Metabolism of the Herbicide Diflufenican in the Soil of Field Wheat Crops

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The fate of diflufenican (1) was studied in the soil of field wheat crops grown in different regions and during two crop seasons. Diflufenican soil metabolism was also studied in sugar beet replacement crops. When diflufenican was soil applied in the early spring, the time for 50% loss of initial diflufenican in soil was shorter than when it was applied in the autumn of the preceding year. The rate of diflufenican soil metabolism thus was greater during the spring/summer seasons than during the winter. The rate of diflufenican soil metabolism was also related to the climate. Diflufenican was transformed in soil into 2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxylic acid (2), *N*-(2,4-difluorophenyl)-2-hydroxy-3-pyridinecarboxamide (3), and 2-hydroxy-3-carboxypyridine (4). No 2,4-difluoroaniline (5) or 3-(trifluoromethyl)phenol (6) was detected in the soil; no diflufenican (1), its metabolites 2–4, compounds 5 and 6 were detected in the wheat grain or in the root or leaves of the sugar beet.

INTRODUCTION

Diflufenican [1; *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide] is a contact and persistent herbicide widely and efficiently used for protection of winter cereals, especially winter wheat and winter barley. Its solubility in water is 0.05 mg/L, and its vapor pressure is 0.07 mPa. Because of its soil persistence, wide-spectrum herbicide activity, and selectivity, diflufenican gives good protection against weeds during the long period starting from the autumn—during which sowing is made—until the end of the spring of the next year (Kyndt and Turner, 1985). Diflufenican controls the many autumn- and winter-germinating broad-leafed species (*Galium aparine, Veronica hederifolia, Veronica persica, and Viola arvensis*) which have proved more difficult than the traditional spring-germinating species and which are resistant to the substituted-urea herbicides isoproturon and chlortoluron (Cramp et al., 1985, 1987). These last, however, are widely used for controlling grass weeds, especially *Alopecurus myosuroides*. For that reason, diflufenican is mainly used in mixture with isoproturon. Diflufenican is a bleaching herbicide; its mode of action is the inhibition of the desaturation reaction of carotenoid biosynthesis, causing the accumulation of phytoene in place of the normal colored carotenoids (Britton et al., 1987). The reported diflufenican soil half-lives in wheat fields were between 2 and 6 months (Cramp et al., 1987; Deleu et al., 1987). To our knowledge, nothing so far has been published about the soil metabolism of diflufenican. In the present work, we studied the persistence and the metabolism of diflufenican in the soil of nine crops, among which were mainly wheat crops.

In the areas where diflufenican is used—by application just after cereal sowing in November—the triennial rotation is usually practiced, i.e., sugar beet (sowing in April; sensitive to diflufenican) or maize (sowing in April; not sensitive to diflufenican) is grown during the first year, and cereals (wheat and barley, successively) are grown during the second and third years. One of the aims of the present work thus was to know the kinetics of diflufenican soil biodegradation, to determine if persistent diflufenican soil residues could hinder the normal following crop or the replacement crop sown in April after failure of the cereal crop sown in November of the preceding year.

EXPERIMENTAL PROCEDURES

Field Crops and Diflufenican Treatments. *Wheat and Barley Crops: Assay 1.* A winter wheat crop was grown in 1987–1988 at Melle (clay 11%, silt 66%, sand 23%, organic matter 2.5%, pH 6.5, silt loam type). Wheat (cv. Fidel) was sown on 20-10-1987 (day-month-year). Soil was treated with 188 g of diflufenican (the active ingredient)/ha by spraying the emulsion of Javelin (500 g of isoproturon + 62.5 g of diflufenican/L) in water (750 L/ha; the same amount of water was used for all the assays described in this work; the locations of all these assays were in Belgium) on 21-10-1987. Harvest was made on 1-8-1988.

Assay 2. A winter wheat crop was grown in 1988–1989 at Melle (clay 10%, silt 65%, sand 25%, organic matter 2.3%, pH 6.8, silt loam type). Wheat (cv. Capitaine) was sown on 26-10-1988. Soil was treated with 200 g of diflufenican (ai)/ha by spraying the emulsion of Cougar (500 g of isoproturon + 100 g of diflufenican/L) in water on 28-10-1988. Harvest was on 1-8-1989.

Assay 3. A winter wheat crop was grown in 1988–1989 at Gijzenzele (clay 3%, silt 77%, sand 20%, organic matter 1.8%, pH 6.2, silt loam type). Wheat (cv. Castell) was sown on 3-11-1988. Soil was treated with 200 g of diflufenican (ai)/ha by spraying the emulsion of Cougar (500 g of isoproturon + 100 g of diflufenican/L) in water on 8-11-1988. Harvest was made on 24-7-1989.

Assay 4. A winter barley crop was grown in 1988–1989 at Houtem-Veurne (clay 18%, silt 56%, sand 26%, organic matter 2.2%, pH 7.2, silt loam type). Barley (cv. Corona) was sown on 4-10-1988. Soil was treated with 175 g of diflufenican (ai)/ha by spraying the emulsion of Cougar (500 g of isoproturon + 100 g of diflufenican/L) in water on 5-10-1988. Harvest was made on 10-7-1989.

Assay 5. A winter wheat crop was grown in 1988–1989 at Gijzenzele (same soil composition as in assay 3); wheat sowing was made in the fall of 1988, but the diflufenican soil treatment was

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made in the spring of 1989. Wheat (cv. Castell) was sown on 3-11-1988. Soil was treated with 140 g of diflufenican (ai)/ha by spraying the emulsion of Javelin (500 g of isoproturon + 62.5 g of diflufenican/L) in water on 14-3-1989. Harvest was made on 24-7-1989.

Sugar Beet Replacement Crops. These assays were made to observe the effects of the persistence of diflufenican in soil onto the sugar beet sensitive replacement crops sown after the failure of the first crop (which had been soil treated with diflufenican some days after sowing). In the assay model system, the sugar beet replacement crops were sown in April, either 5 months after the diflufenican soil treatment of the first crop made in the fall of the preceding year, as for winter cereal crops, or 1.2 months after the soil diflufenican treatment of the first crop made in the early spring.

Assays of 1987–1988. Sugar beet replacement crops (cv. Monohil) were grown at Melle in 1988 (clay 10%, silt 65%, sand 25%, organic matter 2.2%, pH 6.0, silt loam type soil). The crops were sown on 25-4-1988, on a soil just tilled in its 0–10-cm surface layer and which had been previously treated with diflufenican according to one of the following two procedures (assay 6 or 7).

Assay 6. Soil treatment was done with 250 g of diflufenican (ai)/ha, using the experimental formulation Exp 4005 (500 g of diflufenican/L) in emulsion in water, 4.9 months (3-12-1987) before sowing and in the fall of the preceding year (as is done with cereal crops), on a soil previously normally prepared (ploughing in the 0–40-cm soil layer) as for sowing. The soil was left fallow during the winter, i.e., during the 4.9 months between diflufenican soil treatment and sugar beet sowing.

Assay 7. Soil treatment was done with 200 g of diflufenican (ai)/ha (using the same Exp 4005 formulation) 1.1 months (22-3-1988) before sowing, on a soil previously prepared as for sowing. The soil was left fallow during the 1.1 months between diflufenican soil treatment and sugar beet sowing.

Assays of 1988–1989. The same sugar beet replacement crops were made again in 1989 at Melle. The crops were sown on 25-4-1989 on the soil which previously had been treated with diflufenican according to one of the following procedures.

Assay 8. Soil treatment was done with 250 g of diflufenican (ai)/ha (using the same Exp 4005 formulation) on 21-11-1988 (5.2 months before sowing). The soil was left fallow during the 5.2 months between diflufenican soil treatment and sugar beet sowing.

Assay 9. Soil treatment was done with 200 g of diflufenican (ai)/ha (using the same Exp 4005 formulation) on 20-3-1989 (1.2 months before sowing). The soil was left fallow during the 1.2 months between diflufenican soil treatment and sugar beet sowing.

