THE ENHANCED BIODEGRADATION OF FENAMIPHOS IN SOILS FROM PREVIOUSLY TREATED SITES AND THE EFFECT OF SOIL FUMIGANTS

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Abstract—The application of fenamiphos either alone or in combination with soil fumigants is a common practice in greenhouses and potato-cultivation areas in Greece. However, repeated applications of fenamiphos in the same field for a number of years can lead to the development of enhanced biodegradation of the nematicide. Studies in previously treated greenhouse sites and potato field sites in Greece were employed in order to investigate the development of enhanced biodegradation of fenamiphos and the respective effect of soil fumigants on the development of the phenomenon. Enhanced biodegradation of fenamiphos in a soil from a previously treated greenhouse site from the area of Aggelohori in Northern Greece was observed using both incubation and bioassay studies with nematodes. Fumigation of the enhanced soil with methyl bromide (MeBr) only temporarily inhibited degradation of fenamiphos unlike metham sodium (MS) whose application significantly reduced microbial degradation of fenamiphos. Similarly, enhanced biodegradation of fenamiphos was evident in soil from potato fields that had a history of previous exposure to fenamiphos. The slow rates of fenamiphos degradation observed in soils from the previously treated sites after sterilization with broad-spectrum antibiotics and also in soils from previously untreated sites suggested that soil microorganisms were responsible for its rapid degradation. The inhibition of enhanced biodegradation of fenamiphos in soil from the previously treated greenhouse site caused by the antibiotic penicillin probably indicates that Gram+ or other bacteria sensitive to penicillin are responsible for the rapid degradation of fenamiphos in this soil. No cross-adaptation was observed between fenamiphos and other nematicides registered in Greece for the control of root-knot and potato cyst nematodes, including cadusafos, ethoprophos, and oxamyl. According to our results, applications of MS followed by fenamiphos or in rotation with other registered nematicides are the most promising means for minimizing the risk of development of enhanced biodegradation of fenamiphos in soils.

Keywords—Fenamiphos Fumigants Enhanced biodegradation Nematicides

INTRODUCTION

Application of nematicides remains the most common strategy for the control of plant parasitic nematodes. However, the involvement of methyl bromide (MeBr) in the depletion of the ozone layer and its potential ban beginning January 1, 2005, will increase reliance of growers on alternative chemicals. Recent findings have documented that there is no single fumigant or nonfumigant nematicide that solely could replace MeBr [1,2]. Consequently, nematode control will rely on the frequent use of nonfumigant nematicides in combination with less frequent applications of soil fumigants other than MeBr. However, the frequent and repeated application of nonfumigant nematicides have resulted in drastic reductions of their biological efficacy. This phenomenon has been called enhanced or accelerated degradation and occurs when soil microflora adapts to a chemical after its repeated application [3]. Most of the registered nonfumigant nematicides for the control of root-knot nematodes (Meloidogyne spp.) in protected crops have been reported as labile to enhanced biodegradation, including fenamiphos [4–6], cadusafos [7], aldicarb, and oxamyl [8,9].

In open field crops like potatoes, control of potato cyst nematodes (Globodera rostochiensis Woll, G. pallida Stone) depends solely on nonfumigant nematicides. However, their annual application in such potato monoculture systems have resulted in drastic reduction of their biological efficacy. Studies in Greece and The Netherlands have associated enhanced biodegradation of fenamiphos [10], ethoprophos [11], aldicarb, and oxamyl [12] with their failure to control nematode infestations in potato areas.

