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Soil Biodegradation and Leaf Transfer of Insecticide Imidacloprid Applied in Seed Dressing in Sugar Beet Crops

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Imidacloprid (Gaucho; 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine) is a new systemic insecticide used efficiently in pelleted seed dressing against soil insects (Atomaria lineatus, Blaniulus guttulatus, Agriotes lineatus, Pegomya hyoscyami) and leaf insects (aphids Myzus persicae and Aphis fabae) in sugar beet (Elbert et al. 1990; Schmeer et al., 1990). In that way it gives a long lasting control of virus yellows. Imidacloprid acts on cholinergic receptors of an identified insect motor neurone (Bai et al., 1991). At our knowledge, nothing so far has been published about imidacloprid soil biodegradation in sugar beet crop, and about its transfer in the sugar beet leaf. This is the purpose of the present study. The influence of the recent organic fertilizer treatments and of the old soil humus on that soil biodegradation is simultaneously studied.

MATERIALS AND METHODS

A sugar beet trial was made in 1992 at Lubbeek (clay 10%, silt 65%, sand 25%, silt loam, pH(KCl) 7.00, organic matter 2.16%), Belgium. On 2-3-1992, each plot of the field was treated with one of the organic fertilizers cow manure (40 tons ha^{-1}), or pig slurry (40 tons ha^{-1}), or with the mixture of both (20+20 tons ha^{-1}). Moreover, there were control plots not treated with organic fertilizers. The field was tilled at 20-25 cm depth. Sugar beet (cv. Victoria G) was sown on 8-4-1992. Imidacloprid was applied in seed dressing at the rate of 90 g ha^{-1} (Kleinwanzlebener Saatzucht, Einbeck, Germany). There were 4 replicate plots for each organic fertilizer treatment. At intervals during the trial (Table 1), samples were taken separately (and analyzed once separately) in the soil half-sphere 8 cm diameter around the sugar beet root or, after 31-5-1992, in the 4 cm-thick and at 0-10 cm depth soil envelope around the sugar beet root. This was done in each of the four replicate plots, of each of the three organic fertilizer treatments and control. In addition, on 18-5-1992 samples were taken separately in each of the four replicate (and analyzed once separately) from the 10-20 cm soil layer (8 cm diam) under the young sugar beet plant. The same was done on

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Table 1. Soil concentrations of imidacloprid in the organic fertilizers trial in sugar beet made in 1992 at Lubbeek.

Sampling date[a]	Days after sowing	Cumulative rainfall, mm	Organic fertilizer (OF) treatment			
			Control (no OF)	Cow manure	Pig slurry	Cow manure+pig slurry
			mg imidacloprid kg^{-1} dry soil[b]			
8-4	0	0	7.2±0.4	7.2±0.4	7.2±0.4	7.2±0.4
21-4	13	23	5.7±0.3	6.9±0.3	6.4±0.3	6.3±0.3
2-5	24	63	5.0±0.3	6.9±0.3	6.2±0.3	6.3±0.3
18-5	40	79	3.6±0.2	5.9±0.3	5.6±0.3	6.0±0.3
31-5	53	96	2.4±0.1	5.8±0.3	5.4±0.3	5.2±0.3
14-6	67	157	0.2±0.1	0.9±0.1	0.5±0.1	0.6±0.1
29-6	82	183	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1
30-7	113	251	nd	nd	nd	nd
Corr. coeff.[c]			-0.9906	-0.8873	-0.9184	-0.8933
Y intercept[c]			7.09	7.31	7.03	7.05
Slope, mg kg^{-1} $days^{-1}$ [c]			-0.08839	-0.02940	-0.03340	-0.03267
Soil half-lives, days[c]			40±2	124±6	105±5	108±5

a. Sampling date, day-month, year 1992.
b. Soil concentrations of imidacloprid (mg imidacloprid kg^{-1} dry soil) in the soil half-sphere 8 cm diameter around the sugar beet root or, after 31-5-1992, in the 4 cm-thick and at 0-10 cm depth soil envelope around the sugar beet root. Means of 4 replicates ± s.d. nd=Not detected.
c. For the first 53 days crop period, linear regression y=kt+b of the imidacloprid soil concentrations (y=mg kg^{-1} dry soil) in the soil half-sphere 8 cm diameter around the sugar beet root, against time t (days) following sowing: correlation coefficient, y intercept, and slope. The imidacloprid soil half-lives with their 95% confidence intervals were obtained using the SAS logical CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC 27512).