In each of the replacement crop assays (assays 6–9), there were plots whose soil had not been treated with diflufenican (control plots). Moreover, besides the sugar beet crops, there were other plots in which other crops were grown separately: wheat (cv. Minaret), barley (cv. Apex), oat (cv. Alfred), maize (cv. Frida), potato (cv. Bintje), beans (cv. Maxime), pea (cv. Finale), turnip (cv. Leielander), Savoy cabbage (cv. Winterkoning), scorzonera (cv. Donia superlong), lettuce (cv. Hilde), carrot (cv. Ivor), spinach (cv. Nobel), chicory (cv. Brussels Laat), and onion (cv. Rijnsburger). These other replacement crops were sown on the same day as sugar beet. Residue analyses were made in the soil of these crops, but without replicates and at only two dates during each crop; their soil residue values were similar to the ones obtained in the sugar beet replacement crops.

Experimental Design, Soil and Plant Tissue Sampling. For all the wheat and barley crop assays, the field of each assay was divided into four replicate plots. The size of each replicate plot was about 20 m × 20 m. At intervals during the trials (Table I), samples were taken separately (and analyzed once separately) from the 0–10-cm soil layer of each of the four replicate plots. In addition, at two times during each assay (26-2-88 and 11-5-88 in assay 1; 23-2-89 and 8-6-89 in assay 2; 22-2-89 and 9-6-89 in assay 3; 24-2-89 and 25-5-89 in assay 4; 9-6-89 and 6-7-89 in assay 5), single samples were taken separately (and analyzed once separately) from both the 10–20- and 20–30-cm soil layers of each of two of the four replicate plots. For each soil sample, 15 cores (2.5-cm diameter) were taken from each replicate plot at random points; the cores from each replicate plot were bulked together and then stored at −25 °C until analyzed. Soil was not dried before analysis. At harvest, an aliquot of 1 kg of cereal grain was taken at random from each crop, sampling being made in the four replicate plots, but bulking together the samples from each replicate plots. Grain samples were stored at −25 °C until analyzed. Four replicate analyses were made on the cereal grain from each assay field.

Soil and plant samplings in the sugar beet replacement crops assays were made in the same way as with the wheat crop trials. Each of the sugar beet replacement crop fields was divided into four discrete replicate plots. The size of each replicate plot was 10 m × 5 m. At intervals, samples were taken separately (and analyzed separately) from the 0–10-cm soil layer of each of the four replicate plots of each sugar beet field trial. In addition, at two times during each assay (26-2-88 and 11-5-88 in assay 6; 29-4-88 and 11-5-88 in assay 7; 23-2-89 and 8-6-89 in assay 8; 21-4-89 and 8-6-89 in assay 9) single samples were taken separately (and analyzed once separately) from both the 10–20- and 20–30-cm soil layers of each of two of the four replicate plots. At harvest, an aliquot of sugar beet root (about 20 kg) and foliage (about 15 kg) was taken at random from each crop, sampling being made in the four replicate plots, but bulking together the samples from each replicate. The roots and foliage were separately cut into small pieces which were mixed, and an aliquot of each of them was separately stored at −25 °C until analyzed. Four replicate analyses were made on each of the root and foliage from each sugar beet assay field.

Thin-Layer (TLC) and Gas–Liquid (GLC) Chromatographies. Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass (MS) Spectrometries. TLC was made by using silica gel 60F254 20 × 20 cm, 0.2 mm thick, plates from Merck. The sample solution was applied as a band. Standards were applied on another part of the TLC plate, next to the band of the sample solution.

Diflufenican (1) and compound **3** were analyzed as such by GLC using the Varian 2700 apparatus. Compound **2** was analyzed by GLC after methylation with diazomethane. Compound **4** was analyzed by GLC after successive trifluoroacetylation of the aromatic OH and diazomethane methylation of the carboxylic acid OH. 2,4-Difluoroaniline (**5**) and 3-(trifluoromethyl)phenol (**6**) were analyzed by GLC after trifluoroacetylation. Detection was by ^{63}Ni electron capture. Conditions: injection at 280 °C, detection at 250 °C; glass column 1.80 m × 2 mm i.d.; 5% SE30 or 3% Carbowax 20M on Gas Chrom Q 80–100 mesh; nitrogen carrier gas at 40 mL/min. Compound, column temperature, retention time: (1) with 5% SE30, diflufenican, 220 °C, 5.3 min; compound **2** (CO_2CH_3), 175 °C, 3.2 min; compound **3**, 220 °C, 4.8 min; compound **4** [2-(CF_3COO)-3-(CH_3OCO)-pyridine], 185 °C, 3.1 min; compound **5** (N-trifluoroacetylated), 90 °C, 2.6 min; compound **6** (O-trifluoroacetylated) 80 °C, 3.4 min; (2) with 3% Carbowax 20M, compound **5** (N-trifluoroacetylated), 125 °C, 4.2 min; compound **6** (O-trifluoroacetylated), 115 °C, 4.7 min.

IR spectra were recorded with the Perkin-Elmer 297 apparatus (KBr disks; cm^{-1}). ^{1}H NMR spectra of diflufenican and its metabolites (in $CDCl_3$ or DMSO-d_6) were recorded with the Varian XL 200 apparatus, using tetramethylsilane as internal standard; absorption bands were in the 6.3–8.7 ppm (δ relative to TMS) region of the aromatic protons with additional OH and NH broad proton bands. MS were recorded with the VG Micromass 7070F and VG 70S spectrometers at 70 eV used in the electron impact (spectra indicated here) or chemical ionization (NH_3) modes; m/e, relative abundance, %. Frequently diflufenican and its metabolites extracted from soil were analyzed by MS; diflufenican and its metabolites **2–4** were analyzed as such by MS; compounds **5** and **6** were analyzed by MS as such or as their trifluoroacetyl derivatives.

Standards for Analysis. 2,4-Difluoroaniline (**5**) and 3-(trifluoromethyl)phenol (**6**) were obtained from Janssen Chimica. Diflufenican was isolated from a formulation made for assays. Compounds **2–4** were synthesized; reactions were monitored by TLC and GLC.

Diflufenican (1). The liquid formulation of diflufenican (100 g; 500 g of diflufenican/L, for assays only, obtained from Rhone-Poulenc) was concentrated to dryness in a vacuum rotary evaporator (30 °C); the residue was extracted (20 °C, 1 h, stirring)

Table I. Concentrations of Diflufenican (1) and Its Metabolites 2–4 in the 0–10-cm Surface Soil Layer of Field Crops