Fenamiphos (O-ethyl-O-[3-methyl-4-(methylthio)phenyl]-isopropyl-amido-phosphate) is an organophosphorous nematicide registered in Greece for the control of root-knot and potato cyst nematodes. Fenamiphos is rapidly oxidized in soils initially to fenamiphos sulfoxide (FSO) and subsequently to fenamiphos sulfone (FSO2), which also possess strong nematicidal activity [13,14]. Therefore, determination of the concentrations of fenamiphos, FSO, and FSO2 is essential in order to assess the total biologically active residues of the pesticide remaining in soil. The sum of the concentrations of fenamiphos, FSO, and FSO2 is called total toxic residues (TTR). Although there are several reports related to enhanced biodegradation of fenamiphos, no studies have been reported on the development of this phenomenon in Greek soils and its significance under the local agricultural strategies that vary among crops. The common agricultural practice applied in greenhouses in Greece for the control of root-knot nematodes is soil fumigation with MeBr or metham sodium (MS) once a year with the subsequent use of nonfumigant nematicides, including fenamiphos, within the cropping season two to three times. On the contrary, in potato cultivations fenamiphos is applied only once per year at planting time.
Table 1. Physicochemical and biological properties of the soils from the Aggelohori greenhouse site (Northern Greece). Values of fluorescein diacetate (FDA) and soil carbon microbial biomass (SCMB) are the mean of three replicates ± the standard deviation of the mean.

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>FDA* (μg g⁻¹)</th>
<th>SCMB* (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfumigated</td>
<td>6.97</td>
<td>2.32</td>
<td>62.9</td>
<td>14.1</td>
<td>23</td>
<td>1.18 ± 0.05</td>
<td>129.3 ± 2.5</td>
</tr>
<tr>
<td>Methyl bromide (MeBr)-fumigated</td>
<td>7.31</td>
<td>2.00</td>
<td>61.0</td>
<td>15.1</td>
<td>23.9</td>
<td>0.61 ± 0.02</td>
<td>64.3 ± 5.1</td>
</tr>
<tr>
<td>Metham sodium (MS)-fumigated</td>
<td>7.15</td>
<td>2.18</td>
<td>65.7</td>
<td>12.4</td>
<td>21.9</td>
<td>0.54 ± 0.02</td>
<td>55.0 ± 6.8</td>
</tr>
<tr>
<td>Control—untreated</td>
<td>8.02</td>
<td>1.50</td>
<td>59.2</td>
<td>5.0</td>
<td>35.8</td>
<td>1.02 ± 0.04</td>
<td>95.9 ± 4.1</td>
</tr>
</tbody>
</table>

* FDA: Measured as μg fluorescein/g dry soil.
* SCMB: Measured as μg C/g dry soil.
Table 2. Physicochemical and biological properties of the soils from the potato monoculture areas of Drama, Greece. Values of fluorescein diacetate (FDA), arylsulfatase, and soil carbon microbial biomass (SCMB) are the mean of three replicates ± the standard deviation

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>FDA (μg/g)</th>
<th>SCMB (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato field A</td>
<td>7.6</td>
<td>0.87</td>
<td>73.0</td>
<td>5.0</td>
<td>22.0</td>
<td>0.28 ± 0.04</td>
<td>33.3 ± 1.1</td>
</tr>
<tr>
<td>Potato field B</td>
<td>5.6</td>
<td>0.90</td>
<td>72.4</td>
<td>2.8</td>
<td>24.8</td>
<td>1.10 ± 0.01</td>
<td>33.7 ± 8.5</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.6</td>
<td>1.07</td>
<td>50.4</td>
<td>34.8</td>
<td>14.8</td>
<td>1.46 ± 0.01</td>
<td>29.7 ± 6.0</td>
</tr>
</tbody>
</table>

at a rate of 30°C/min where it was maintained for 3 min and subsequently increased at a rate of 30°C/min to 230°C where it was held for 2 min. Retention times for cadusafos and ethoprophos were 3.5 and 4.9 min, respectively. The limit of detection for these pesticides was set at 0.01 μg/g. Recoveries of cadusafos and ethoprophos were 86.4 to 94.2% and 92.1 to 97.2%, respectively.

