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Received for review February 28, 1988. Accepted August 22, 1988. Michigan Agriculture Experiment Station Journal Article No. 12567. This paper is based on work supported, in part, by U.S. Department of Agriculture—Agricultural Research Service Grants CWU 3607-20300-004-01 S and 58-5114-7-1002 and by a grant from the Shell Development Co., Modesto, CA 95352. Portions of this work were previously given in poster presentations (Shafer and Bukovac, 1985; Shafer et al., 1987).

Fate of the Fungicide Furalaxyl in the Nutrient Solution of Tomato Crops by the Nutrient Film Technique

Jean Rouchaud,* Marc Metsue, Frans Benoit, Norbert Ceustermans, and Alfons Vanachter

The fungicide furalaxyl [methyl *N*-(2,6-dimethylphenyl)-*N*-(2-furanylcarbonyl)-DL-alaninate] was incorporated into the nutrient solution of tomato crop grown according to the nutrient film technique (NFT). Incorporation into the refreshed nutrient solution was made several times at different plant growth stages. Furalaxyl was decomposed in the nutrient solution into *N*-(2,6-dimethylphenyl)-*N*-(2-furanylcarbonyl)-DL-alanine (**2**) and *N*-(2,6-dimethylphenyl)-DL-alanine (**4**) by enzymatic processes. Half-lives in the nutrient solution of furalaxyl and of the total furalaxyl plus compounds **2** and **4** at the beginning of the crop were 2.5 and 5.2 days, respectively. With starting of the continuous recirculation of the nutrient solution, they increased to 6 and 12 days; with plant development, they decreased progressively to 3.5 and 6.5 days. No furalaxyl nor compounds **2** or **4** were detected in tomato fruit, the limit of sensitivity being 0.02 ppm (of fresh weight).

Present-day horticultural production of tomatoes in Northern Europe is now based almost entirely on artificial substrates rather than soil, the common practice until 15 years ago, basically due to the cost of sterilization of the soil due to the large increase in the cost of oil and the lack of good soil and the cost of resoiling houses when the soil is exhausted (Wilson, 1986). Artificial substrates have the following advantages: They are disease and weed free and are light in weight. Repetitive mixes have the same composition. They show quicker growth and higher yields. Tomato yields have increased in the last 30 years mainly due to monocropping systems and growing out of the soil (Wilson, 1983). However, plant protection problems begin to arise now in soilless culture of tomato, especially from the fungi *Pythium* and *Phytophthora nicotianae* (Vanachter et al., 1986). One or two fungicide treatments are made during one crop, in commercial practice. One treatment is always made at the beginning of the crop, when the damage made to the plant roots during plantation makes the plants very susceptible to the fungi. The second treatment is made when symptoms of sickness arise (Benoit and Ceustermans, 1987). Furalaxyl is a fungicide widely used in the soilless culture of tomato. It gives very good plant protection and does not give residues in the fruits at harvest.

The residual behavior of furalaxyl has been studied in greenhouse tomatoes grown on soil sprayed with furalaxyl (Cabras et al., 1985). To our knowledge, nothing has been published about the uptake and translocation of furalaxyl by plants and about its metabolism in biological systems. However, the uptake and translocation of the fungicide metalaxyl has been studied in sunflower plants (Marucchini et al., 1983). Metalaxyl has the chemical structure of furalaxyl in which the 2-furanylcarbonyl group has been replaced by 1-methoxyacetyl. Furalaxyl thus should also be systematic. The metabolism of metalaxyl has been studied in lettuce and sunflower (Businelli et al., 1984).

In the present work, we studied the metabolism of furalaxyl in the nutrient solution of tomato NFT crop grown on a semicommercial scale.

Laboratoire de Phytopathologie, Université Catholique de Louvain, Louvain-la-Neuve, Belgium (J.R., M.M.), Proefstation voor de Groenteteelt, St Katelijne-Waver, Belgium (F.B., N.C.), and Laboratorium voor Fytopathologie en Plantenbescherming, Katholieke Universiteit van Leuven, Belgium (A.V.).