date[a]	days after treatment	cumulative rainfall, mm	diflufenican and its metabolites (as equivalents of diflufenican) concentrations (ppb, i.e., 10^{-3} mg kg^{-1} dry soil) in the 0–10-cm soil layer ±SD[b]				linear regression $y = kt + b$ of diflufenican soil concentrations (y = ppb in dry soil) against time t (days)[d]			
			1	2	3	4	k ± SD, ppb day^{-1}	b, ppb	r (corr coeff)	$t_{1/2}$,[e] days
1. Wheat Crops: Assays 1–5										
assay 1[f]										
21-10-87	0	0	139[c]				−0.3925 ± 0.0121	129.3	−0.9875	149 ● 6
26-10-87	5	3	124 ± 6	ND	ND	ND				
21-12-87	61	113	116 ± 5	5 ± 1	ND	ND				
28-1-88	99	242	92 ± 5	4 ± 1	5 ± 1	5 ± 1				
26-2-88	128	321	73 ± 3	8 ± 1	9 ± 1	11 ± 1				
29-3-88	159	477	68 ± 3	7 ± 1	8 ± 1	11 ± 1				
29-4-88	190	484	52 ● 3	13 ± 1	11 ± 1	13 ± 1				
11-5-88	202	520	46 ± 2	14 ± 1	13 ± 1	16 ± 1				
31-5-88	222	550	37 ± 2	16 ± 1	14 ± 1	16 ± 1				
30-6-88	252	561	32 ± 2	15 ± 1	15 ± 1	17 ± 1				
1-8-88[o]	284	657	25 ± 1	15 ± 1	18 ± 1	14 ± 1				
assay 2[g]										
28-10-88	0	0	148[c]				−0.3805 ± 0.0119	141.6	−0.9938	176 ± 6
4-11-88	7	0	134 ± 6	ND	ND	ND				
28-11-88	31	19	138 ± 7	ND	ND	ND				
25-1-89	89	120	104 ± 5	9 ± 1	6 ± 1	ND				
23-2-89	118	155	98 ± 5	5 ± 1	6 ± 1	7 ± 1				
31-3-89	154	241	85 ± 4	8 ± 1	9 ± 1	10 ± 1				
21-4-89	175	318	71 ± 3	14 ± 1	13 ± 1	11 ± 1				
8-6-89	223	366	56 ± 3	14 ± 1	18 ± 1	14 ± 1				
7-7-89	252	413	47 ± 2	18 ± 1	17 ± 1	18 ± 1				
1-8-89[o]	277	460	37 ± 2	18 ± 1	21 ± 1	20 ± 1				
assay 3[h]										
8-11-88	0	0	148[c]				−0.4528 ± 0.0124	145.3	−0.9903	154 ± 6
16-11-88	8	5	134 ± 7	ND	ND	ND				
2-12-88	24	45	140 ± 6	ND	ND	ND				
23-1-89	76	78	119 ± 6	11 ± 1	8 ± 1	ND				
22-2-89	106	155	100 ± 5	7 ± 1	6 ± 1	5 ± 1				
14-3-89	126	253	81 ± 4	9 ± 1	10 ± 1	11 ± 1				
17-4-89	160	299	71 ● 4	10 ± 1	12 ± 1	12 ± 1				
9-6-89	213	366	46 ● 3	15 ± 1	19 ± 1	17 ± 1				
6-7-89	240	403	34 ± 2	16 ± 1	23 ± 1	20 ± 1				
24-7-89[o]	258	430	34 ± 1	15 ± 1	22 ± 1	19 ± 1				
assay 4[i]										
5-10-88	0	0	130[c]				−0.3645 ± 0.0130	125.6	−0.9908	165 ± 6
27-10-88	22	58	123 ± 6	ND	ND	ND				
14-12-88	70	149	95 ● 5	5 ± 1	ND	ND				
27-1-89	114	195	86 ± 4	5 ± 1	5 ± 1	4 ± 1				
24-2-89	142	229	74 ± 4	7 ± 1	6 ± 1	7 ± 1				
21-3-89	167	305	64 ± 3	8 ± 1	9 ± 1	9 ± 1				
19-4-89	196	383	49 ● 3	11 ± 1	14 ± 1	12 ± 1				
25-5-89	232	407	36 ± 2	15 ± 1	15 ± 1	14 ± 1				
24-6-89	262	439	35 ± 2	12 ± 1	12 ± 1	15 ± 1				
10-7-89[o]	278	507	28 ± 1	13 ± 1	15 ± 1	16 ± 1				
assay 5[j]										
14-3-89	0	0	104[c]				−0.4319 ● 0.0288	94.3	−0.9969	98 ± 8
14-3-89	0	0	94 ± 5	ND	ND	ND				
17-4-89	34	87	80 ± 4	8 ± 1	ND	ND				
9-6-89	87	154	58 ± 3	8 ± 1	7 ± 1	7 ± 1				
6-7-89	114	191	42 ± 2	9 ± 1	12 ± 1	11 ± 1				
24-7-89[o]	132	218	39 ± 2	9 ± 1	12 ± 1	10 ± 1				
2. Replacement Crops: Assays 6–9										
assay 6[k]										
3-12-87	0	0	185[c]				−0.5939 ± 0.0131	177.6	−0.9896	141 ● 5
21-12-87	18	14	170 ± 8	ND	ND	ND				
28-1-88	56	143	152 ± 8	10 ± 1	ND	ND				
26-2-88	85	242	130 ± 7	7 ± 1	6 ± 1	ND				
29-3-88	116	378	101 ± 5	11 ± 1	13 ± 1	11 ± 1				
29-4-88	147	385	88 ± 5	12 ± 1	15 ± 1	13 ± 1				
11-5-88	159	421	81 ± 4	13 ± 1	17 ± 1	14 ± 1				
31-5-88	179	451	62 ± 3	16 ± 1	22 ± 1	19 ± 1				
30-6-88	209	462	46 ± 2	19 ± 1	24 ± 1	21 ± 1				
1-8-88	241	558	38 ± 2	18 ± 1	26 ± 1	22 ± 1				
1-9-88	272	594	28 ± 2	19 ± 1	24 ± 1	23 ± 1				

Table I. (Continued)

date[a]	days after treatment	cumulative rainfall, mm	diflufenican and its metabolites (as equivalents of diflufenican) concentrations (ppb, i.e., 10^{-3} mg kg^{-1} dry soil) in the 0–10-cm soil layer ±SD[b] 1	2	3	4	linear regression $y = kt + b$ of diflufenican soil concentrations (y = ppb in dry soil) against time t (days)[d] $k \pm$ SD, ppb day^{-1}	b, ppb	r (corr coeff)	$t_{1/2}$,[e] days
assay 7[l]										
22-3-88	0	0	148[c]				−0.6412 ± 0.0237	130.4	−0.9785	86 ± 5
29-3-88	7	46	135 ± 7	ND	ND	ND				
29-4-88	38	53	109 ± 5	7 ± 1	6 ± 1	ND				
11-5-88	50	89	91 ± 5	9 ± 1	10 ± 1	10 ± 1				
31-5-88	70	119	82 ± 4	9 ± 1	12 ± 1	11 ± 1				
30-6-88	100	130	56 ± 3	16 ± 1	19 ± 1	18 ± 1				
1-8-88	132	226	47 ± 3	16 ± 1	20 ± 1	18 ± 1				
1-9-88	163	262	34 ± 2	18 ± 1	22 ± 1	20 ± 1				
assay 8[m]										
21-11-88	0	0	185[c]				−0.5230 ± 0.0143	177.1	−0.9944	158 ± 5
28-11-88	7	3	170 ± 8	ND	ND	ND				
25-1-89	65	104	148 ± 7	7 ± 1	ND	ND				
23-2-89	94	139	126 ± 7	11 ± 1	9 ± 1	ND				
31-3-89	130	225	115 ± 6	7 ± 1	9 ± 1	8 ± 1				
21-4-89	151	302	92 ± 5	13 ± 1	15 ± 1	14 ± 1				
8-6-89	199	350	77 ± 4	13 ± 1	19 ± 1	16 ± 1				
7-7-89	228	397	53 ± 3	20 ± 1	24 ± 1	22 ± 1				
1-8-89	253	444	46 ± 2	18 ± 1	25 ± 1	21 ± 1				
assay 9[n]										
20-3-89	0	0	148[c]				−0.6995 ± 0.0309	150.0	−0.9939	108 ± 5
31-3-89	11	12	144 ± 7	ND	ND	ND				
21-4-89	32	89	129 ± 6	12 ± 1	6 ± 1	ND				
8-6-89	80	137	87 ± 5	10 ± 1	9 ± 1	9 ± 1				
7-7-89	109	184	75 ± 4	8 ± 1	15 ± 1	12 ± 1				
1-8-89	134	231	59 ± 3	13 ± 1	16 ± 1	15 ± 1				