**Microbial measurements**

The size and activity of the soil microflora was measured in order to determine possible effects that fumigant and non-fumigant nematicides might have on soil microorganisms. For this purpose, arylsulfatase and fluorescein diacetate activity in soil as well as the soil carbon microbial biomass (SCMB) were determined. The size of SCMB was measured using a modification of the Jenkinson and Powlson method [18] as proposed by Mele and Carter [19]. Thus, ninhydrin-reactive nitrogen released during fumigation of soil with chloroform (essentially amino acids and ammonium nitrogen) was measured and the following Equation 1 was used for the calculation of soil carbon microbial biomass (μg C/g dry soil).

\[ \text{SCMB} = 21 \times \text{ninyhdrin-reactive nitrogen} \] (1)

The activity of the soil microbial biomass was estimated using a modification of the fluorescein diacetate activity method [20], recently proposed by Adam and Duncan [21]. In addition, the activity of arylsulfatase also was determined using a modified method recently proposed by Elsgaard et al. [22].

**Effect of soil fumigants on the development of enhanced biodegradation of fenamiphos**

Soil samples (30 kg) were collected in May 2002 from the surface layer (0–15 cm) of a commercial greenhouse from the area of Aggelohori near Thessaloniki, Greece. The greenhouse was in a tomato–cucumber rotation and the grower had applied fenamiphos three times in the last two years through the drip irrigation system.

Three 10-kg bulk samples of the previously treated field were weighted. The first sample was placed in a 10-m² (2 × 5 m) microplot and fumigated with MeBr using the quantity of formulation (98% w/w, Bromine Compounds, Beer Sheva, Israel) indicated per 10 m². Methyl bromide was applied to the soil in the plot using a 690-ml bottle and the plot was covered with clear polyethylene plastic film in a way similar to the MeBr-treated plot. The plastic films were maintained in place for the next 15 d. Subsequently, plastics were removed and the soils were mixed and aerated for 2 d to release any remaining fumes. Complete degradation of residues of the fumigants in the corresponding fumigated soils was verified after analysis of soil subsamples (20 g) by gas chromatography with an electron capture detector as described by Verhagen et al. [24]. During fumigation, the third sample (10 kg) was maintained in the laboratory to serve as previously treated but nonfumigated control.

Fumigated and nonfumigated soils were partially air-dried overnight, passed through 3-mm mesh sieve, and subsamples were used for the determination of moisture content and MWHC. Amounts of 6.4 kg from each of the three bulk samples were separated and subsequently divided into 64 subsamples of 100 g. Each of those subsamples received the appropriate amount of an aqueous solution of fenamiphos (5 ml, 100 mg/L) corresponding to a dose of 5 μg/g. Additional water was added to soils to adjust moisture content to 45% of their MWHC. Subsequently, soils were mixed by hand to ensure uniform distribution of pesticide and placed into airtight plastic bags incubated at 25°C. Immediately before incubation and at regular intervals during the next 70 d, eight subsamples from each treatment were removed from the incubator. Three of the eight replicates were used for chromatographic analysis and the remaining five were used in bioassays. A similar number of samples from the previously treated site, which received the same amount of water without fenamiphos, also were incubated along with the fenamiphos-treated samples, but were used only in the bioassay measurements to assess the basic survival of the nematode population in the soil when no fenamiphos had been added.

The involvement of microorganisms in degradation of fenamiphos was verified in chloramphenicol-treated (1.2 kg) samples from fumigated and nonfumigated soils, which were considered as corresponding sterilized samples. Those samples received the appropriate amount of an aqueous solution of the antibacterial antibiotic chloramphenicol (40 ml; 1,500 mg/L) corresponding to a dose of 50 μg/g dry soil and incubated at 25°C for 48 h. Subsequently, soils were divided into 24 subsamples of 50 g, which were treated with a dose of 5 μg/g of fenamiphos (2.5 ml; 100 mg/L) and incubated at 25°C. Immediately before incubation and at regular intervals during the next 70 d triplicate samples from each treatment were analyzed for pesticide residues. Metabolism of fenamiphos also was determined in soil samples (1.2 kg) collected from an adjacent field with no known history of fenamiphos application. Samples were prepared and treated in an identical way as described previously. The moisture content in all soil incubations was maintained constant with the addition of appropriate amounts of water when needed.

