiral due to the presence of the stereogenic center in the alkyl moiety (**Figure 1**) and consists of a pair of enantiomers, *S*(+) and *R*(−) (*7*). A comparative study of the biochemical effects of the stereoisomers of metalaxyl indicated that the *R*- and *S*-enantiomers have the same mode of action, but show considerable differences in their effectiveness in reaching or binding to the receptor (*8*). Biological in vitro tests against *Phythophthora infestans* and *Pythium ultimum* showed that the *R*-enantiomer was approximately 1000 times more active than the *S*-enantiomer, but in vivo the activity of the *R*-enantiomer was still 3−10 times

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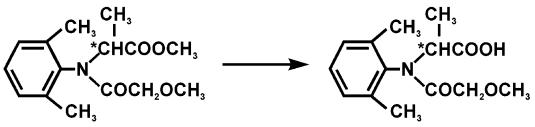

Figure 2. Hydrolysis reaction of metalaxyl to its acid metabolite.

more than the *S*-enantiomer. The *S*-enantiomer of metalaxyl is inactive in the herbicide test, and therefore metalaxyl, as the technical racemate, is not phytotoxic. Nevertheless, the implications of stereoisomerism for the biological activity of fungicides are difficult to characterize, since the receptors and the metabolic pathways must be considered in both the pathogens and the host plant (*9*).

Biodegradation of metalaxyl in different soils and plants has been extensively studied (*10*−*14*), and many studies have demonstrated that the degradation of this chemical in soil is a microbiologically mediated process (*15*). The biological processes generally tend to stereospecifically degrade chiral organic compounds. Indeed, a enantiospecific microbial degradation of racemic metalaxyl in two media (soil and sewage sludge) was reported (*16*), but these studies provided no information on the fate of the individual enantiomers of either the parent compound or their chiral hydrolysis products.

In our previous study (*17*), we demonstrated that the biodegradation of metalaxyl and metalaxyl-M in soil is stereospecific; likewise, it can be expected that the behavior and the reactions of these compounds with chiral structures (protein such as metabolizing enzymes) of biological systems (plants) are stereospecific.

In light of the recent recommendations by Sukul and Spiteller (*15*) that precautions be taken in the continued application of metalaxyl to crops, the purpose of this work is to investigate the behavior of (*R*,*S*)-metalaxyl and of the pure active *R*-enantiomer (metalaxyl-M) in infected sunflower plants as well as to verify and monitor the possible chiral switch of the racemic mixture and/or of the single enantiomer. Moreover, because both in soil and in plants metalaxyl undergoes a hydrolysis reaction, we investigated the rate of the formation of the acid metabolite (**Figure 2**) and the stereochemistry of this reaction. Indeed, a stereospecific biodegradation may lead either to enantiomeric ratios of parent compounds and metabolites which differ from the 1:1 ratio (when the pesticide is applied as a racemic mixture) or to a racemization of the pesticide applied as a pure enantiomer.

MATERIALS AND METHODS

Chemicals. All reagents, Analar or Hipersolv grade, were obtained from BDH. The certified standard of metalaxyl [(*R*,*S*)-metalaxyl)] (chemical purity greater than 99.3%) was purchased from Dr. Ehrenstorfer G.m.b.H. (Germany). The certified standard of metalaxyl-M *R*(−)enantiomer (chemical purity greater than 94.7%), the commercial products Apron SD35 (35% of metalaxyl), and CGA329351 ES350 (35% of metalaxyl-M) were provided by Syngenta (formerly Novartis).

Biological Materials. All experiments were carried out with susceptible sunflower plants (*Helianthus annuus* L. cv. Ala). Isolate of *Plasmopora helianthi* (race 1 or "European race") was used for the induction of infection.