EXPERIMENTAL SECTION

Tomato Crop with the Nutrient Film Technique (NFT): Furalaxyl Treatments. Tomato plants were grown in semicommercial installations. Seeds of tomato cv. Concreto were sown in peat–sand on 11-21-86. Seedlings were transferred to cubes of rockwool (10 × 10 × 17 cm) on 12-10-86 and placed in the NFT gullies on 1-28-87 at the Proefstation voor de Groenteteelt, St Katelijne-Waver, Belgium, when the buds of the first truss were just visible. Plant interdistance in the same row was 60 cm; interdistance between each row was 1 m; there were six rows in a glasshouse that was 6.5 m wide. During the propagation stage (until 2-16-1987), the Cooper nutrient solution (Cooper, 1979) was circulated for five 15-min periods every 24 h (four periods during daylight hours and one period during darkness). Subsequently, the nutrient solution was continuously recirculated. The nutrient solution was maintained at pH 5.8. The conductivity of the nutrient solution was continuously monitored by two electrodes and was regulated by separate addition of water and of a concentrate solution of the Cooper mixture; this last was made up instantaneously by pumping from two separate tanks, the one containing the calcium nitrate solution and the other containing the solution of the other salts. The pH of the nutrient solution was also continuously monitored by two electrodes and was adjusted by pumping from two tanks of acid and basic solutions. The

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Table I. Relative Mobilities (R_f) of Furalaxyl and Its Derivatives on Silica Gel Thin-Layer Plates

solvent system	R_f 1	2	3	4
diethyl ether	0.64	0.18	0.84	0.71
tetrahydrofuran	0.83	0.85	0.91	0.86
chloroform	0.52	0	0.70	0
benzene	0	0	0.57	0
ethyl acetate	0.82	0.30	0.94	0.76
acetone	0.92	0.68	0.87	0.82

whole system, including the temperature control, was automatized and regulated by computer. The conductivity of the nutrient solution was adjusted to 4 mS until 2-26-87, to 3.5 until 3-18-87, to 4 until 4-7-87, and then to 4.5 until the end of the crop. The temperature of the nutrient solution was 23 °C until 2-15-87 (development of the roots). Then, the temperature was progressively lowered to 19 °C. Tomato plant development was limited to 11 trusses. Tomato harvest was from 4-21-87 to 7-7-87.

Fungarid 25 WP (formulation containing 25g% of furalaxyl) was incorporated into the nutrient solution at the rate of 3 ppm of furalaxyl several times during the crop. It was always done just after replacement with fresh nutrient solution.

Thin-Layer (TLC) and Gas-Liquid (GC) Chromatographies and Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass (MS) Spectrometries. TLC was made using DC-Plastikfolien Kieselgel 60F254 silica gel plates, 20 × 20 cm, 0.2 mm thick, Merck. The sample was applied as a band to the origin; standards were applied to a separate part of the TLC plate.

Furalaxyl was analyzed as such by GC with the Varian 2700 gas chromatograph equipped with a flame ionization detector. Conditions of analysis: injector and detector temperature, 250 °C; glass column 1.80 m × 2 mm (i.d.) filled with 5% OV17 on 80–100-mesh Gas Chrom Q; nitrogen carrier gas at 40 mL/min. With column oven at 210 °C, the retention time of furalaxyl was 4.2 min; at 135 °C, it was 3.6 min for compound **3**.