**Bioassays with nematodes**

The persistence of fenamiphos and its oxidation products in the different soils described in the previous section also was
evaluated using bioassays with nematodes. Therefore, at each sampling date, five soil samples of 100 g each were removed from the incubator and placed into plastic pots. Samples were inoculated with 1,000 newly hatched second-stage juveniles of Meloidogyne spp. and left for 24 to 36 h at 25°C to ensure uniform distribution of nematodes. Subsequently, a tomato seedling (Lycopersicon esculentum Mill, cv Tiny Tim) at the four-leaf stage was transplanted into each pot. The plants were placed in a growth room at 30°C and, 35 d later, were uprooted and the stems were removed. The roots were washed free of soil and females on roots were counted using a stereoscope at a ×12.5 magnification.

Effect of antibiotics on the biodegradation of fenamiphos

The group of soil microorganisms responsible for the rapid biodegradation of fenamiphos in the soil from the previously treated greenhouse (Aggelohori, Thessaloniki) was identified using specific antibiotics that selectively inhibit certain groups of microorganisms. Four soil samples of 840 g were separated and treated with the appropriate amounts of aqueous solutions (42 ml; 1,000 mg/L) of the antibiotics chloramphenicol, cycloheximide, polymixin B, and kanamycin corresponding to a dose of 50 μg/g dry soil. Another soil sample (840 g) received an aliquot of a solution of penicillin in methanol (16.8 ml; 1,000 mg/L) corresponding to a final dose of 20 μg/g. A final sample (840 g) received the same amount of water without antibiotic to serve as control. All samples subsequently were handled as described before and divided into 21 subsamples of 40 g. Each of the subsamples was treated individually with the appropriate amount of an aqueous solution of fenamiphos (1.6 ml; 100 mg/L) corresponding to a dose of 4 μg/g dry soil, and their moisture content was adjusted to 45% of soil MWHC. Samples were mixed briefly and placed into plastic bags that were incubated in the dark at 25°C. Immediately before incubation and at regular intervals for the next 42 d, triplicate samples from each treatment were removed from the incubator and analyzed as described above.

Cross-enhancement between fenamiphos and other nematicides

Cross-adaptation between fenamiphos and other registered nematicides, including the organophosphates cadusafos, ethophrophos, and the carbamate oxamyl, was studied in the soil from the previously treated greenhouse and also in soil from a previously untreated adjacent field. Four soil samples (840 g) from each field site were divided into 21 subsamples of 40 g. Each one of the group of paired samples received a dose of 4 μg/g of a nematicide including fenamiphos, cadusafos, oxamyl, and ethophrophos. The former three were applied as aqueous solutions of their respective formulations (1.6 ml; 100 mg/L) unlike ethophrophos, which was applied as a methanol solution (1.6 ml; 100 mg/L). In the latter case, samples were left for 2 h for the solvent to evaporate and water was then added to adjust the moisture content to 45% of their MWHC. Samples were mixed briefly and then placed into plastic bags and incubated at 25°C. Immediately before incubation and at regular intervals for the next 70 d, triplicate samples from each pesticide–soil combination were removed and analyzed for pesticide residues as described before.

Enhanced biodegradation of fenamiphos in soil from previously treated potato fields

Soil samples were collected during November 2002 from two commercial potato fields (fields A and B) in the area of Drama, Greece, which had been treated annually with fenamiphos for the last four years. Soil samples also were collected from an adjacent field that was not treated with fenamiphos for the last 10 years. After sampling, soils were handled as before and subsamples were used for the determination of soil properties (Table 1). Duplicate soil samples of 840 g from each field site were separated. The first sample received a dose of 50 μg/g (42 ml; 1,000 mg/L) of the antibiotics chloramphenicol and cycloheximide in order to impair microbial activity. These samples were incubated for 48 h at 25°C. Subsequently, both untreated and antibiotic-treated samples were divided into 21 subsamples (40 g), which received a dose of 4 μg/g fenamiphos (1.6 ml; 100 mg/L). Additional water was added in order to adjust water content to 45% of MWHC and samples were mixed by hand. Samples were then incubated at 25°C. Immediately before incubation and at subsequent intervals for the next 56 d, triplicate samples from each treatment were analyzed for residues as described before. The moisture content of the soils was maintained constant with regular addition of soil water when needed.