Fungicides Application, Plant Care, and Pathogen Inoculum. In the first experiment, the fungicides Apron SD35 and CGA 329351 ES 350 were applied as a seed dressing to 5 g of sunflower seeds at the rate of 6 and 3 g/kg of seed, respectively. The sunflower seeds were sown (15 seeds/box) in boxes (20 × 40 × 10 cm) each containing 1.5 kg of a sterilized sandy−peat mixture (1:1, v:v). The isolate of *P. helianthi* was maintained on susceptible sunflower plants by inoculating pregerminated seeds and growing them in pots containing perlite in a separate growth chamber. After 2 weeks, sporulation was induced by covering the infected plants with plastic bags for 48 h to provide a saturated atmosphere (*18*). Zoosporangia (and zoosporangiophores) were gently brushed from the cotyledons and leaves into distilled water; their concentration was determined with a haemocytometer and adjusted as necessary with distilled water. Seven days after sowing, the infection of the experimental plants was obtained by distributing the zoosporangia suspension (700 zoosporangia/g of soil) over the soil surface (20 mL/box). The controls consisted of plants treated with the same chemicals without the inoculation of *P. helianthi*, plants treated with water, and untreated plants inoculated with *P. helianthi*.

In the second experiment, the seeds were treated with the fungicides in the same way as experiment 1, but without pathogen inoculum.

Plants of all experiments were grown in a greenhouse at an alternating day−night temperature of 20/18 ± 2 °C, 60−70% day/night relative humidity with 12 h of daylight (180 μE/m^{-2} s^{-1}) for 80 days. Plants were fertilized one per week with 20−5−10 (N−P−K). These experiments were repeated 4 times, and 7 days after planting the plant material was sampled periodically.

Evaluation of Systemic Infection by *P. helianthi*. Plants from the first experiment were also placed for 48 h in a saturated atmosphere to assess fungal sporulation on hypocotyls, cotyledons, and true leaves. Plants were removed from the soil, and sporulation of *P. helianthi* was assessed visually with or without a dissecting microscope. All hypocotyls that seemed to be healthy were cut into sections 2−3 cm long and were then incubated on moist filter paper in Petri dishes at 20 °C for 24−48 h in the dark to induce sporulation. Percentages of infected plants were therefore determined by counting plants with systemic infection (showing chlorosis, stunting, and sporulation on cotyledons and true leaves) and plants with sporulation on hypocotyls.

Plant Samples Preparation. Metalaxyl, metalaxyl-M, and their acid metabolites were extracted from plant leaves (of the second experiment) following this procedure. Five grams of vegetable matter was homogenized in a high-speed blender with methanol (2 × 20 mL) for 15 min. The supernatant was filtered through filter paper and the filtrate evaporated to 5 mL by using a rotary evaporator at 45 °C; 25 mL of distilled water was added to the residue, and this solution was extracted with ethyl-acetate (3 × 30 mL). Organic phases were collected, dried with 2 g of anhydrous sodium sulfate, filtered, and evaporated under vacuum at 40 °C to dryness. The residue, dissolved in 2 mL of hexane, was transferred into an SPE column (1 g of silica) for the cleanup step; impurities were washed out by rinsing with 20 mL of hexane, hexane−ethyl acetate 5% v/v, hexane−ethyl acetate 10% v/v, and hexane−ethyl acetate 15% v/v. The desorption of compounds was achieved using 30 mL of hexane−ethyl acetate 20% v/v. The eluate was evaporated to dryness.

Preparation of the Comparison Compound Metalaxyl Acid Metabolite [*N*-(2,6-Dimethylphenyl)-*N*-(methoxyacetyl)-alanine]. One gram of metalaxyl or metalaxyl-M was added to 100 mL of 10% sulfuric acid solution at 100 °C by refluxing for 10 h. Fifty milliliters of water was added to the cool solution, the pH of which was adjusted to 9−10 with 1 N NaOH, and the solution was then extracted with ethyl-acetate (3 × 100 mL). The aqueous phase was adjusted to pH 2 with 2 N HCl and further extracted with ethyl-acetate (3 × 100 mL). Organic phases were collected, dried with 2 g of anhydrous sodium sulfate, and the solution was then filtered and evaporated in vacuo to dryness. The resulting white crystals were collected and washed with water. The identity of the acid was confirmed by NMR data. The NMR spectra were obtained with a Bruker DRX 400 (400 MHz, ^{1}H; 400 MHz ^{13}C) spectrometer equipped with a 5-mm probe.