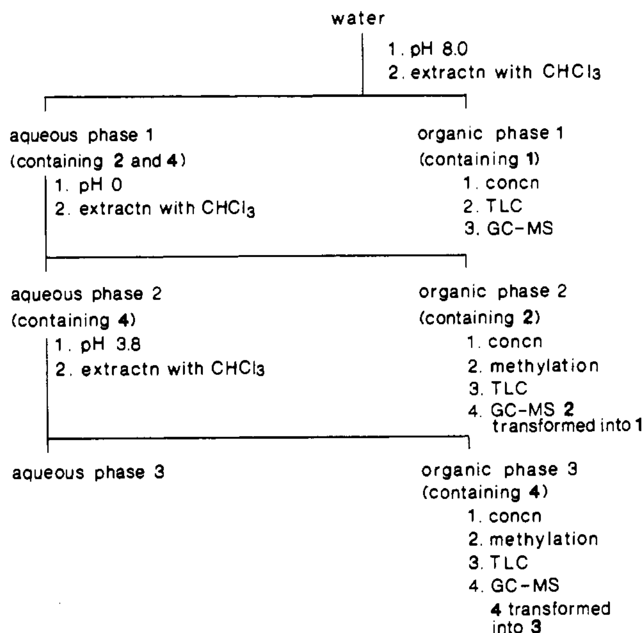
IR spectra were recorded with the Perkin-Elmer 297 apparatus (KBr disks; cm^{-1}). ^1H NMR ($CDCl_3$, δ, TMS) spectra were recorded on a Varian XL-200 spectrometer with tetramethylsilane as internal standard. MS were recorded with the Varian MAT (70-eV) mass spectrometer in the electron impact mode (m/e; relative abundance, %). Several times, furalaxyl and its metabolites isolated from the water of the NFT culture were analyzed by MS.

Standards of Furalaxyl and Its Metabolites. Synthesis reactions were monitored by TLC (Table I) and by GC.

1. Furalaxyl (*1*). Fungarid 25 WP (50 g; 25% furalaxyl) was stirred (30 min) with methylene chloride (200 mL). The mixture was filtered, and the extraction was repeated. The gathered filtrates were evaporated to dryness in a vacuum evaporator and the oily residue crystallized (11.2 g) in hexane–methylene chloride after one night in the cold, giving furalaxyl >98% pure, as shown by GC and TLC. IR: 2800, 1770, 1650, 1480, 1410, 1280, 1210, 1130, 1030, 1000, 790, 775. MS: 301 ($M^{\bullet+}$, 100, 269 (M – CH_3OH, 28), 268 (M – CH_3OH – H, 32), 242 (M – CO_2CH_3, 95), 206 (M – furoyl, 29), 180 (M – $C_6H_3(CH_3)_2NH_2$, 85).

2. N-(2,6-Dimethylphenyl)-N-(2-furanylcarbonyl)-DL-alanine (*2*). Furalaxyl (7 g) and Fungarid (0.7 g) were heated to reflux (3 h, stirring) in water (100 mL) containing potassium hydroxide (4 g). The cooled mixture was brought to a pH <1 by addition of hydrochloric acid. Compound **2** was filtered and recrystallized in acetone–chloroform (1:1, v/v): yield 6.2 g, >98% pure; mp (uncorrected) 196–197 °C. IR: 3400, 3000, 1725, 1650, 1570, 1480, 1420, 1360, 1320, 1285, 1250, 1230, 1200, 1160, 1130, 1110, 1090, 1030, 940, 890, 870, 850. MS: 287 ($M^{\bullet+}$, 3), 243 (M – CO_2, 100), 228 (243 – CH_3, 8), 214 (243 – CH_2CH_3, 9), 166 (OCHCHCHCCOC(CH_2)CO_2H, 32), 148 ($C_6H_3(CH_3)_2NCH_2CH_3$, 34), 146 ($C_6H_3(CH_3)_2NCHCH_2$, 37).

Scheme I. Procedure for Analysis of the Water from the NFT Culture



3. Methyl N-(2,6-Dimethylphenyl)-DL-alaninate (*3*). 2,6-Dimethylaniline (52 g), methyl DL-2-bromopropionate (30 g), and toluene (100 mL) were heated to reflux (8 h, stirring). The cooled mixture was filtered and washed with dilute acid (pH 4.0). The solvent was removed, and hexane (90 mL) was added to the oily residue. The precipitate was filtered and discarded, and the filtrate was concentrated in a vacuum evaporator, giving the oily compound **3** (29 g, >95% pure), which was used as such in the next reaction. IR: 3000, 1745, 1560, 1480, 1375, 1335, 1290, 1260, 1200, 1145, 1100, 1060, 980, 770. ^1H NMR: 1.37 (d, 3 H, $CHCH_3$), 2.28 (s, 6 H, $Ar(CH_3)_2$), 3.57 (s, 3 H, OCH_3), 3.93 (m, 1 H, $CHCH_3$), 6.83 (m, 3 H, aromatic), 7.15 (s, 1 H, NH). MS: 207 ($M^{\bullet+}$, 95, 192 (M – CH_3, 3), 148 (M – CO_2CH_3, 100), 132 ($C_6H_3(CH_3)_2CHCH_2$, 31), 121 ($C_6H_3(CH_3)_2NH_2$ 87).