[a] Sampling date, day-month-year. [b] Means of four replicates ± SD; ND, not detected; 1, diflufenican; 2, 2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxylic acid; 3; *N*-(2,4-difluorophenyl)-2-hydroxy-3-pyridinecarboxamide; 4, 2-hydroxy-3-carboxypyridine. [c] Calculated initial soil concentrations. [d] Time for 50% loss of initial diflufenican in soil. Calculated by means of the SAS logical (1984, 1986, SAS Institute Inc., Cary, NC 27512). [e] Diflufenican soil half-lives ($t_{1/2}$, days) with their 95% confidence intervals; the confidence intervals were evaluated—by inverse regression—from the 95% confidence bands for the diflufenican soil concentrations (Figures 2–6; Draper and Smith, 1981). [f] Wheat sown at Melle on 20-10-1987, 188 g of diflufenican/ha on 21-10-1987. [g] Wheat sown at Melle on 26-10-1988, 200 g of diflufenican/ha on 28-10-1988. [h] Wheat sown at Gijzenzele on 3-11-1988, 200 g of diflufenican/ha on 8-11-1988. [i] Winter barley sown at Houtem-Veurne on 4-10-1988, 175 g of diflufenican/ha on 5-10-1988. [j] Wheat sown at Gijzenzele on 3-11-1988, 140 g of diflufenican/ha on 14-3-1989. [k] 250 g of diflufenican/ha at Melle on 3-12-1987; sugar beet sown on 25-4-1988. [l] 200 g of diflufenican/ha Melle on 22-3-1988; sugar beet sown on 25-4-1988. [m] 250 g of diflufenican/ha at Melle on 21-11-1988; sugar beet sown on 25-4-1989. [n] 200 g of diflufenican/ha at Melle on 20-3-1989; sugar beet sown on 25-4-1989. [o] Harvest date.

with methylene chloride (2 × 300 mL), and the methylene chloride solution was washed with a concentrated solution of NaCl in water (200 mL), dried with Na_2SO_4, and concentrated to dryness in a vacuum rotary evaporator. The residue was recrystallized in methylene chloride + hexane 1 + 1 mL/mL, giving diflufenican (yield 93%, purity >99%). Spectra of diflufenican: IR 3370, 1670, 1610, 1590, 1555, 1450, 1435, 1410, 1350, 1330, 1310, 1280, 1230, 1180, 1130, 1090, 1070, 965, 920, 890, 860, 820, 800, 770, 725; MS 394 (M^+, 28), 375 (M − F, 3), 266 (M − $NHC_6H_3F_2$, 100), 246 (266 − HF, 9), 238 (266 − CO, 4), 218 (238 − HF, 12).

2-[3-(Trifluoromethyl)phenoxy]-3-pyridinecarboxylic Acid (2). Diflufenican (4 g), water (100 mL), and KOH (8 g) were heated together to reflux (60 h, stirring). The mixture was cooled overnight at 6 °C and filtered, giving the solid untransformed diflufenican (2.9 g, 73%). The alkaline aqueous phase was extracted with methylene chloride; the methylene chloride solution was evaporated to dryness, giving 2,4-dichloroaniline (0.3 g). The alkaline aqueous phase was brought to pH 1 with concentrated hydrochloric acid; the precipitate was filtered and recrystallized in acetone + water, giving compound 2 (0.6 g, 21%): mp 151–152 °C; IR 3200–2800, 1740, 1600, 1520, 1440, 1330, 1290, 1240, 1190, 1140, 1130, 1110, 1090, 1070, 925, 900, 850, 775; MS 283 (M^+, 22), 282 (M − 1, 25), 264 (M − F, 28), 239 (M − CO_2, 100), 218 (M − CO_2H − HF, 24), 212 (M − H_2CF_3, 28), 170 (239 − CF_3, 57). Anal. Calcd (found) for $C_{13}H_8F_3NO_3$ (283.05): C, 55.11 (55.46); H, 2.85 (2.71); F, 20.14 (20.53). Spectra of 2,4-difluoroaniline were similar to those of the commercial product.

N-(2,4-Difluorophenyl)-2-hydroxy-3-pyridinecarboxamide (3) *and 2-Hydroxy-3-carboxypyridine* (4). Diflufenican (4 g), acetic acid (75 mL), and concentrated hydrochloric acid (37% HCl in water; 35 mL) were heated together to reflux (48 h, stirring). The cooled mixture was evaporated to dryness in a vacuum rotary evaporator (60 °C). Acetone (200 mL) was added to the solid residue, and the solution was again concentrated to dryness. The solid residue was extracted (10 min, 20 °C, stirring) with 5 g % (w/v) of KOH in water (50 mL); the insoluble product was filtered. GLC analysis indicated that it did not contain any untransformed diflufenican; after recrystallization in acetone + water, it gave compound 3 (0.7 g, 28%). The aqueous alkaline filtrate was extracted with methylene chloride, and the methylene chloride solution was evaporated to dryness in a vacuum rotary evaporator at 25 °C, giving the oily 2,4-difluoroaniline (0.6 g). The aqueous alkaline filtrate was brought to pH 1; the precipitate was filtered. GLC analysis indicated that it did not contain any compound 2; after recrystallization in ethanol + water, it gave compound 4 (0.8 g, 57%). The acid aqueous filtrate was extracted with methylene chloride, and the methylene chloride solution was concentrated to dryness, giving the oily 3-(trifluoromethyl)phenol (1.1 g).

Compound 3: mp 243–244 °C; IR 3300–2600, 1680, 1645, 1600, 1550, 1500, 1475, 1430, 1320, 1250, 1240, 1200, 1135, 1090, 955, 900, 835, 770, 720; MS 250 (M^+, 89), 233 (M − OH, 2), 222 (M − CO, 3), 210 (M − 2HF, 2), 130 ($C_6H_3F_2NH_3$, 30), 122 (HOC_5H_3-NCO, 100), 94 (HOC_5H_3N, 45). Anal. Calcd (found) for $C_{12}H_8F_2N_2O_2$ (250.06): C, 57.59 (57.23); H, 3.22 (3.39); F, 15.20 (15.38).

Compound 4: mp 255–256 °C; IR 3240, 3200–2800, 1750, 1640, 1610, 1550, 1490, 1450, 1420, 1325, 1240, 1130, 1090, 1060, 1000, 890, 785, 730; MS 139 (M^+, 78), 122 (M − OH, 22), 121 (M − H_2O, 24), 95 (M − CO_2, 100), 94 (M − CO_2H, 72). Spectra of 3-(trifluoromethyl)phenol were similar to those of the commercial product.

N-(2,4-Difluorophenyl)trifluoroacetamide (5′). Trifluoroacetic anhydride (7.5 g, 36 mmol) was added dropwise during 30 min to ice-cooled 2,4-difluoroaniline (2 g, 16 mmol). The mixture was heated to reflux (5 min), the excess trifluoroacetic anhydride was evaporated, and the solid residue was recrystallized in methylene chloride + hexane, yielding *N*-(2,4-difluorophenyl)trifluoroacetamide (5′; 3.4 g, 94%): MS 225 (M^+, 91), 206 (M − F, 3), 177 (M − CO − HF, 3), 156 (M − CH_3, 37), 138 (156 − H_2O, 2), 128 ($NHC_6H_3F_2$, 100), 101 ($C_5H_3F_2$, 63).

3-(Trifluoromethyl)phenyl Trifluoroacetate (6′). Trifluoroacetic anhydride (13.5 g, 64 mmol) and 3-(trifluoromethyl)phenol (6 g, 37 mmol) were heated together to reflux (10 h, stirring). The cooled mixture was diluted with methylene chloride (60 mL), washed with 4 g % (w/v) of KOH in water, dried (Na_2SO_4), and concentrated in a vacuum rotary evaporator, yielding 3-(trifluoromethyl)phenyl trifluoroacetate (6′; 8.6 g, 90%): MS 258 (M^+, 82), 239 (M − F, 28), 230 (M −CO, 73), 211 (230 − F, 12), 189 (M − CF_3, 4), 161 (M − CF_3CO, 18), 145 (M − CF_3CO_2, 59), 133 (145 − C, 47), 114 (CF_3CO_2H, 37).

