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Restoration of the Slow Biodegradation of Isoxaben in the Soil of a Fruit Orchard After Interruption of Repeated Isoxaben Applications

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The accelerated biodegradation of the thiocarbamate herbicide EPTC in crop soils has been observed when EPTC was applied repeatedly in the past (Harvey et al., 1986). The thiocarbamate herbicide butylate also gives enhanced biodegradation in soil after repeated applications in the past (Harvey, 1987). The accelerated biodegradation was also observed with the carbamate carbetamide (Hole and Powles, 1997), the sulfonylurea chlorsulfuron (Ravelli et al., 1997), and the sulfonamide bentazon (Wagner et al., 1996). On the other hand, the α-chloro-acetanilides alachlor and metolachlor, and the triazine simazine did not show accelerated biodegradation after repeated applications in the past (Dowler et al., 1987; Kotoula-Syka et al., 1997; Walker and Welch, 1991). The chemical structure of the herbicide thus influences the occurrence of accelerated biodegradation, as not all the herbicides generate it. High herbicide doses and repeated applications over a long period are favorable for generating accelerated biodegradation. The accelerated biodegradation may reduce herbicide efficiency. On the other hand, it decreases the concern about the accumulation of herbicide in soil after repeated past applications.

In fruit orchards, the herbicides usually are applied at high doses, and the application of the same herbicide is frequently repeated annually during many years. The occurrence of the accelerated soil biodegradation is thus especially possible in fruit crops. After the annual applications of 0.5 kg isoxaben ha^{-1} repeated during 9 years in the past in a pear tree orchard, the soil biodegradation of isoxaben was highly accelerated (Rouchaud et al., 1997). The accelerated biodegradation of the ureas diuron and chlorotoluron, and of the amide propyzamide was observed in the soil of the same orchard where these treatments were applied annually and repeatedly since 11 years, each plot receiving always one and the same herbicide (Rouchaud et al., 2000). In the same trial, simazine generated only a weak accelerated soil biodegradation. On the other hand, after 3 years of annual and repeated applications of diflufenican in an orchard, diflufenican showed no enhanced biodegradation in soil.

Only few publications study the time during which it is necessary to interrupt the repeated applications of an herbicide, so that the accelerated soil biodegradation

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disappears, and the slow biodegradation of the herbicide in soil is restored. Harvey (1991) observed that the interruption during one year of the repeated annual applications of EPTC was sufficient to cancel its enhanced biodegradation in soil.

In the vineyards and the fruit orchards, the accelerated biodegradation of isoxaben in soil may considerably reduce the efficiency of its herbicide protection. In this work, we studied during what time it was necessary to interrupt the annual and repeated applications of isoxaben, in order to restore its slow and normal biodegration in soil. This was done in the pear tree orchard where we previously observed the accelerated biodegradation of isoxaben on account of repeated annual applications since 1987 (Rouchaud et al., 1997).