RESULTS

Effect of soil fumigants on the enhanced biodegradation of fenamiphos

A rapid degradation of the TTRs of fenamiphos was observed in the nonsterilized soil collected from the previously treated greenhouse site. In this soil, fenamiphos degraded rapidly but only trace amounts of FSO and FSO₂ were formed (Fig. 2a). Sterilization of this soil with the antibiotic chloramphenicol only temporarily impaired the degradation of TTRs. Degradation of the parent compound was coupled with the concurrent formation of FSO and small amounts of FSO₂ (Fig. 2a). Total toxic residues of fenamiphos disappeared rapidly in the nonsterilized samples that were fumigated with MeBr where no TTRs were detected 21 d after application. Fenamiphos in these soils degraded rapidly but only trace amounts of FSO and FSO₂ were measured (Fig. 2b). In comparison, chloramphenicol significantly reduced fenamiphos degradation in the MeBr-fumigated samples that was slowly transformed to FSO, resulting in the accumulation of approximately 90% of the applied fenamiphos as FSO at the end of the incubation (Fig. 2b). Fumigation with MS significantly impaired degradation of TTRs of fenamiphos (Fig. 2c). An even slower degradation of TTRs of fenamiphos was observed in the MS-treated samples that also were sterilized with the antibiotic. Therefore, 4.3 and 5 μg/g of TTRs of fenamiphos still were present in the nonsterilized and sterilized MS-treated samples, respectively, 70 d after application. As before, disappearance of fenamiphos was coupled with the gradual accumulation of FSO. A significantly slower degradation of TTRs was observed in the soil from the previously untreated field where approximately 0.7 μg/g of TTRs still were present in the soil 42 d after application. A rapid degradation of fenamiphos was observed in the soil from the previously untreated field (Fig. 2d). However, this rapid degradation was followed by the immediate formation of significant amounts of FSO and lower amounts of FSO₂, which both slowly dissipated thereafter.

Significantly (p < 0.05) lower enzymatic activity and SCMB were measured in samples from the previously treated greenhouse site after fumigation with MeBr or MS compared with the corresponding values in the nonfumigated samples.
Enhanced degradation of fenamiphos/soil fumigants effects

**Fig. 2.** Degradation of fenamiphos (■), fenamiphos sulfoxide (●), fenamiphos sulfone (♦), and total toxic residues (▲) in nonsterilized (closed symbols) and sterilized soil samples (open symbols, dotted lines) from soil (a) previously treated from greenhouse (Aggelohori, Northern Greece), (b) previously treated but fumigated with methyl bromide, (c) previously treated but fumigated with metham sodium, and (d) previously untreated with fenamiphos. Each value is the mean of three replicates with error bars representing the standard deviation of the mean.

from the previously treated greenhouse site and in the soil from the previously untreated field (Table 1).

**Bioassays with nematodes**

Throughout the incubation period, significantly (p <0.05) higher numbers of female nematodes were counted in tomato roots grown in the soil of the previously treated greenhouse that was subjected to fenamiphos treatment (5 μg/g) than in the soils of the same greenhouse subjected to fumigation with either MeBr or MS (Fig. 3). Similarly, significantly (p <0.05) lower numbers of nematodes were counted in plants grown in MS- than in MeBr-fumigated soils. Furthermore, 28 d after fenamiphos application there was no significant difference (p >0.05) in the number of nematodes counted in tomato roots grown in the soil of the previously treated greenhouse subjected to fenamiphos treatment (5 μg/g) and the soil from the same field that was not subjected to fenamiphos (0 μg/g) or any other treatment.