Soil and Plant Analyses. Soil (100 g, moist) was refluxed with stirring for 20 min in a mixture of acetone + water 8 + 2 mL/mL (200 mL). The mixture was filtered, and the extraction was repeated. The filtrates were combined, water (80 mL) was added, the acetone was evaporated in a vacuum rotary evaporator (30 °C), NaCl (15 g) was added to the aqueous solution, and this was extracted two times with methylene chloride (200 + 150 mL) (extract 1). The methylene chloride solution was dried (Na_2SO_4) and concentrated to 40 mL in a vacuum rotary evaporator (30 °C) and to 0.5 mL by a slow stream of nitrogen (20 °C); it was applied onto a TLC plate, along with the standard of diflufenican. Elution with methylene chloride + hexane 1 + 1 mL/mL gave diflufenican (R_f 0.72); this was scraped off, and the silica gel was put in a small (6 mm i.d.) glass chromatography column. This was eluted with ethyl acetate (40 mL); the extract was concentrated to 0.5 mL by a slow stream of nitrogen (20 °C). The extract was purified by a second TLC, with methylene chloride + hexane 1 + 2 mL/mL as elution solvent [R_f(diflufenican) 0.35]; the band of diflufenican was isolated, extracted, and analyzed by GLC and, occasionally, MS.

The soil, already extracted by the mixture of acetone + water, was refluxed for 20 min with stirring in a solution of 4 g % (w/v) of KOH in water (200 mL). The cooled mixture was filtered, the filtrate was brought to pH 1 with concentrated hydrochloric acid, NaCl (15 g) and dodecyl sulfate (0.1 g) were added, and the acid aqueous solution was extracted two times with ethyl acetate (200 + 150 mL), giving the aqueous acid extract 2 and the ethyl acetate extract. The ethyl acetate extract was concentrated to 40 mL in a vacuum rotary evaporator (30 °C) and to 5 mL by a slow stream of nitrogen (20 °C). Methylene chloride (45 mL) was added, the mixture was filtered, the precipitate was discarded (otherwise, it would consume large amounts of diazomethane during the futher methylation procedures), and the filtrate was washed with 0.1 N HCl in water (25 mL), dried (Na_2SO_4), and concentrated first to 30 mL in a vacuum rotary evaporator (30 °C) and then to 5 mL (extract 3) by a stream of nitrogen (20 °C). After concentration to 0.5 mL with a stream of nitrogen, the extract was applied onto a TLC plate. Elution with ether + hexane 1 + 2 mL/mL gave a band at R_f 0–0.20 which contained compounds **2** (R_f 0.20), **3** (R_f 0.0), and **4** (R_f 0.0); that band was separated, extracted with ethyl acetate, and concentrated to 5 mL (extract 4) with a stream of nitrogen (20 °C).

For compound **2** analysis, to an aliquot of extract 4 were added successively methanol (3 mL) and diazomethane in ether; the mixture was concentrated to 0.5 mL with a stream of nitrogen (20 °C) and applied onto a TLC plate. Elution with ethyl acetate + hexane 1 + 2 mL/mL gave a band containing methylated compound **2** (R_f 0.84); that band was isolated, extracted, and analyzed by GLC for compound **2**. For the occasional MS analyses of compound **2** extracted from soil, an aliquot of extract 4 was purified by a second TLC using ether + hexane 1 + 2 mL/mL; the band at R_f 0.20 was isolated, extracted, and analyzed by MS.

For compound **3** analysis, an aliquot of extract 4 was applied onto a TLC plate; elution with 1-butanol + concentrated ammonia (30% NH_3 in water) 6 + 1 mL/mL gave a band at R_f 0.65 containing compound **3**; the band was isolated, extracted, and analyzed for compound **3** by GLC and, occasionally, MS.

For compound **4** analysis, an aliquot of extract 4 was applied onto a TLC plate; elution with ethanol + concentrated ammonia 1 + 1 mL/mL gave a band at R_f 0.81 containing compound **4**. That band was isolated, extracted with ethyl acetate, and concentrated to 0.5 mL with a stream of nitrogen (20 °C). That extract was occasionally analyzed for compound **4** by MS. For GLC analysis, benzene (10 mL) and trifluoroacetic anhydride (2 mL) were added and the mixture was heated to reflux (30 min); after cooling, it was concentrated to dryness with a stream of nitrogen (20 °C). The residue was dissolved in methanol (5 mL), diazomethane (from *N*-nitroso-*N*-methylurea) in ether was added, and the mixture was concentrated with nitrogen (20 °C) and analyzed by GLC.

For compound **6** analysis, an aliquot of extract 3 was brought to 25 mL with benzene, trifluoroacetic anhydride (2 mL) was added, and the mixture was heated to reflux (30 min, stirring); after cooling, it was washed with water, dried (Na_2SO_4), concentrated to 0.5 mL with a stream of nitrogen (20 °C), and applied onto a TLC plate. Elution with benzene gave a band containing 3-(trifluoromethyl)phenyl trifluoroacetate (6′; R_f 0.35); the band was isolated, extracted, and concentrated, and the TLC was repeated. The final extract was analyzed by GLC and, occasionally, MS.

For compound **5** analysis, the acid aqueous extract 2 was brought to pH 12 with KOH in water and extracted with benzene (150 mL). The benzene solution was dried (Na_2SO_4), trifluoroacetic anhydride (2 mL) was added, and the mixture was heated to reflux (30 min, stirring); after cooling, it was washed with water, dried (Na_2SO_4), concentrated to 0.5 mL with a stream of nitrogen (20 °C), and applied onto a TLC plate. Elution with benzene gave a band containing *N*-(2,4-difluorophenyl)trifluoroacetamide (5′; R_f 0.62); the band was isolated, extracted, and concentrated, and the TLC was repeated. The final extract was analyzed by GLC and, occasionally, MS.

Each recovery experiment was completely made four times. At the 25 ppb level in soil, the recoveries (± SD) were diflufenican (1) 90 ± 4%, compound **2** 88 ± 5%, compound **3** 86 ± 5%, compound **4** 80 ± 7%, compound **5** 81 ± 8%, and compound **6** 82 ± 5%. Recovery experiments, made separately with each of the compounds, indicated that none of them was transformed into another during the analytical process. The analytical limits of sensitivity for diflufenican and its metabolites in soil were (relative to the dry soil) diflufenican 1 ppb (1×10^{-3} mg kg^{-1} = 1 μg g^{-1} = 0.001 ppm), compounds **2**–**4** 2 ppb, and compounds **5** and **6** 5 ppb.

Grain and the root and leaves of the sugar beet were analyzed in the same way as soil; as with soil, plant parts were not dried before analysis. However, roots and leaves were first cut into small pieces; grains were first transformed into flour. The pieces of sugar beet and the flour were extracted at 20 °C in the Sorvall omnimixer. At the 25 ppb level (relative to the fresh weight) in the grain flour and in the root and leaves of sugar beet, the recoveries of diflufenican and its metabolites (including their SD) were similar to the corresponding ones in the soil; their analytical limits of sensitivity in the grain flour and the roots and leaves of sugar beet (relative to the fresh weight) were similar to the corresponding values in the soil (relative to the dry soil).

Incubation in the Laboratory of Diflufenican in Sterilized Water Buffer or Soil. The stirred mixture of 200 ppb of diflufenican (using the formulation Exp 4005) in a water buffer at pH 6.5 was incubated at 18–20 °C in sterilized conditions. After 3.8 months, more than 94% of the diflufenican was recovered. On the other hand, diflufenican (using the formulation Exp 4005) was incorporated into sterilized soil at the concentration of 200 ppb (relative to dry soil), and the mixture was incubated in sterilized conditions, water being added to maintain the soil at 70% of its maximum water capacity; after 3.8 months of incubation, more than 95% of the initial diflufenican was recovered unchanged.