MATERIALS AND METHODS

A 22-year-old pear tree orchard with cv. *Doyenné du Comice* planted according to the single-row planting system (4 m x 1.75 m) at the Fruits Research Station of Gorsem, Belgium (clay 12%, silt 75%, sand 13%, loam soil, organic matter 3.1%, pH(H_2O) 6.5) was divided into plots (bands 30 m x 2 m, with the trees at the center). Since 13 years (since1987), each orchard plot was treated annually and without interruption at the beginning of April with one and always the same treatment, i.e. 0.5 kg isoxaben ha^{-1} (treatment 1). On some of these plots however, the repeated treatment with isoxaben was interrupted during the latest two years (1998 and 1999)(treatment 2); on some other of these plots, the repeated treatment with isoxaben was interrupted during the latest four years (1996, 1997, 1998 and 1999)(treatment 3). Moreover, there were control plots onto which isoxaben was never applied in the past (treatment 4) (Table 1). On March 23, 2000, all the plots were treated with 0.5 kg isoxaben ha^{-1} by spraying the emulsion in water (300 L/ha) of the formulation AZ 500 (50 wt% isoxaben, Dow AgroSciences). For each treatment, there were four replicate plots. At several delays after the application of isoxaben, soil samples were taken separately (and analyzed separately) in the 0-8 cm surface soil layer in each of the four replicate plots of each of the treatments (Table 1). Soil samples were also taken in the 8-15 and 15-20 cm surface soil layers, with samples at each depth from two replicate field plots being mixed to give duplicate samples for analysis, for each treatment and depth. Before the application of isoxaben made on March 23, 2000, on March 10, 2000, soil samples were also taken in the plots of the four treatments and at each of the depths. 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 analysis of isoxaben was made according to a previously described procedure which was slightly modified (Rouchaud et al., 1993). Soil (100 g) was extracted by heating to reflux for 15 min in the mixture acetone/water 8/2 vol./vol. (200 ml). The mixture was filtered and the extraction repeated. The filtrates were combined, water (100 ml) was added, and the acetone was removed in a vacuum rotary evaporator (30°C). NaCl (15 g) was added to the aqueous solution, which was

Table 1. Dissipation of isoxaben in the 0-8 cm surface soil layer in the pear tree orchard after application on all plots of 0.5 kg isoxaben ha^{-1} on March 23, 2000.

Sampling date, day-month in 2000	Days after isoxaben application in 2000	Cumulative rainfall, mm	Isoxaben applications made repeatedly in the past			
			Control: no application in the past (treatment 4)	Isoxaben applied annually and repeatedly since 1987		
				Without interruption (treatment 1)	With two years interruption in 1998 and 1999 (treatment 2)	With four years interruption in 1996, 1997, 1998 and 1999 (treatment 3)
			Isoxaben soil concentration (µg kg^{-1} dry soil) in the 0-8 cm surface soil layer; means of 4 replicates ± SD (± 95% confidence intervals)[a]			
24-3	1	3	442±22	486±24	462±23	481±24
11-4	19	25	421±21	315±16	355±18	379±19
17-5	55	83	307±15	185±9	277±14	281±14
7-6	76	154	282±14	159±8	196±10	254±13
26-6	95	168	216±11	86±4	178±9	207±10
19-7	118	273	212±11	63±3	111±6	188±9
14-8	144	416	161±8	42±2	100±5	142±7
11-9	172	477	83±4	37±2	55±3	72±4
Corr. coeff.[b]			-0.9812	-0.9921	-0.9858	-0.9878
Y intercept[b]			6.13	6.15	6.13	6.13
Slope, $days^{-1}$ [b]			-7.0994 10^{-3}	-1.6815 10^{-2}	-1.0909 10^{-2}	-8.0426 10^{-3}
Isoxaben soil half-life, days, ±95% confidence intervals[b]			97.6±4.9	41.2±2.1	63.5±3.2	86.2±4.3

[a] Isoxaben was not detected in the 0-8, 8-15 and 15-20 cm surface soil layers in the plots of the four treatments on March 10, 2000. After the application of isoxaben on March 23, 2000 and until September 11, 2000, isoxaben was not detected in the 8-15 and 15-20 cm surface soil layers.

[b] For the 144 days period following the isoxaben treatment made on March 23, 2000.

then partitioned two times against dichloromethane (2 x 200 ml). The dichloromethane solutions were gathered, dried (Na_2SO_4), concentrated successively to 40 and 15 ml in a vacuum rotary evaporator (at 30 and 20°C, and in a 1 L and 50 ml flasks, respectively), and then concentrated to 0.5 ml under a