**Effect of selective antibiotics on the enhanced biodegradation of fenamiphos**

During laboratory incubation studies, the degradation of fenamiphos and its oxidation products proceeded rapidly in the soil from the greenhouse site previously exposed to fenamiphos (Fig. 4a). Similar degradation patterns for fenamiphos and its oxidation products were observed in samples of the same soil subjected to treatments with the antibiotics cycloheximide (Fig. 4b), kanamycin (Fig. 4c), and polymixin B (Fig. 4d). In contrast, application of the antibiotic chloramphenicol resulted in a temporary inhibition of fenamiphos metabolism for the first 10 d of the incubation (Fig. 4e). Thereafter, degradation of fenamiphos and its oxidation products was very rapid and no TTR were measured 28 d after application. Penicillin was the only antibiotic that irreversibly inhibited degradation of the TTR of fenamiphos resulting in only a negligible degradation of TTR for the duration of the experiment (Fig. 4f). A gradual degradation of fenamiphos in the penicillin-treated samples was coupled with the accumulation of almost 80% of the initially applied nematicide in the form of FSO.

**Cross-enhancement between fenamiphos and other nematicides**

Similarly to the previous experiment, a very rapid degradation of the TTR of fenamiphos was observed in the soil from the previously treated greenhouse and no residues of fenamiphos and its oxidation products were detected 21 d after application (Fig. 5a). Rapid degradation of fenamiphos was
Fig. 3. Numbers of Meloidogyne spp. females in tomato roots after inoculation with 1,000 second-stage juveniles in nonfumigated soils from a previously treated greenhouse (Aggelohori, Northern Greece) subjected to fenamiphos (5 μg/g) treatment (●), previously treated but fumigated with methyl bromide prior to fenamiphos application (●), previously treated but fumigated with metham sodium prior to fenamiphos application (●). Samples from the previously treated soil that had been neither treated with fenamiphos nor fumigated were included in the study to serve as controls (▫). The least significant difference of the mean of the different treatments is presented.

Fig. 4. Degradation of fenamiphos (●), fenamiphos sulfoxide (●), fenamiphos sulfone (●), and total toxic residues (●) in soil from a previously treated greenhouse not subjected to any treatment (a), and in the same soil treated with the antibiotic cycloheximide (b), kanamycin (c), polymixin B (d), chloramphenicol (e), and penicillin (f). Each value is the mean of three replicates with error bars representing the standard deviation of the mean.

Enhanced biodegradation of fenamiphos in soil from previously treated potato fields

Very rapid degradation of the TTR of fenamiphos was observed in laboratory incubation studies carried out with soils collected from two previously treated potato fields, field A and field B (Fig. 6). No detectable TTR of fenamiphos were detected 14 d after application in the soil collected from field A; however, in the same soil, which was sterilized before the application of fenamiphos, no detectable TTR of fenamiphos were found after the 30th day postapplication (Fig. 6a and b). Similarly, rapid degradation of the TTR of fenamiphos was observed in the soil collected from field B, in which 28 d postapplication no detectable residues were found; however, in the respective soil sterilized before application of fenamiphos, only 30% of the initially applied nematicide degraded during the same incubation period (Fig. 6c and d). A much slower degradation of fenamiphos and its oxidation products was evident in the soils collected from a previously untreated potato field of the same potato growing area regardless of sterilization (Fig. 6e and f). Evidently, in this latter soil, the slow degradation of TTRs was due to abiotic reactions. Fenamiphos degraded to nondetectable residue level within the 14-d postapplication period with the concomitant appearance of an equivalent concentration of FSO that slowly decreased as the concentration of FSO slowly increased. Consequently, both oxidized products attaining approximately the same concentration at the end of the incubation period.

No significant differences in the size and activity of the soil microflora were observed in soils from previously treated and the previously untreated potato fields (Table 2). The only exception was the lower fluorescein diacetate hydrolytic activity measured in the previously treated potato field A (Table 2).