^{1}H NMR ($CDCl_3$). δ 1.14 (d, 3H, J = 7.4 Hz); 2.2 (s, 3H); 2.41 (s, 3H); 3.37 (s, 3H); 3.56 (qAB, 2H, J = 15.6 Hz); 4.59 (q, 1H, J = 7.4 Hz); 7.2 (m, 3 ArH); 10.64 (bs, OH).

^{13}C NMR ($CDCl_3$). δ 14.74, 18.62, 18.98, 56.28, 59.28, 129.33, 129.68, 129.89, 135.72, 139.14, 137.56, 171.7, 176.4.

Methylation with Diazomethane. The synthesized acid and the plant extracts containing the acid metabolite were converted into their corresponding methyl ester to confirm their identity. The dry residue was dissolved in 1 mL of $CHCl_3$, and 1 mL of CH_2N_2 (in $CHCl_3$) was added. Diazomethane was produced by the reaction of potassium hydroxide dissolved in diethylene glycol and a solution of *N*-nitro-*N*-methyl-*p*-toluensulfonamide in diethyl ether. After methylation, the

solution was evaporated to dryness and the residue dissolved in 1 mL of hexane for GC−MS analysis.

High Performance Liquid Chromatography (HPLC). A modular instrumentation comprising a solvent delivery system constaMetric 4100 (LDC Analytical), a variable UV wavelength detector spectroMonitor 3200 (Thermo Separation Products), a Rheodyne injection system with a 10 μL loop, and a Alltima Alltech C_{18} column (5 μm 150 × 4.6 mm) was used to monitor the disappearance of the parent metalaxyl and the production of its acid metabolite. Peak area was integrated using a computer with a data system Chrom-Card. The operating parameters were as follows: solvent A (H_2O) and solvent B (CH_3CN) with a gradient system (0−10 min 50% A and 50% B and 10−20 min 10% B), and a flow rate of 0.8 mL/min. Ten microliters of the samples, redissolved in 1 mL of the same mobile phase, were injected and detected at a wavelength of 254 nm. Calibration was performed daily using external standards and linear regression analysis.

Chiral High Performance Liquid Chromatography (HPLC). Enantiomers of metalaxyl were separated using the HPLC apparatus previously described equipped with a OJ Chiralcel column with cellulose derivative as the stationary phase (Daicel, 250 × 4.6 mm). The operating conditions for the determination of (*R*,*S*)-metalaxyl and (*R*)-metalaxyl were as follows: mobile phase *n*-hexane/2-propanol (85:15 by volume), flow rate 0.8 mL/min, and detector wavelength 254 nm. The metalaxyl peak was split into two components, the enantiomers (*R*) and (*S*), whose elution order was established by comparison with the retention time of the *R*-pure enantiomer.

The operating conditions for the determination of corresponding acid metabolites were as follows: mobile phase *n*-hexane/2-propanol/formic acid (95:5:0.5 by volume), flow rate 0.8 mL/min, and detector wavelength 254 nm. The enantiomers were identified as described above.

Gas Chromatography−Mass Spectroscopy (GC-MS). A Varian Star 3400 gas chromatograph equipped with a split-splitless injector was combined by direct coupling to a Varian Saturn II mass spectrometer, operating in the electron impact mode (EI), equipped with a multiple-ion detector. A CP-Sil8CB capillary column (Chiralpack 25 m × 0.25 mm, F.t. 0.25 μm) was used. The chromatographic conditions were as follows: the temperature was programmed from 60 °C (1 min) to 260 °C (15 min) at 20 °C min^{-1}. In this condition, the metalaxyl retention time was 14 min 38 s. The carrier gas was helium at the flow rate of 1 mL min^{-1}; the temperatures of the injector port and of the ion source were 220 and 280 °C, respectively; the emission current was 10 μA.