30-7-1992 in the 4 cm-thick soil envelope around the sugar beet root at a 10-20 cm soil depth. For each soil sample, the soil corresponding to 15 sugar beets was taken from each replicate plot at random points; the soil from each replicate plot was bulked together and then stored at -25°C until analyzed. The same was done with the sugar beet foliage.

A sugar beet crop trial was made in 1992 at Remicourt (clay 12%, silt 79%, sand 9%, silt), Belgium, in the same way as at Lubbeek,

Table 2. Soil concentrations of imidacloprid in the sugar beet crop made in 1992 at Remicourt on a field divided in two parts.

Sampling date[a]	Days after sowing	Cumulative rainfall, mm	Organic fertilizer (OF) treatment	
			No OF treatment; high soil OM concentration	Cow manure each 3 year; low soil OM concentration
			mg imidacloprid kg^{-1} dry soil	
11-4	0	0	7.2±0.4	7.2±0.4
24-4	13	25	5.8±0.3	7.0±0.4
2-5	21	59	5.4±0.3	6.4±0.3
18-5	37	82	4.3±0.2	5.7±0.3
31-5	50	98	2.9±0.1	5.2±0.3
14-6	64	184	0.2±0.1	0.4±0.1
28-6	78	251	0.1±0.1	0.1±0.1
13-7	93	301	nd	nd
Corr. coeff.[c]			-0.9840	-0.9437
Y intercept[c]			7.10	7.35
Slope, mg kg^{-1} $days^{-1}$ [c]			-0.08165	-0.04313
Soil half-lives, days[c]			44±2	85±4

a,b,c: As in Table 1. OM=soil organic matter.

except the following. The field was divided in two parts. In the first one, the soil organic matter concentration was of a usual level (2.4%); for 18 years, cow manure (40 tons ha^{-1}) was applied every 3 years in the autumn preceding the sowing in April of the sugar beet crop, which is the first crop of the crop rotation cycle; the latest cow manure treatment thus had been made in November 1991, i.e. 6 months before sugar beet sowing. The second part of the field had a high soil organic matter concentration (4.3%); it corresponded to a meadow which had been ploughed 18 years ago; no organic fertilizer at all had been applied since then. In both field parts, the soil pH(KCl) was 6.9. Sugar beet with imidacloprid in pelleted seed dressing was sown on 11-4-1992 (Table 2). Additional soil samples in the 10-20 cm soil layer were taken on 31-5 and 13-7-1992.

Thin-layer chromatography (TLC) was carried out with silicagel 60F254 20x20 cm, 0.2 mm thick plates. The sample solution was applied as a band; imidacloprid standard was applied on another part of the TLC plate. Imidacloprid extracted from soil and plant was analyzed by gas-liquid chromatography (GLC) after trifluoroacetylation. Trifluoroacetic anhydride (2 ml; Janssen Chimica, Belgium) was added to the soil or plant TLC cleaned-up final extract in ethylacetate (1 ml). The mixture was carefully heated to boiling until concentrated to 0.1 ml (~5 min), ethylacetate was added, and the mixture analyzed by GLC. Electron capture detection. Injection and detection at 225°C. Glass column 1.80 m x 2 mm i.d., 5% SE30 on Gas Chrom Q 80-100 mesh, nitrogen as carrier gas at 40 ml min^{-1}. With column oven at 225°C, retention times for both isomers of trifluoroacetylated imidacloprid were 2.1 and 5.3 min; the surfaces of both peaks were added. Frequently imidacloprid extracted from soil and plant was further analyzed by mass spectrometry (MS) by direct introduction in the MS apparatus of the non-trifluoroacetylated imidacloprid. Infrared (IR) spectra were recorded with KBr disks (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were recorded in $CDCl_3$ at 200 MHz with tetramethylsilane as internal standard: δ, ppm. MS spectra were recorded at 70 eV in the electron impact mode; m/e, relative abundance, %.