4. N-(2,6-Dimethylphenyl)-DL-alanine (*4*). Compound **3** (10 g) was heated to reflux (3 h, stirring) in water (100 mL) containing potassium hydroxide (4 g). The cooled mixture was washed with toluene to eliminate remaining 2,6-dimethylaniline. The aqueous phase was brought to pH 3.8, and compound **4** was filtered (8.2 g) and recrystallized in acetone–chloroform (1:1, v/v) >98% pure; mp (uncorrected) 93–94 °C. IR: 3395, 3000, 1730, 1635, 1600, 1490, 1470, 1415, 1360, 1300, 1265, 1220, 1155, 1110, 1065, 1040, 1000, 925, 905, 845, 835. ^1H NMR: 1.32 (d, 3 H, $CHCH_3$), 2.27 (s, 6 H, $Ar(CH_3)_2$, 3.95 (m, 1 H, $CHCH_3$), 5.17 (s, 1 H, CO_2H), 6.87 (m, 3 H, aromatic), 7.90 (s, 1 H, NH). MS: 193 ($M^{\bullet+}$ 31), 148 (M – CO_2H, 100), 132 ($C_6H_3(CH_3)_2NCH$, 14), 105 ($C_6H_3(CH_3)_2$, 16).

Analysis of the Water of the NFT Culture. Water (100 mL) from the tank of the NFT culture was brought to pH 8 by addition of potassium hydroxide, NaCl (10 g) was added, and the resulting solution was extracted two times with chloroform (2 × 200 mL), giving organic phase 1 containing furalaxyl and aqueous phase 1 (Scheme I). Organic phase 1 was dried (Na_2SO_4), concentrated to 40

Table II. Biodegradation of Furalaxyl in the Nutrient Solution of the Tomato NFT Crop

sampling date[a]	delay,[c] days	concn of 1 (ppm) and metabolites (ppm of equiv of 1) in the nutrient soln[d]				half-life, days	
		1	2	4	total	1	total 1 and 2 + 4
		First Sampling Period[e]					
30-1[b]	0	2.6 ± 0.2	nd	nd	2.6		
2-2	4	1.2 ± 0.05	0.3 ± 0.02	0.3 ± 0.02	1.8		
5-2	7	0.8 ± 0.03	0.1 ± 0.01	0.2 ± 0.01	1.1		
12-2	14	0.5 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.6		
17-2	19	0.6 ± 0.03	0.05 ± 0.01	nd	0.6	2.5 ± 0.1	5.2 ± 0.3
		Second Sampling Period[f]					
20-2[b]	0	2.7 ± 0.2	nd	nd	2.7		
23-2	3	1.8 ± 0.1	0.5 ± 0.2	0.3 ± 0.02	2.6		
27-2	7	1.5 ± 0.1	0.4 ± 0.02	0.2 ± 0.02	2.1		
2-3	10	1.3 ± 0.05	0.3 ± 0.02	0.1 ± 0.02	1.7	6.0 ± 0.3	12.0 ± 0.6
		Third Sampling Period[g]					
6-3[b]	0	2.4 ± 0.2	nd	nd	2.4		
9-3	3	1.8 ± 0.1	0.3 ± 0.02	0.1 ± 0.02	2.2		
		Fourth Sampling Period[h]					
11-3[b]	0	2.8 ± 0.2	nd	nd	2.8		
13-3	2	1.9 ± 0.1	0.5 ± 0.02	0.1 ± 0.02	2.5		
17-3	6	1.3 ± 0.1	0.5 ± 0.02	0.3 ± 0.01	2.1		
23-3	12	1.1 ± 0.05	0.2 ± 0.02	0.1 ± 0.02	1.4	4.5 ± 0.2	12.0 ± 0.5
		Fifth Sampling Period[i]					
5-6[b]	0	2.6 ± 0.2	nd	nd	2.6		
6-6	1	2.4 ± 0.1	0.05 ± 0.01	nd	2.4		
7-6	2	1.8 ± 0.1	0.2 ± 0.01	0.1 ± 0.02	2.1		
8-6	3	1.6 ± 0.1	0.4 ± 0.02	0.3 ± 0.02	2.3		
12-6	7	1.0 ± 0.05	0.1 ± 0.02	0.2 ± 0.01	1.3	3.5 ± 0.1	6.5 ± 0.3