stream of nitrogen (20°C). The concentrate was applied as a band on a TLC plate (thin-layer chromatography plate 20 x 20 cm, 0.2 mm-thick silica gel 60F254; Merck), along with the isoxaben analysis standard next to the band of the sample solution and as a spot in a separate lane. Elution was made with dichloromethane/hexane 3/1 vol./vol. Complete elution with this solvent system was made three times, after each elution the TLC plate was air dried at room temperature for about 1 min, and then again placed into the same TLC elution tank for a new elution; after the third elution, the isoxaben band was at $R_f = 0.18$. The band next to the isoxaben standard (the standard being visualized by fluorescence) was scraped off, the silica gel extracted with ethyl acetate (40 ml), the extract concentrated to 0.5 ml under a stream of nitrogen (20°C) and applied onto a second TLC plate. Elution was made with diethyl ether/hexane 1/1.5 vol./vol. After the first elution, the plate was air dried and eluted a second time with the same solvent system; after the second elution, the isoxaben band was at $R_f = 0.48$. The band was scraped off, the silica gel extracted with ethyl acetate (40 ml), the extract was concentrated, and analyzed by GC (gas chromatography). Several samples were analyzed further by GC-MS (GC combined with mass spectrometry) for confirmation.

The GC conditions were the following. Detection was by flame ionization. Inlet and detector were set at 250°C. The glass column used was 1.8 m x 2 mm internal diameter, and contained 5% SE30 on 80-100 mesh Chromosorb W-HP. Nitrogen was the carrier gas at 50 ml min^{-1}. With the column oven at 170°C, the isoxaben retention time was 3.6 min.

Frequently, the isoxaben extracted from soil was further analyzed by GC-MS (VG AutoSpec mass spectrometer; Fisons GC 8065) with a 15 m capillary column, 0.45 mm i.d., containing SE 54 at 1.0 µm film thickness; column oven temperature program was 50°C (3 min) increasing to 250°C at 20°C min^{-1}. Electron impact ionization (EI) was at 30 eV, and the source temperature was 200°C. The MS spectrum of isoxaben was (m/e, relative abundance, %): 332 (M^+, 37), 303 (M-CH_2CH_3, 31), 222 (M-$CNC(CH_3)(CH_2CH_3)_2$ + H, 87); 166 ($C_6H_3(OCH_3)_2CHO$, 100); 151 (166-CH_3, 43).

At the levels of 20, 5 and 2 (analytical limit of sensitivity) µg isoxaben kg^{-1} dry soil, recoveries (from isoxaben untreated soil sampled in the orchard) were 82-96, 78-89 et 71-83%, respectively (4 replicates at each level).

For the 4.8 months period which followed the application of isoxaben, the linear regression $\ln y = kt + b$ was applied between the naperian logarithms of the isoxaben soil concentrations (y = µg isoxaben kg^{-1} dry soil) in the 0-8 cm surface soil layer and the time t (days) following isoxaben treatment. The isoxaben soil half-lives with their 95% confidence intervals were obtained using the SAS logicial 6.12 (Jan 2, 1997)(SAS Institute Inc., Cary, NC 27513). The preparation of the isoxaben analysis standard from the commercial formulation has been described (Rouchaud et al., 1993).

RESULTS AND DISCUSSION

No isoxaben was detected in the soil samples taken on March 10, 2000, in each of the replicate plots of each of the four treatments and at each depth, some days before the application of isoxaben on March 23, 2000. The treatments with isoxaben made in the past thus did not generate the accumulation of isoxaben residues in soil. The enhanced biodegradation of isoxaben in soil after repeated applications in the past contributed to the complete disparition of isoxaben. The heavy rains and the soft temperatures during the autumn and the beginning of the spring generated the high soil microbial activity and the complete dissipation of isoxaben.

During the trial, since the application of isoxaben on March 23, 2000 until the mid of September 2000, isoxaben was not detected in the 8-15 and 15-20 cm surface soil layers. This indicated that isoxaben was strongly adsorbed onto the soil and its organic matter; isoxaben remained in the 0-8 cm surface soil layer where it was progressively metabolized and mineralized (Rouchaud et al., 1993).