For synthesis of imidacloprid standard for analysis, sugar beet pelleted seeds (175 g) were ground in a mortar to a fine powder, extracted four times (20°C, stirring) with acetone (4x750 ml), filtered, the gathered filtrates were filtered again, acetone was evaporated in vacuo, the solid residue was recrystallized in dichloromethane+hexane 1+1 ml/ml, giving imidacloprid (4.71 g, 91%) whose purity was greater than 99.5% as shown by TLC and GLC. IR: 3372(NH), 3052, 2895, 1570,1451, 1393, 1289, 1235, 1148, 1107, 1051, 1026, 943, 812, 783, 718. ^1H-NMR: 3.46, 3.51, 3.56, (t, 2H, NCH_2CH_2N); 3.76, 3.81, 3.85(t, 2H, NCH_2CH_2N); 4.53(s, 2H, Pyr$\underline{CH_2}$N); 7.27, 7.32, 7.36(m, 1H, pyridine H); 7.66, 7.68, 7.70, 7.72 (m, 1H, pyridine H); 8.21(br, 1H, NH); 8.31(s, 1H, pyridine H). MS: 255(M+, 6); 257(255+2, 2); 239(M-O, 2.4); 241(239+2, 0.8); 209(M-NO_2, 100); 211(209+2, 33); 194(M-NNO_2-H, 5); 196(194+2, 2); 180(209-$(CH_2)_2$-H, 7); 182(180+2, 2); 173(209-HCl, 52); 126($C_5H_3NClCH_2$, 45); 128(126+2, 15).

For field soil samples analyses, in the air-dried soil, complete or pieces of seed pellets were taken off, and analyzed separately in the same way as soil; they were observed during the 50 days period following sowing, and corresponded to less than 20% of the total imidacloprid soil residue; their imidacloprid contents were added to the ones of soil. The dry soil then was finely ground and homogenized in a Krups robot omnimixer. Soil (100 g) was refluxed for 10 min with stirring in 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 removed in a vacuum rotary evaporator (30°C). NaCl (15 g) was added to the aqueous solution, which was then extracted with dichloromethane (200+150 ml). The dichloromethane solution was dried (Na_2SO_4), concentrated to 40 ml in a vacuum rotary evaporator (30°C), and then concentrated further to 0.5 ml under a slow stream of nitrogen (20°C). The concentrate was applied to a TLC plate. Elution with ethylacetate gave imidacloprid at Rf=0.42. The band was scraped off, the silicagel extracted with ethylacetate (40 ml), and the extract was concentrated to 0.5 ml under a slow stream of nitrogen (20°C). When the clean-up was insufficient, the TLC was repeated one or two times. In several cases, the extract was analyzed by MS for imidacloprid as such. The final extract then was trifluoroacetylated and analyzed by GLC. At the 0.1 mg imidacloprid kg^{-1} level in soil, the recovery was 83-96%. The analytical limit of sensitivity for imidacloprid was 0.01 mg kg^{-1} dry soil. Sugar beet foliage and root were analyzed in the same way as soil. At the 0.1 mg kg-1 fresh weight level, the recoveries of imidacloprid were similar to the ones in the soil. The limit of detection of imidacloprid in the sugar beet leaves and roots was 0.01 mg kg-1 fresh weight.

RESULTS AND DISCUSSION

In all the organic fertilizers treated and untreated control plots in the trial at Lubbeek, and in both field parts in the trial at Remicourt, no imidacloprid was detected in the 10-20 cm soil layer under and around sugar beet. There was thus no apparent leaching of this compound in soil. No residues of imidacloprid were detected in the roots and the leaves of sugar beet at harvest.

In all field plots of both trials at Lubbeek and Remicourt, during the first two months of the sugar beet crops there was a negative linear relationship between the imidacloprid soil concentrations and the time elapsed since sowing (Tables 1 and 2). In the trial at Lubbeek, the organic fertilizers had been applied one month before sowing. Imidacloprid soil half-lives in the organic fertilizers untreated control plots, and in the cow manure, pig slurry and cow manure+pig slurry treated plots were 40, 124, 105 and 108 days, respectively (Table 1).