RESULTS AND DISCUSSION

Diflufenican is active against broad-leafed weeds species (*G. aparine*, *V. hederifolia*, *V. persica*, *V. arvensis*) which are resistant to the substituted-urea herbicides (isoproturon and chlortoluron). These urea herbicides are active

for controlling grass weeds (*A. myosuroides*); against these grass weeds, the efficiency of diflufenican is insufficient. For that reason, diflufenican is developed in mixtures with the traditional cereal herbicides (especially isoproturon) to enable a single autumn application to ensure effective control of the wide spectrum of competitive weeds associated with the long growing cycle of the winter cereal crop (Cramp et al., 1985; Kyndt et al., 1985). The soil half-life time of isoproturon is only about 2 months; it has no phytotoxicity at all on the replacement crops (Van Himme and Bulcke, 1987, 1988, 1989); it is completely separated from diflufenican and its metabolites during the soil analytical procedure, by, among others, the TLC and GLC separation techniques. Isoproturon thus did not affect in the present work the analyses and bioassays of diflufenican and its metabolites.

In the soil layers at 10–20- and 20–30-cm depths, diflufenican and its metabolites **2–4** generally were not detected, their soil concentrations there being thus lower than about 1 or 2 ppb, which is the limit of the analytical sensitivity (data not shown). Diflufenican and its biodegradation products thus remained in the 0–10-cm surface soil layer, no leaching occurring; the same was true when there were heavy rains, as during the winter of 1987–1988. This very low mobility of diflufenican in soil should be related to its very low solubility in water and its high lipophilicity. 2,4-Difluoroaniline (**5**) and 3-(trifluoromethyl)phenol (**6**) were never observed in soil. Diflufenican and its metabolites **2–4** were observed in the soil of the field crops.

Diflufenican and its metabolites **2–4** and compounds **5** and **6** were searched separately in the grain obtained after harvest of each of the wheat crops of assays 1–5 (four replicate separate analyses of the grain from each crop). None of these compounds was ever detected in the flour; their concentrations in the grain thus was lower than their sensitivity limits, i.e., 1 ppb (relative to the fresh weight) for diflufenican, 2 ppb for compounds **2–4**, and 5 ppb for compounds **5** and **6**. Similarly, diflufenican and compounds **2–6** were never detected in the root or in the leaves of the sugar beet harvested at the end of October in each of the assays 6–9.

After about 4 months of diflufenican incubation in a water buffer or in sterilized soil in the laboratory, more than 95% of the initial diflufenican was recovered unchanged. In the soil of the field crops, between 40 and 60% of the initially soil applied diflufenican was transformed after 4 months, no leaching occurring (Table I). This suggested that the observed soil biodegradation of diflufenican in crop fields was mainly due to the soil microbial and enzymatic activities.

The rates of diflufenican soil metabolism were of zero order against diflufenican soil concentrations; indeed, diflufenican soil concentrations showed linear correlations against time following soil application (Table I; Figures 2–6). At Melle, the time for 50% loss of the initial diflufenican in soil was shorter in the wheat crop of 1987–1988 (assay 1) than in the one of 1988–1989 (assay 2). This should be related to the rains which were heavier during the 1987–1988 crop season than during the 1988–1989 season; on the other hand, during both seasons, temperatures were similar (Figure 7). In the wheat crop at Gijzenzele (assay 3), the time for 50% loss of the initial diflufenican in soil was shorter than it was in the wheat crop at Melle (assay 2) during the same 1988–1989 crop season. In the winter barley crop at Houtem-Veurne (assay 4), the time for 50% loss of the initial diflufenican in soil was close to the one in the wheat crop at Melle (assay 2)

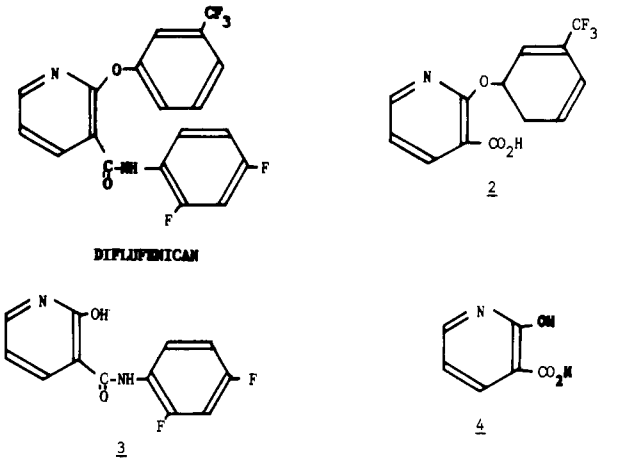

Figure 1. Diflufenican (1) and its soil metabolites **2–4**.

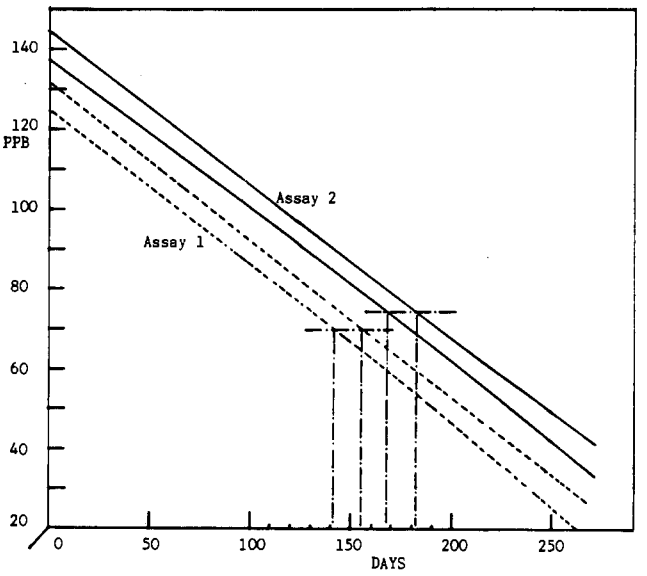

Figure 2. Relationship between diflufenican soil concentrations (ppb, i.e. 10^{-3} mg kg^{-1} dry soil) and the time (days) following diflufenican soil treatment in assays 1 and 2: 95% confidence bands for the diflufenican soil concentrations and 95% confidence intervals for the diflufenican soil half-lives (Draper and Smith, 1981; computer calculations using the SAS logical CMS SAS 5.18; 1984, 1986, SAS Institute Inc., Cary, NC 27512). In Table I are the values of the slopes (±SD), y intercepts, and correlation coefficients of the regression lines and the diflufenican soil half-lives with their 95% confidence intervals.

grown during the same crop season. When diflufenican was soil applied during the spring in a wheat crop (assay 5), the time for 50% loss of the initial diflufenican in soil was only 63% of its value when soil treatment was made in November of the preceding year (assay 3).

In the sugar beet replacement crop sown in April 1988 on a soil treated with diflufenican in December 1987 (assay 6), the time for 50% loss of initial diflufenican in soil was similar to the one observed in the winter wheat crop made during the same period of time (assay 1) (Table I). About the same was observed in 1988–1989 with both assays 8 and 2.

In the sugar beet replacement crop sown in April 1988 on a soil treated with diflufenican 1.2 months before (assay 7), the time for 50% loss of initial diflufenican in soil was similar to the one observed during the spring/summer period of 1989, in the wheat crop of assay 5 (Table I); the same was observed with the spring/summer sugar beet crop of 1989 (assay 9). The rate of diflufenican soil biodegradation thus was always greater during the spring/

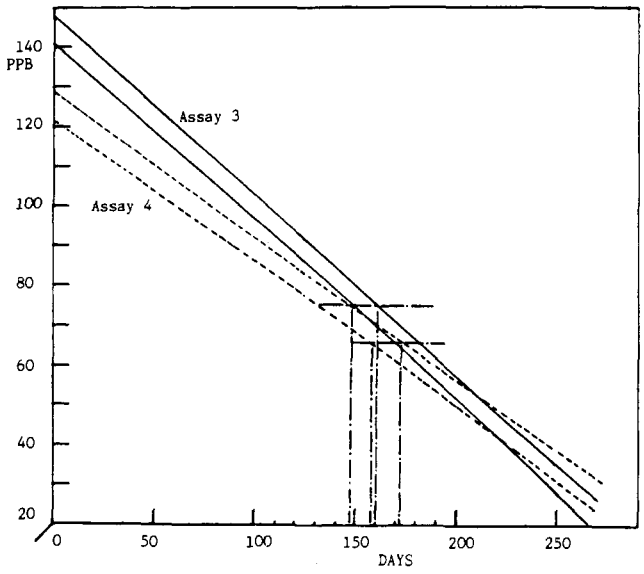

Figure 3. Diflufenican soil concentrations (ppb) and the time (days) following diflufenican soil treatment in assays 3 and 4: 95% confidence bands for the diflufenican soil concentrations and 95% confidence intervals for the diflufenican soil half-lives. In Table I are the values of the slopes (±SD), y intercepts, and correlation coefficients of the regression lines and the diflufenican soil half-lives with their 95% confidence intervals.