RESULTS AND DISCUSSION

Response of Sunflower Plants to Metalaxyl-M in Comparison to Metalaxyl. Plants treated with the two fungicides did not show any symptom of disease (chlorosis, stunting, and sporulation on cotyledons and true leaves): the percentage of infection was 0%; therefore, Apron SD35 and CGA329351 fungicides showed the same activity toward the pathogen. Conversely, the percentage of infection observed in control plants untreated with fungicides, ranged from 78 to 81% (average 79%).

Biodegradation of Metalaxyl and Metalaxyl-M in Sunflower Plants. The analytical method used to determine the residue of pesticides in sunflower plants was fast and reliable. The recoveries of pesticides from vegetables treated with standard solutions of metalaxyl and metalaxyl-M were 80% ± 0.71 (standard error of four replicates) for a concentration of 1 ppm and 75% ± 0.48 for a concentration of 0.01 ppm. Quantitative analyses of these compounds were carried out by comparing peak area and retention time of the residue with the authentic standard. Concerning the behavior of metalaxyl and metalaxyl-M in sunflower plants, both compounds were absorbed by the target plant and accumulated in leaves, but we did not observe any difference regarding the rate of translocation. Their maximum concentrations were achieved 38 and 31 days after seed treatment, and these concentrations declined from 2.66 to 0.87 ppm and from 1.82 to 0.33 ppm, for metalaxyl and metalaxyl-M, respectively, 85 days after treatment (**Table 1**). The biodegradation of both fungicides appeared to follow a pseudo first-order kinetic reaction, and the degradation rate constants were determined using regression plots of ln (c/c_0) versus time (t). The half-life time values ($t_{1/2}$) of 23 and 28 days for metalaxyl and metalaxyl-M, respectively, were observed (**Figure 3**).

Table 1. Concentrations of Metalaxyl and Metalaxyl-M in Sunflower Leaves over Time

days after treatment	ppm metalaxyl	ppm metalaxyl-M	days after treatment	ppm metalaxyl	ppm metalaxyl-M
7	0.50		44	1.89	1.44
10	0.93		50	1.58	1.00
14	1.28	0.60	56	1.24	0.96
17	1.50	0.70	60	1.14	0.92
21	1.68	0.81	66	1.07	0.70
25	1.70	1.28	75	0.96	0.67
31	1.73	1.82	85	0.87	0.33
38	2.66	1.49			

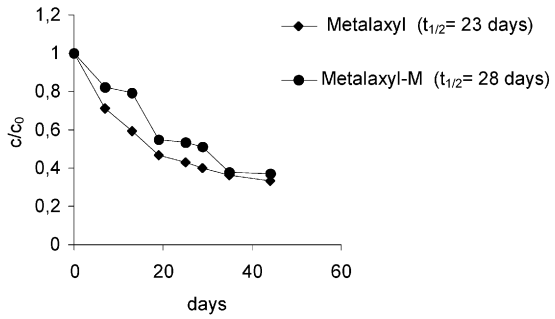

Figure 3. Biodegradation of metalaxyl and metalaxyl-M in sunflower leaves over time. (The starting point is the maximum value of concentration.)