In the trial at Remicourt, in the field part not treated with cow manure, but containing high concentrations of old humus, imidacloprid soil half-life was 44 days (Table 2). In the field part recently treated with cow manure, it was 85 days.

After the first two months crop period, the imidacloprid soil concentrations diminished at increased rates, leaving no soil residue at sugar beet harvest.

The recent organic fertilizer treatments thus decreased -relative to the organic fertilizers untreated control plots- the rate of imidacloprid soil biodegradation, same if the insecticide was applied in seed dressing. The pelleted seed dressing probably acts according to a slow release process. Probably in the same way, the young soil organic matter -from the recent organic fertilizer treatments- decreased the rate of imidacloprid

Table 3. Concentration of imidacloprid (mg kg^{-1} fresh weight) in the sugar beet foliage of the trial made at Lubbeek in 1992.

Days after sowing	Foliage fresh weight $plant^{-1}$, g	Organic fertilizer (OF) treatment			
		Control (no OF)	Cow manure	Pig slurry	Cow manure +pig slurry
		mg imidacloprid kg^{-1} foliage fresh weight[a]			
40	2.4	3.6±0.1	5.7±0.2	4.6±0.2	4.1±0.2
53	21	1.5±0.1	2.1±0.1	1.9±0.1	1.8±0.1
67	162	0.9±0.1	1.3±0.1	1.2±0.1	1.2±0.1
82	900	0.8±0.1	1.5±0.1	1.3±0.1	1.4±0.1
113	1400	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1

a. Means of 4 replicates ± s.d. On 31-8-1992, no more imidacloprid was detected in the sugar beet foliage.

Table 4. Concentration of imidacloprid (mg kg^{-1} fresh weight) in the sugar beet foliage of the trial made at Remicourt in 1992.

Days after sowing	Foliage fresh weight $plant^{-1}$, g	Organic fertilizer (OF) treatment	
		No OF treatment; high soil OM concentration	Cow manure each 3 year; low soil OM concentration
		mg imidacloprid kg^{-1} foliage fresh weight[a]	
37	0.6	10.8±0.5	12.4±0.6
50	14	4.1±0.2	5.6±0.3
64	112	1.5±0.1	2.3±0.1
78	750	0.7±0.1	1.2±0.1
93	1400	0.2±0.1	0.2±0.1

a. As in Table 3.

soil biodegradation during the first crop period (which is the most important for the plant protection against insects). Both effects should add to decrease the rate of imidacloprid soil biodegradation, and probably to increase the efficiency of the insecticide protection. The effect of the recent organic fertilizer treatments could be due to the increase -relative to the organic fertilizers untreated control plots- of imidacloprid adsorption on the young soil organic matter (Rouchaud et al., 1991). This should protect in some way imidacloprid against the soil microbial activity which metabolizes it. On the other hand, in the trial at Remicourt, the old humus -in spite of its high soil concentration- had no significant effect on imidacloprid soil biodegradation. Differences between the young and old soil organic matters -as to their effects on imidacloprid soil biodegradation and insecticide adsorption power- should be due to the differences as to their chemical structures (Honnay, 1991).

High imidacloprid concentrations were observed in the sugar beet leaves, especially of the young plants (Tables 3 and 4). They should be due to the application technique in the seed dressing, which gives a high proximity between the insecticide and the young plant. They were also due to the high systemicity of imidacloprid. To these great imidacloprid sugar beet leaf concentrations correspond the very efficient protection given against aphids (Elbert et al., 1990; Schmeer et al., 1990; Wauters, 1993). With plant growth (which dilutes the leaf residue) and imidacloprid metabolism in the leaf, the imidacloprid concentration in sugar beet leaf progressively decreased when the time after sowing increased. That leaf imidacloprid concentration however was equal or greater than 1 mg kg^{-1} fresh weight during a period as long as 80 days after sowing. The imidacloprid leaf concentrations were greater in the plots recently treated with organic fertilizers. The soil of these plots indeed contained higher concentrations of imidacloprid, which should generate a greater imidacloprid transfer into the leaves.

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