[a] Day-month, year 1987. [b] Incorporation of furalaxyl into the nutrient solution that has just been changed and thus has not been treated previously with furalaxyl. [c] Delay since furalaxyl incorporation into the nutrient solution. [d] Means of four replicates (±SD); nd = none detected. [e] Discontinuous recirculation of the nutrient solution at 23 °C, development of the first truss. [f] Nutrient solution at 19 °C and continuous recirculation, development of the second truss. [g] Nutrient solution at 19 °C, continuous recirculation, development of the third truss. [h] Nutrient solution at 19 °C, continuous recirculation, development of the fourth truss. [i] Nutrient solution at 19 °C, continuous recirculation, plant completely developed.

mL in a vacuum evaporator (30 °C) and to 0.3 mL with a slow current of nitrogen, and applied as a band onto a TLC plate, along with the standard of furalaxyl; elution with diethyl ether gave furalaxyl at R_f 0.64. The silica gel area opposite to the authentic compound was scraped off, extracted in a small column with ethyl acetate, concentrated with a slow current of nitrogen, and analyzed by GC and MS.

Aqueous phase 1 was made 1.2 N in hydrochloric acid (pH ≈0) by addition of hydrochloric acid. The mixture was extracted two times with chloroform (2 × 200 mL), giving aqueous phase 2 and organic phase 2 containing compound 2. Aqueous phase 2 contained compound 4. Organic phase 2 was dried and concentrated successively to 40 mL in a vacuum evaporator (30 °C) and to 5 mL with a current of nitrogen. Diazomethane in ether was added; the mixture was concentrated to 0.5 mL with a current of nitrogen, applied as a band onto a TLC plate along with the standard of furalaxyl, and analyzed further as furalaxyl.

Aqueous phase 2 was brought with sodium carbonate to pH 3.8 and extracted two times with chloroform (2 × 200 mL), giving organic phase 3 containing compound 4. Organic phase 3 was dried and concentrated to 40 mL in a vacuum evaporator (30 °C) and to 5 mL with a current of nitrogen. Diazomethane in ether was added: the mixture was concentrated to 0.5 mL with a current of nitrogen and applied as a band on a TLC plate along with the standard of compound 3. Elution with chloroform–hexane–acetic acid (15:85:1, v/v) gave the band of compound 3 at R_f 0.35. The band was scraped off, extracted in a small column with ethyl acetate, concentrated with a slow current of nitrogen, and analyzed by GC and MS.

Tomato fruits were analyzed in the same way as water. However, the extraction (100 g of tomato fruits) was made with acetone (200 mL) in a Sorvall Omnimixer, the mixture was filtered, water (80 mL) was added to the filtrate, the acetone was evaporated in a vacuum evaporator, and the remaining water solution was analyzed as described above for the nutrient solution. At least two consecutive TLC were necessary in order to clean the final extracts; they were made with the solvents indicated in Table I. Recoveries in the tomato fruit at the 0.1 ppm level of furalaxyl and of compounds 2 and 4 were, respectively, 81–95%, 78–89%, and 71–94%.

RESULTS AND DISCUSSION

During the analysis of the water of the NFT culture (Scheme I), the differences of the acid and basic properties of each of the furalaxyl and of its metabolites were used for their separation. Water of the NFT culture was first brought to pH 8; extraction with chloroform gave furalaxyl in organic phase 1, whereas compounds 2 and 4 remained in aqueous phase 1 as the carboxylate salts.

Aqueous phase 1 was brought to pH 0; at that pH compound 4 was N-protonated at the opposite of compound 2. Extraction by chloroform thus gave compound 2 in organic phase 2, whereas compound 4 remained in aqueous phase 2.