In view of these different residual concentrations of metalaxyl and metalaxyl-M, we decided to study and monitor the formation of acid compound as one of the metabolic products of this chemical. This acid metabolite is the main breakdown product, both for metalaxyl and metalaxyl-M, arising from ester hydrolysis (*15*). The disappearance of both fungicides and the formation of their acid metabolites were monitored by HPLC analysis as described above. The identity of the acid metabolite of metalaxyl was confirmed by comparing the residue retention time with authentic standards and by following the methylation procedure described by Businelli et al. (*19*). The GC-MS data are according to those of the synthesized compound. The retention time and the MS fragmentation of the acid metabolite after methylation are the same as that of the parent metalaxyl (M^+ at m/z 280 and significant m/z values at 248, 220, 160) (**Figure 4**). Quantitative analyses of these compounds were carried out, and the HPLC profiles of residues of metalaxyl show that the decrease in the unchanged parent compound was accompanied by an increase in the concentration of the acid metabolite. This acid metabolite did not appear until 21 days after treatment as seed dressed, and its concentration increases about 2-fold after 60 days, but the complete disappearance of metalaxyl ester was not observed at least up to 90 days (**Figure 5**).

Concerning the formation of acid metabolite from metalaxyl-M, we observed that the compound appeared later than the previous acid compound (38 days after treatment) and also its concentration increased about 2-fold at the end of the experiment (**Figure 6**). These observations are confirmed by the very similar values of formation rate of the acid metabolite; in fact, k is 0.0147 for the metalaxyl acid metabolite and 0.0149 for the metalaxyl-M acid metabolite (**Figure 7**).

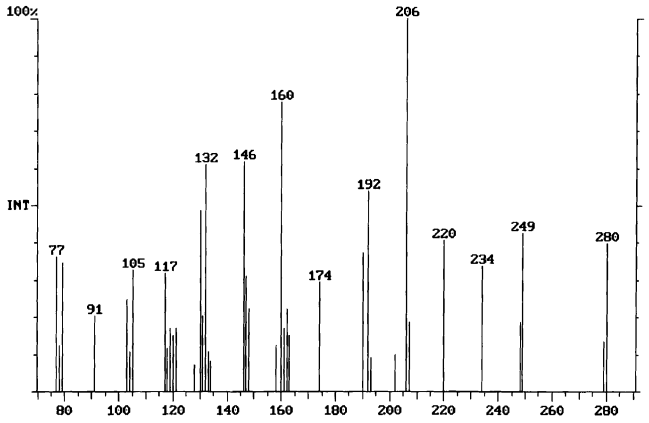

Figure 4. Mass spectrum of metalaxyl ester.

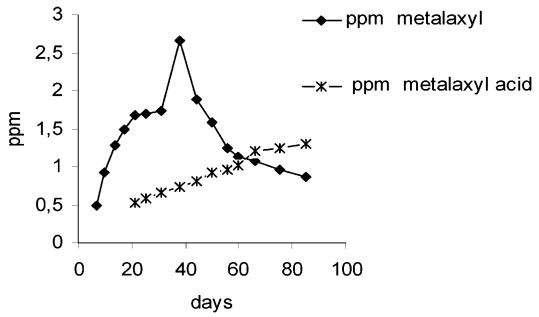

Figure 5. Concentration of metalaxyl and its acid metabolite in sunflower leaves over time.

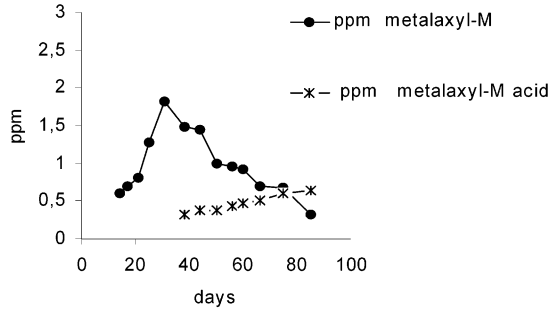

Figure 6. Concentration of metalaxyl-M and its acid metabolite in sunflower leaves over time.