The dissipation of isoxaben in the 0-8 cm surface soil layer followed a first order kinetics. In the control plots -which were not treated with isoxaben in the past-, the isoxaben soil half-life was 98 days (treatment 4, Table 1). In the plots treated annually and repeatedly without interruption with isoxaben since 1987 (since 13 years; treatment 1), the isoxaben soil half-life was 41 days, i.e. 57 days less than the soil half-life in the control plots of treatment 4. The accelerated biodegration observed here had an intensity similar to the one observed previously (Rouchaud et al., 1997).

On some of the plots treated annually and repeatedly with isoxaben since 1987, the treatment was interrupted during the latest four years (1996, 1997, 1998 and 1999; treatment 3); the soil half-life of isoxaben in these plots was 86 days, i.e. 12 days less than with the control treatment 4. The interruption of the repeated treatments during the latest 4 years thus restored for 79% (1 - 12/57) x 100) the normal slow biodegradation of isoxaben in soil. On some of the plots treated annually and repeatedly with isoxaben since 1987, the treatment was interrupted during the latest two years (1998 and 1999; treatment 2); the soil half-life of isoxaben in these plots was 64 days, i.e. 34 days less than with the control treatment 4. The slow and normal biodegradation of isoxaben in these plots was restored for only 40% (1 - 34/57) x 100).

The restoration of the normal biodegradation of isoxaben in soil thus was slow. She was however faster than the installation of the enhanced biodegradation. Indeed, in the same orchard, some plots were treated annually in the past with isoxaben only during two years; no significant enhanced biodegradation was observed for the isoxaben applied on these plots one year later (unpublished results). The installation and the disappearance of the enhanced biodegradation thus was slower for isoxaben than with the thiocarbamate EPTC (Harvey, 1991).

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REFERENCES

Dowler CC, Marti LR, Kvien CS, Skipper HD, Gooden DT, Zublena JP (1987) Accelerated degradation potential of selected herbicides in the southeastern United States. Weed Technol 1: 350-358.

Harvey RG, McNevin GR, Albright JW, Kozak ME (1986) Wild proso millet control with thiocarbamate herbicides on previously treated soils. Weed Sci 34: 773-780

Harvey RG (1987) Herbicide dissipation from soils with different herbicide use histories. Weed Sci 35: 583-589

Harvey RG (1991) Effect of herbicide rotations on enhanced herbicide biodegradation and wild-proso millet control. Weed Sci 39: 607-613

Hole SJW, Powles SB (1997) Reduced efficacy and enhanced degradation of carbetamide after repeated application in Australia. Weed Res. 37: 165-170

Kotoula-Syka E, Hatzios KK, Berry DF, Wilson HP (1997) Degradation of acetanilide herbicides in history and nonhistory soils from eastern Virginia. Weed Technol 11: 403-409

Ravelli A, Pantani O, Calamai L, Fusi P (1997) Rates of chlorsulfuron degradation in three Brazilian oxisols. Weed Res. 37: 51-59

Rouchaud J, Gustin F, Callens D, Van Himme M, Bulcke R (1993) Soil metabolism of the herbicide isoxaben in winter wheat crops. J Agr Food Chem 41: 2142-2148

Rouchaud J, Neus O, Bulcke R, Callens D, Dekkers T (1997) Isoxaben soil biodegradation in pear tree orchard after repeated high dose application. Arch Environ Contam Toxicol 33: 247-251

Rouchaud J, Neus O, Bulcke R, Eelen H, Dekkers T (2000) Soil dissipation of diuron, chlorotoluron, simazine, propyzamide, and diflufenican herbicides after repeated applications in fruit tree orchards. Arch Environ Contam Toxicol 39: 60-65

Wagner SC, Zablotowicz RM, Gaston LA, Locke MA, Kinsella J (1996) Bentazon degradation in soil: influence of tillage and history of bentazon application. J Agr Food Chem 44: 1593-1598

Walker A, Welch SJ (1991) Enhanced degradation of some soil-applied herbicide Weed Res 31: 49-57