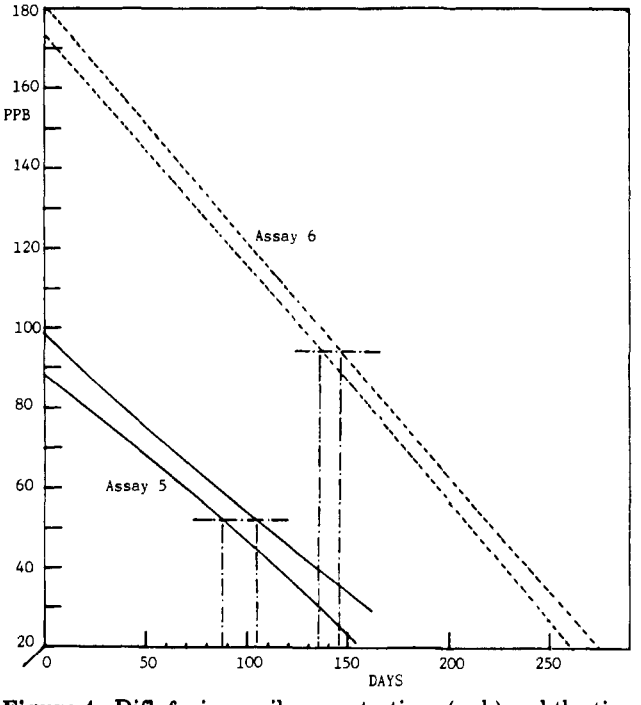

Figure 4. Diflufenican soil concentrations (ppb) and the time (days) following diflufenican soil treatment in assays 5 and 6: 95% confidence bands for the diflufenican soil concentrations and 95% confidence intervals for the diflufenican soil half-lives. In Table I are the values of the slopes (±SD), y intercepts, and correlation coefficients of the regression lines and the diflufenican soil half-lives with their 95% confidence intervals.

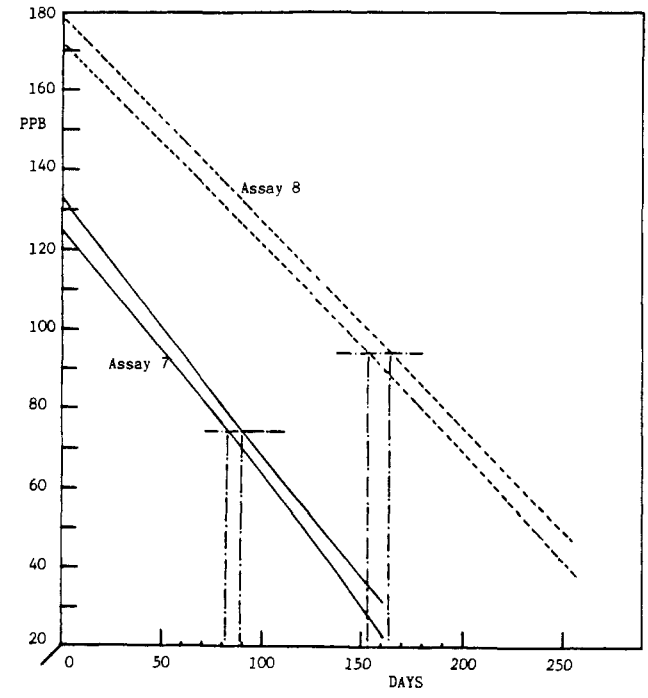

Figure 5. Diflufenican soil concentrations (ppb) and the time (days) following diflufenican soil treatment in assays 7 and 8: 95% confidence bands for the diflufenican soil concentrations and 95% confidence intervals for the diflufenican soil half-lives. In Table I are indicated the slopes (±SD), y intercepts, and correlation coefficients of the regression lines and the diflufenican soil half-lives with their 95% confidence intervals.

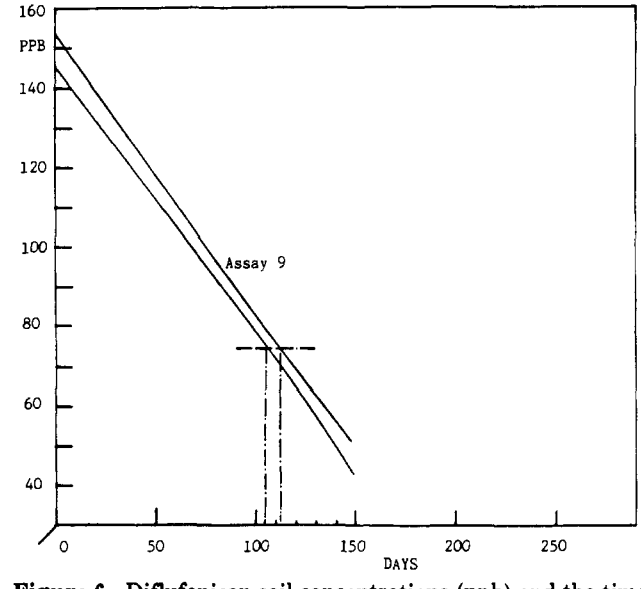

Figure 6. Diflufenican soil concentrations (ppb) and the time (days) following diflufenican soil treatment in assay 9: 95% confidence bands for the diflufenican soil concentrations and 95% confidence intervals for the diflufenican soil half-lives. In Table I are indicated the slopes (±SD), y intercepts, and correlation coefficients of the regression lines and the diflufenican soil half-lives with their 95% confidence intervals.

summer period; this should correspond to the greater microbial and enzymatic soil activities during spring/summer, induced by the higher temperatures of that period.

In soil, diflufenican was progressively transformed into compound **2** by hydrolysis of the amide bond (Figure 1). In sterilized water a pH 6.5 and 18–20 °C and in sterilized soil, such process did not occur, after 3.8 months of incubation, at a comparable rate. A long time of heating to reflux (60 h) and a high KOH concentration [8 g % (w/v)] in water were necessary to transform only 27% of the diflufenican and to get 21% of compound **2**. This indicated that the diflufenican soil biodegradations in the crop fields occurred by microbial and enzymatic processes.