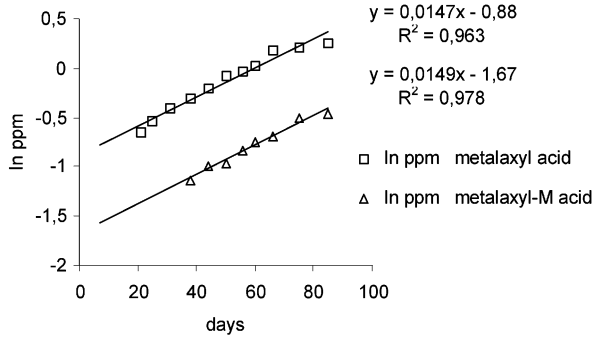

Figure 7. Rate of formation of metalaxyl acid and metalaxyl-M acid.

In order to compare the fate of the two enantiomers of metalaxyl, the ratios of *R*:*S* enantiomers in the plant extracts containing *rac*-metalaxyl were investigated. The degradation rate constants and the half-life values of the *S*- and *R*-enantiomers were calculated. The data showed that the two enantiomers degraded at slightly different rates: the *R*-enantiomer ($t_{1/2}$ = 24 days) degrades more slowly than the *S*-enantiomer ($t_{1/2}$ = 21 days). The data of the residual concentrations of the two enantiomers were used for estimating the enantiomeric ratio (ER) values during this experiment. The ER was defined as the peak area of the first eluting *R*-enantiomer divided by the peak area of the later eluting *S*-enantiomer (*20*). The initial formulation (Apron) containing (*R*,*S*)-metalaxyl has an ER = 1.02 showing that the two enantiomers are present in the same proportion. In **Table 2** are shown the ER values in plant leaves over time; these values concurred with the enantiospecific degradation observed from the kinetic data and are more revealing of the enantiospecificity than the values of the half-life. The vegetable extracts were initially rich in *S*-enantiomer, whereas 25 days after treatment there was a rise in the concentration of *R*-enantiomer. The ER values ranged from 0.49 (composition $S > R$) to 4.80 (composition $R \gg S$). The change of ER indicated a initially predominance of *S*-enantiomer in comparison to *R*-enantiomer, but with time the *S*-enantiomer disappeared faster than *R*-enantiomer. This steady decrease of *S*-form concentration in comparison to the *R*-form could indicate a preferential biotransformation of *S*-isomer by the plant's enzymatic systems when this isomer is applied as a racemic mixture. On the other hand, it is known that the *R*-enantiomeric form is biologically more active than the *S*-form, so its higher concentration and its greater fungicide activity help in the protection against *Plasmopora* diseases in sunflower plants. In turn, the decrease of *S*-enantiomer could be a consequence of the chiral inversion ($S \rightarrow R$), while it is not so surprising that the biotransformation process of chiral fungicides could lead to an inversion of configuration, but it was observed from the beginning of this study that there was no chromatographic evidence of an inversion of the *R* absolute configuration of metalaxyl-M to the inactive *S*-form and probably the reverse is the case.

We compared the relative concentrations of the *R*- and *S*-acid isomers (enantiomer peak areas) of metabolite arising from (*R*,*S*)-metalaxyl. These data showed little changes in the 1:1 ratio of the racemic mixture of acid metabolites; at any given moment the *R*-isomer peak is always slightly smaller than *S*-isomer with a practically constant enantiomeric ratio (ER = 0.94−0.98). The study of the formation of the two enantiomers of metalaxyl acid has showed that the *S*-enantiomer appeared

Table 2. Enantiomeric Ratio (ER = *R*/*S*) of (*R*,*S*)-Metalaxyl and (*R*,*S*)-Metalaxyl Acid Metabolite in Sunflower Leaves

days after treatment	ER *R*,*S*-metalaxyl	ER *R*,*S*-metalaxyl-M	days after treatment	ER *R*,*S*-metalaxyl	ER *R*,*S*-metalaxyl-M
7	0.75		44	3.20	0.97
10	0.67		50	3.38	0.95
14	0.75		56	3.13	0.98
17	0.70		60	3.38	0.96
21	0.49	0.92	66	3.86	0.98
25	1.04	0.93	75	3.80	0.98
31	1.19	0.94	85	4.80	0.94
38	3.33	0.97			

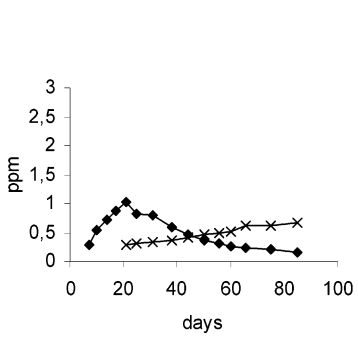

Figure 8. Concentration of *S*-enantiomer of metalaxyl and its acid metabolite in sunflower leaves over time.