DISCUSSION

Enhanced biodegradation of fenamiphos was confirmed in soils from greenhouses and potato fields from Greece with a history of previous exposure to fenamiphos using both analytical and bioassay methods. Fenamiphos usually is applied twice a year in greenhouse cultivations, unlike in potato cultivation where it is applied only once at the beginning of the growing season. Therefore, the possibility for the development of enhanced biodegradation is higher in greenhouse soils, although application of fenamiphos usually is accompanied by
soil fumigation with MeBr or MS once a year. The data presented here suggest that in the specific greenhouse soil studied, application of the fumigant MeBr resulted in a transient inhibition of the enhanced biodegradation of fenamiphos; this is unlike MS, which significantly impaired the biodegradation of this nematicide. The significantly slower degradation of fenamiphos and its oxidation products in the soil from the previously untreated field suggests that the rapid degradation of the TTR of fenamiphos observed in the soil from the previously treated field could be attributed to soil adaptation. Enhanced biodegradation of fenamiphos also was evident in soils collected from previously treated fields from a potato cultivation area in Drama, Greece. This is further supported by the lower rate of degradation of the TTR of fenamiphos observed in samples from a previously untreated field and in the sterilized samples from the previously treated fields. Degradation of fenamiphos and formation of FSO was very rapid in most of the soils tested. Oxidation of fenamiphos was so rapid that during the short time between application, sampling, and analysis, fenamiphos was partially transformed into FSO, as indicated by some data at time zero (Figs. 5a and 6). Similar observations have been reported previously for fenamiphos [13]. Enhanced biodegradation of fenamiphos was accompanied by the formation of small amounts of FSO that probably was rapidly hydrolyzed by soil microorganisms as soon as it was formed (Fig. 2a). In contrast, in soils where no microbial adaptation occurred, degradation of fenamiphos was accompanied by the formation of high amounts of FSO that slowly degraded to other products or oxidized to FSO$_2$ (Fig. 6e). Similar degradation patterns for fenamiphos in enhanced and nonenhanced soils have been reported before [5,6,12,17].

Application of the antibiotic chloramphenicol significantly inhibited degradation of fenamiphos in the soil from the previously treated greenhouse that were fumigated with MeBr or MS. However, chloramphenicol had less of an impact in the nonfumigated soil than the previously treated field. Chloramphenicol is a broad-spectrum antibiotic whose activity is bacteriostatic, meaning that it inhibits growth and proliferation of sensitive bacteria solely for the time it is still present in the soil [25]. Therefore, by the time chloramphenicol has dissipated from soil, it is possible that the population of the specialized fenamiphos-degrading soil bacteria rapidly recovered and metabolized the pesticide. In contrast, the lower microbial biomass measured in the soils fumigated with MeBr or MS was more vulnerable to the antibiotic whose effects persisted for longer. The lack of significant correlation between biological measurements and enhanced biodegradation of fenamiphos was expected because such measurements are not specific

Fig. 5. Degradation of (a) fenamiphos (●), fenamiphos sulfoxide (□), fenamiphos sulfone (●), and total toxic residues (▲); (b) oxamyl, (c) cadusafos, and (d) ethoprophos in soil from a greenhouse site previously exposed to fenamiphos (closed symbols) and an adjacent site previously untreated with fenamiphos (open symbols, dotted lines). Each value is the mean of three replicates with error bars representing the standard deviation of the mean.
to the specialized pesticide-degrading microflora. The utilization of those measurements in our study was aimed at obtaining a coarse knowledge of the microbial properties of the soil. In retrospect, estimation of the size of the specific fenamiphos-degrading microbial population in both soils may well have provided a better understanding of the microbial dynamics involved in the degradation of fenamiphos.