Compound **2** has already been reported as a diflufenican soil metabolite (Rhône-Poulenc, 1988) (Figure 1); its soil concentrations had been reported to be, however, much lower (maximum 3.7% of the diflufenican applied dose) than the ones reported in the present work. On the other hand, to our knowledge compounds **3** and **4** have not

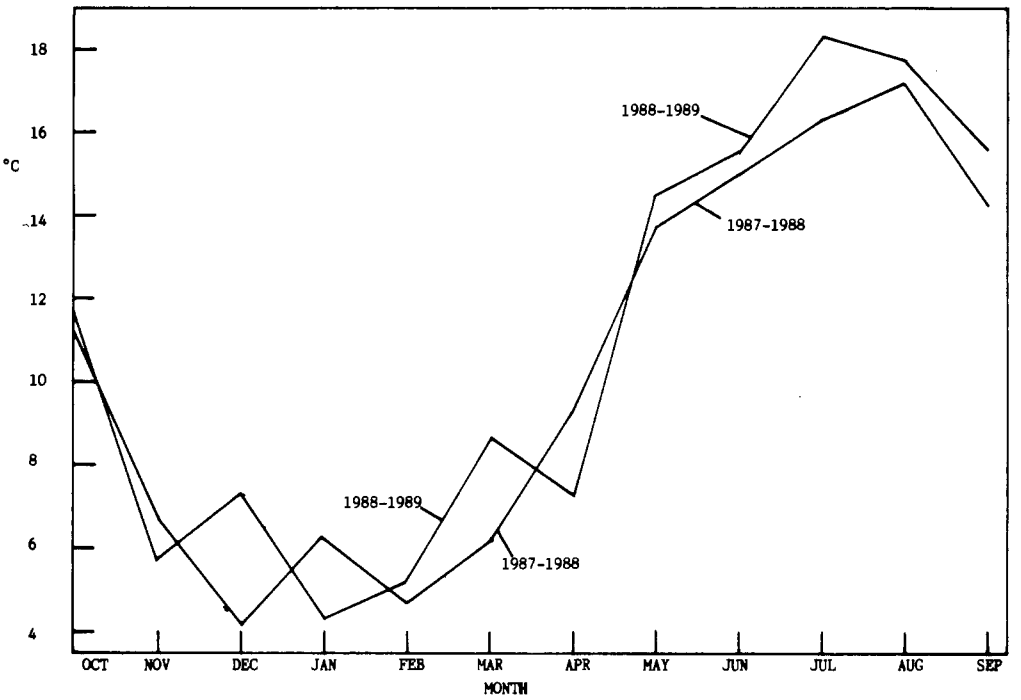

Figure 7. Mean temperatures for each month at Melle during the 1987–1988, and 1988–1989 crop seasons. For each month, the mean was made from each daily mean temperature (mean between the maximum and minimum temperatures of that day of 24 h). Mean temperatures at the other crop locations were similar to the ones at Melle.

been—before the present work—reported as diflufenican metabolites.

Because of the chemical structures of diflufenican and its metabolites **2**–**4**, it seems that compounds **2** and **3** both were generated in soil from diflufenican by parallel and competitive bioreactions (Figure 1). Compounds **2** and **3** both should generate compound **4** in soil. Three months after the diflufenican soil treatment made in autumn, and 2 months after the treatment made in the spring, the soil concentrations of the diflufenican metabolites were generally in the order compound **3** > compound **4** > compound **2**. Compound **4** being generated by biodegradation of both compounds **2** and **3**, the relatively low concentrations of compound **4** should indicate that this was soil biodegraded at a greater rate than diflufenican and compounds **2** and **3**.

2,4-Difluoroaniline (**5**) and 3-(trifluoromethyl)phenol (**6**) were never observed in soil, in spite of the severity of the extraction conditions for analysis [heating soil to reflux with stirring in 4 g % (w/v) of KOH in water during 20 min under air or nitrogen atmosphere]. During diflufenican soil biodegradations, compounds **5** and **6** thus were quickly biodegraded into products of futher decomposition, which either were incorporated into the soil organic matter or were completely oxidized into CO_2.

In each of the replacement crop assays (assays 6–9), there were plots whose soil had not been treated with diflufenican (control plots). Moreover, besides the sugar beet crops, there were other plots onto which other crops were grown separately: wheat, barley, oat, maize, potato, beans, pea, turnip, Savoy cabbage, scorzonera, lettuce, carrot, spinach, chicory, and onion. These other replacement crops were sown on the same day as sugar beet. Residue analyses were made in the soil of these crops but without replicates and only on two dates during each crop; their soil residue values were similar to the ones obtained in the sugar beet replacement crops.

The results of soil biodegradation are consistent with the biological observations made by Van Himme (Van Himme and Bulcke, 1987, 1988). These biological observations may be summarized in the following way. When the replacement crops were *sown in April*, onto a soil which had been treated with diflufenican either 1.2 months before (i.e., in March; assays 7 and 9) or 5 months before (i.e., in November of the preceding year) (in both cases the soil remaining fallow between diflufenican treatment and sowing of the replacement crop), no growth hindering or reducing of yield at harvest were observed for all the replacement crops, except for the sensitive sugar beet, for which slight but not serious growth hindering and yield reducing at harvest were observed. Some of the replacement crops (sugar beet, oat, turnip, and lettuce) were again *sown in July* of the same year; this was done onto the freshly prepared soil (harrowing in the 0–10-cm soil layer) of the plots onto which the cereal replacement crops had been harvested. These cereal replacement crops had been sown in April and were grown on a soil treated with diflufenican either in November of the preceding year (8 months before) or in March of the same year (3 months before) (the soil remaining fallow in winter between diflufenican treatment and wheat sowing). No growth hindering or yield reducing at harvest at all was observed with these July-sown replacement crops, including with the sensitive sugar beet crop.

After cereal winter or spring crops—i.e., respectively about 9 and 5 months after diflufenican soil treatment of the crops—the persistent soil residues of diflufenican and its metabolites which subsist in soil are so low that they could not hinder a following sensitive crop. Moreover, between the harvest of the cereal and the sowing of the next crop in the following autumn, there are 3 months more of summer fast diflufenican soil metabolism; during that period of time, the results obtained in this work suggest that the low soil residues (at cereal harvest) should be further reduced by a factor of 2. Moreover, these very low remaining soil residues are still diluted by a factor of 4 by the ploughing (in the 0–40-cm soil layer) which precedes the sowing of the next crop. On the other hand, the long soil persistence of diflufenican during the cereal crops corresponded to the very good control of weeds which was

observed when diflufenican was soil applied in mixture with isoproturon.

In the areas where diflufenican is used (by application just after cereal sowing in November), the triennial rotation is usually practiced: the sugar beet crop (sown in April) is grown during the first year; a wheat crop (sowing in November and harvest in July of the next year) is grown during the second year; a barley crop or another cereal crop (sowing in November and harvest in July) is made during the third year. Results obtained here indicate that, in the practiced crop rotation system, sugar beet cannot be hindered at all by the diflufenican soil treatment made at the beginning of the preceding cereal crop. If, however, the November-sown cereal crop should fail, the replacement crop sown in April of the next year should preferably not be sugar beet and the same if the negative effects (of the diflufenican and its metabolites persistent soil residues) onto the sugar beet crop are small.

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LITERATURE CITED

Britton, G.; Barry, P.; Young, A. J. The mode of action of diflufenican: its evaluation by HPLC. *Proc. Br. Crop. Prot. Conf.—Weeds* **1987**, *3*, 1015.

Cramp. M. C.; Gilmour, J.; Hatton, L. R.; Hewett, R. H.; Nolan, C. J.; Parnell, E. W. Diflufenican—A new selective herbicide. *Proc. Br. Crop. Prot. Conf.—Weeds* **1985**, *1*, 23.

Cramp, M. C.; Gilmour, J.; Hatton, L. R.; Hewett, R. H.; Nolan, C. J.; Parnell, E. W. Design and synthesis of N-(2,4-difluorophenyl)-2-(3-trifluoromethylphenoxy)-3-pyridinecarboxamide (diflufenican), a novel pre- and early postemergence herbicide for use in winter cereals. *Pestic. Sci.* **1987**, *18*, 15.

Deleu, R.; Copin, A.; Salembier, J. F.; Gomand, M. Relationship between the biological efficiency and the chemical behavior of diflufenican in the soil of cereal crops. Synthesis of two years of assays. *Med. Fac. Landbouww. Rijksuniv. Gent* **1987**, *52*, 653.

Draper, N. R.; Smith, H. *Applied regression analysis*, 2nd ed.; Wiley: New York, 1981; p 48.

Kyndt, C. F. A.; Turner, M. T. F.; Rognon, J. Diflufenican—A new herbicide for use in winter cereals. *Proc. Br. Crop Prot. Conf.—Weeds* **1985**, 1, 29.

Van Himme, M.; Bulcke, R. IRSIA–IWONL Weed Control Research Center Reports 48 (1986–1987), 182 pp, 50 (1987–1988), 172 pp, 52 (1988–1989), 163 pp; State University of Gent, Belgium.

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