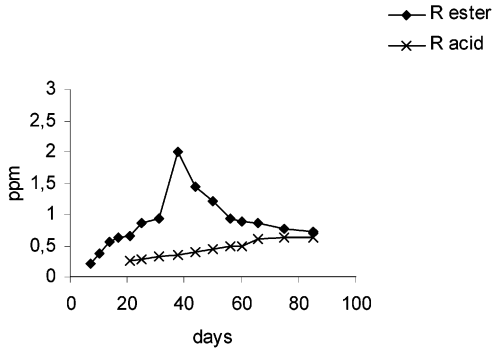

Figure 9. Concentration of *R*-enantiomer of metalaxyl and its acid metabolite in sunflower leaves over time.

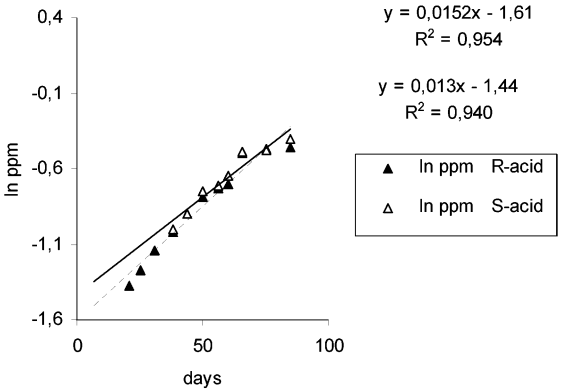

Figure 10. Rate of formation of the two enantiomers of metalaxylacid metabolite in sunflower leaves over time.

after 21 days, and the final concentration of *S*-enantiomer acid metabolite was about four times its parent *S*-ester (0.68 vs 0.15 ppm) (**Figures 8** and **9**), while the *R*-enantiomer acid appeared after 38 days and at the end of the experiment the concentration of *R*-acid was similar to its parent *R*-ester (0.63 vs 0.72 ppm). In this case, the *K* values of (*R*)- and (*S*)-metalaxyl acid metabolite were different: $k_{(R)} = 0.013$ and $k_{(S)} = 0.0152$ (**Figure 10**).

CONCLUSIONS

In our experiment, we have confirmed that the two fungicides, metalaxyl and metalaxyl-M, exhibited the same efficacy against infections by *P. helianthi*. Metalaxyl and metalaxyl-M were readily biodegraded in sunflower plants by ester hydrolysis, so that the major metabolites were the corresponding carboxylic acids. These acid compounds arising from metalaxyl and metalaxyl-M were formed at different times: (*R*,*S*)-metalaxyl acid metabolite appeared before the (*R*)-metalaxyl acid metabolite. The hydrolysis process of the two enantiomers of (*R*,*S*)-metalaxyl occurred at different rates: the *S*-enantiomer was biodegraded faster than the *R*-enantiomer. Furthermore, we have observed that in sunflower plants metalaxyl was converted into its acid metabolite with retention of configuration; in fact, the (*R*,*S*)-metalaxyl was converted into (*R*,*S*)-metalaxyl acid metabolites and metalaxyl-M was converted only into an *R*-acid metabolite without inversion of configuration.

LITERATURE CITED

(1) Williams, A. Opportunities for chiral agrochemicals. *Pestic. Sci*. **1996**, *46*, 3−9.