In general, there was a good agreement between analytical results and bioassays. The rapid degradation of TTRs observed in the soil from the previously treated greenhouse coupled well with the rapid reduction in nematode mortality. Application of MS in the soil from the previously treated field extended the persistence of a subsequent fenamiphos application and only few nematodes survived even after 70 d of application. Our results from incubation and bioassay studies suggest that the combination of MS fumigation with subsequent applications of fenamiphos maximizes the efficacy of fenamiphos by inhibiting microbial degradation and, therefore, increasing its persistence in soil. In contrast, methyl bromide had only a transient effect on the development of enhanced biodegradation of fenamiphos. This result was surprising because MeBr is a broad-spectrum soil fumigant. Measurements of microbial biomass and activity in MeBr-treated soils in our study showed a significant decline in both the size and activity of soil microflora, which is consistent with findings reported by others [26, 27]. However, Ibekwe et al. [27] reported that application of MeBr in an agricultural soil resulted in dramatic reductions in the numbers of fungi, actinomycetes, and Gram− bacteria but had only a transient effect on the Gram+ bacteria that rapidly recolonized the soils. Zelles et al. [28] reported similar results in chloroform-fumigated soils and subsequently suggested that Gram+ bacteria are more resistant to fumigants because of either their different cell wall structure or their ability to produce spores that help them survive under unfavorable conditions. Our findings might be attributed to the presence of specific Gram+ fenamiphos-degrading bacteria that survived MeBr fumigation and rapidly recolonized the MeBr-treated soil. Such a soil is considered an ideal environment for the establishment of such a population after fumigation; there will be relatively large amounts of readily decomposable substrate derived from the original fumigant-killed biomass and competition will be low [29]. Our suggestion is further supported by the results of the antibiotic study where only penicillin significantly inhibited the rapid biodegradation of fenamiphos. Penicillin is a selective antibiotic that is used against various Gram+ cocci and other Gram+ bacteria [25]. In contrast, bactericidies like polymyxin B and kanamycin, which specifically inhibit Gram− bacteria, and the fungicide cycloheximide had no effect on the degradation of TTRs suggesting that Gram− bacteria and fungi are not involved in the enhanced biodegradation of fenamiphos [25].

No cross-adaptation between fenamiphos and the organophosphorous nematicides cadusafos and ethoprophos was observed in the soil from the previously treated greenhouse from Aggelohori. In contrast, degradation of cadusafos and ethoprophos proceeded more rapidly in the soil from the previously untreated field than in the soil from the previously treated greenhouse (Fig. 5). This could be explained by the higher pH of the soil from the previously untreated field (Table 1). Several studies so far have documented the vulnerability of organophosphates to hydrolysis at high soil pH [30, 31]. Our findings are consistent with results reported by Smelt et al. [10] and Anderson and Lafuente, [4] who found no cross-adaptation between fenamiphos and ethoprophos. Similarly, Anderson et al. [32] showed that cadusafos was not subjected to cross-adaptation in a soil from Costa Rica that rapidly degraded fenamiphos. The high specificity of enhanced biodegradation among organophosphorous nematicides has been reported before by several studies [3, 33]. It is possible that similarity in chemical structure may influence the development of enhanced biodegradation. Cadusafos and ethoprophos are not chemically related to fenamiphos and this is probably the reason for the lack of cross-adaptation. However, when isofenphos, an organophosphate chemically related to fenamiphos, was tested, no cross-adaptation was observed [3]. In our study, the nematicides tested for cross-adaptation with fenamiphos were selected according to their use for the control of root-knot or potato cyst nematodes and not according to chemical similarity. Therefore, cadusafos and ethoprophos that are registered in Greece for the control of root-knot and potato cyst nematodes were included in the study. No cross-adaptation between fenamiphos and the oxime carbamate oxamyl was observed. Our findings are consistent with previous findings that also reported no cross-enhancement between organophosphorous and carbamate nematicides and, hence, fenamiphos and oxamyl [12]. Oxamyl is registered in Greece for the control of root-knot nematodes and potato cyst nematodes. Our results suggest that oxamyl, ethoprophos, and cadusafos could be alternated safely with fenamiphos in the specific soil and climatic conditions of Greece without entailing any risks for the development of enhanced biodegradation. However, further studies on the stability of enhanced biodegradation of fenamiphos.
under the specific climatic and agricultural conditions of Greece are required in order to establish a complete management program of enhanced biodegradation of nematicides.

CONCLUSION

Enhanced biodegradation of fenamiphos has developed in soils from commercial field sites in Greece where the nematicide was applied either once (potato fields) or twice (greenhouse fields) a year. Application of MS and, subsequently, fenamiphos according to our study will extend the nematicide with a year. Application of MS and, subsequently, fenamiphos according to our study will extend the nematicide

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