When aqueous phase 2 was brought to pH 3.8, compound 4 was no more N-protonated and the carboxylic group was not ionized; extraction with chloroform thus gave compound 4 in organic phase 3.

The stability of furalaxyl toward acid hydrolysis and of compound 2 toward basic and acid hydrolyses was first studied at the 5-g level. Furalaxyl heated to reflux (3 h, stirring), with some Fungarid as emulsifier, in 10 g % sulfuric acid in water, gave less than 10% of compound 2. Compound 2 heated to reflux (8 h, stirring) in water

containing increasing concentrations of potassium hydroxide gave no compound **4**, same as when the concentration of potassium hydroxide was as high as 30%. Heating to reflux (8 h, stirring) compound **2** in 6 N HCl in water gave no compound **4**.

At the residue level, and using the analytical procedure for the water of the NFT culture, recoveries were made using separately furalaxyl or one of the compounds **2** and **4**. During the analytical procedure, furalaxyl was not transformed at all into compounds **2** or **4** and compound **2** was not transformed at all into compound **4**. Recoveries (four replications) of furalaxyl and of compounds **2** and **4** in the nutrient solution at the 1 ppm level were, respectively, 91 ± 5%, 87 ± 4%, and 84 ± 5%. At the 0.2 ppm level, these recoveries were, respectively, 93 ± 6%, 85 ± 5%, and 79 ± 6%.

Furalaxyl was rapidly decomposed in the nutrient solution. During the period of discontinuous recirculation, the half-live of furalaxyl was 2.5 days (Table II). When continuous circulation started, the half-live increased to 6 days, in spite of the fact that the temperature of the solution was decreased from 23 to 19 °C, which should decrease the rate of pure hydrolysis. Continuous recirculation probably increased the rate of the fungicide absorption by the plant roots and probably decreased the deposit of the product in all the parts of the installation. With plant growth, half-live of furalaxyl in the nutrient solution progressively decreased from 6 to 3.5 days, probably by increasing root absorption of the fungicide.

Furalaxyl was incorporated into the nutrient solution at the concentration of 1 g/L (4 g of Fungarid/L) and kept with stirring in the laboratory (20 °C) without plant growing. Less than 5% of the furalaxyl was decomposed after 15 days. Synthetic assays also indicated that the acid hydrolysis of furalaxyl into compound **2** was very slow; alkaline hydrolysis with heating to reflux was required to obtain compound **2**. The fast decomposition observed here of furalaxyl into compound **2** in the nutrient solution of the tomato NFT crop could correspond to the enhancement of the rate of hydrolysis by some of the components of the nutrient solution arising during plant growth; one should first think of the interaction of plant roots and of their excudation products, with the nutrient solution.

Compound **4** could not be obtained synthetically by the basic or acidic hydrolyses of compound **2**, even with high concentrations of base or of acid, and heating to reflux. This should be related, in compound **2**, to the low basicity of the nitrogen (at pH 0 in water, it is not yet protonated); thus, the acid catalysis for hydrolysis of compound **2** cannot function. For the alkaline hydrolysis of compound **2**, the steric hindrance is too large for the attack of the carbonyl of the furoyl group. Formation of compound **4** in the nutrient solution of the tomato crop thus should also arise by catalysis through compounds of biological origin.

Tomato fruits were harvested for analysis on 6-22-87, i.e., 17 days after incorporation of furalaxyl into the fresh nutrient solution. No residues of furalaxyl or of compounds **2** and **4** were detected in the tomato; the concentrations of these compounds in the fruit thus were lower than the detection limit for these compounds, i.e., 0.02 ppm relative to the fresh weight.

ACKNOWLEDGMENT

This work was supported by the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA), Belgium (Grant No. 4868A). We thank Mr. Moulart (Université Libre de Bruxelles, Bruxelles, Belgium), who recorded the mass spectra, and Mr. Merenyi (Université Catholique de Louvain, Louvain-la-Neuve, Belgium), who recorded the NMR spectra.

Registry No. 1, 57646-30-7; **2**, 118597-19-6; **3**, 52888-49-0; **4**, 67617-64-5.

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Received for review March 14, 1988. Accepted September 6, 1988.