(2) Kurihara, N.; Miyamoto, J.; et al. Chirality in synthetic agrochemicals: bioactivity and safety consideration. *Pure Appl. Chem*. **1997**, *69*, 2007−2025.

(3) Zipper, C.; Suter, M. J. F.; et al. Changes in the enantiomeric ratio of (*R*)- to (*S*)-Mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environ. Sci. Technol*. **1998**, *32*, 2070−2076.

(4) Schmitt, P.; Garrison, A. W.; Freitag, D.; Kettrup, A. Application of cyclodextrin-modified electrokinetic chromatography to the separation of selected neutral pesticides and their enantiomers. *J. Chromatogr. A* **1997**, *797*, 419−429.

(5) Moser, H.; Vogel, C. Preparation and biological activity of the enantiomers of CGA 48988, a new systemic fungicide. Presented at the 4th International Congress on Pesticide Chemistry, Zürich, 1978; Abstract II, p 310.

(6) Hassall, K. A. Systemic fungicides. In *The Biochemistry and Uses of Pesticides: Structure, Metabolism, Mode of Action and Uses in Crop Protection*. VCH: Weinheim, 1990; pp 350−354.

(7) Ariëns E. J.; van Rensen J. J. S.; Welling W. Implication of stereoisomerism in agricultural fungicides. In *Stereoselectivity of Pesticides, Biological and Chemical Problems*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1988; pp 203−209.

(8) Vishunavat, K.; Nashaat, N. I.; Heran, A.; Kolte, S. J. Sensitivity to the racemic mixture and isomeric forms of metalaxyl in Indian and European homotallic and heterotallic isolates of *Peronospora parasitica* in *Brassica* species. *Crop Prot.* **1998**, *17* (6), 543−546.

(9) Ramos Tombo, G. M.; Bellus, D. Chirality and crop protection. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1193−1215.

(10) Droby, S.; Coffey, M. D. Biodegradation process and the nature of metabolism of metalaxyl in soil. *Ann. Appl. Biol*. **1991**, *118*, 543−553.

(11) Sukul, P.; Spiteller, M. Persistence, fate and metabolism of [^{14}C] Metalaxyl in typical Indian soils. *J. Agric. Food Chem.* **2001**, *49*, 2352−2358.

(12) Marucchini, C.; Patumi, M.; Zazzerini, A. Uptake and persistence of Metalaxyl in sunflower plants. *J. Agric. Food Chem.* **1983**, *31*, 1124−1125.

(13) Cole, D. J.; Owen, W. J. Metabolism of Metalaxyl in cell suspension cultures of *Lactuca sativa L.* and *Vitis vinifera L. Pest. Biochem. Physiol.* **1987**, *28*, 354−361.

(14) Sharom, M. S.; Edigington, L. V. The adsorption, mobility, and persistence of metalaxyl in soil and acqueous systems. *Can. J. Plant. Pathol*. **1982**, *4*, 334−340.

(15) Sukul, P.; Spiteller, M. Metalaxyl: Persistence, degradation, metabolism and analytical methods. *Rev. Environ. Contam. Toxicol*. **2000**, *164*, 1−26.

(16) Müller, D. M.; Buser, H. R. Environmental behaviour of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environ. Sci. Technol.* **1995**, *29*, 2031−2037.

(17) Marucchini, C.; Zadra, C. Stereoselective degradation of metalaxyl and metalaxyl-M in soil and sunflower plants. *Chirality* **2002**, *14*, 32−38.

(18) Cohen, Y.; Sackston, W. Factors affecting infection of sunflower by *Plasmopara halstedii*. *Can. J. Bot.* **1973**, *51*, 15−22.

(19) Businelli, M.; Patumi, M.; Marucchini, C. Identification and determination of some metalaxyl degradation products in lettuce and sunflower. *J. Agric. Food Chem.* **1984**, *32*, 644−647.

(20) Garrison, A. W.; Shmitt, P.; Martens, D.; Kettrup, A. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ. Sci. Technol*. **1996**, *30*, 2449−2455.

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