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Imidacloprid Insecticide Soil Metabolism in Sugar Beet Field Crops

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Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine, 1) is a new insecticide which is useful for the protection of sugar beet from soil insects (Atomaria, Blaniulus, Agriotes and Pegomya) and leaf insects (aphids). It is applied in pelleted seed dressing. It is a systemic compound which is absorbed by the plant. In the leaves, it gives a long lasting (3 to 4 months) protection against aphids and the virus yellows infections. The metabolism of ^{14}C-imidacloprid was investigated in plant cell suspension cultures (Koester 1992). The main metabolites corresponded to imidacloprid hydroxylation (l-[(6-chloro--3-pyridinyl)methyl]-5-hydroxy-4,5-dihydro-N-nitro-lH-imidazol--2-amine) or dehydrogenation (1-[(6-chloro-3-pyridinyl)methyl] -N-nitro-lH-imidazol-2-amine) of the imidazolidine ring, and to 6-chloronicotinic acid. The nitroso derivative of imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitroso-1H-imidazol-2-amine) corresponded to only 0.1% of the total recovered ^{14}C. The imidacloprid soil metabolism has been studied by incubation in laboratory conditions (Scholz and Spiteller 1992). The metabolites observed in plant cell cultures did not accumulate in soil; their total amount was less than 4% of the total recovered radioactivity during ^{14}C-imidacloprid soil biodegradation. $^{14}CO_2$ was the main product of ^{14}C-imidacloprid soil biodegradation. We previously studied the disappearance of imidacloprid in the soil of a sugar beet, organic fertilizers trial made at Lubbeek in 1992 (Rouchaud et al. 1994). Also, in a sugar beet trial located at Remicourt, one part of the field had not been treated with organic fertilizers for 18 years, but its soil contained a high concentration (4.3%) of soil organic matter. Another part of the field had been treated every 3 years with cow manure for 18 years, but its soil contained a normal concentration (2.4%) of organic matter. In the present work, we studied the imidacloprid soil metabolism in a new organic fertilizers trial made on another field at Lubbeek in 1993. We also searched for the imidacloprid soil metabolites in the trial made in 1992 at Remicourt.

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MATERIALS AND METHODS

The organic fertilizer, sugar beet trial made in 1992 at Lubbeek, Belgium, was repeated in 1993 on another field (clay ll%, silt 63%, sand 26%, silt loam, pH(KC1) 6.81). This field, relative to the field used in 1992, had a greater soil concentration of organic matter (4.4%) as it was a meadow which had been ploughed 4 years earlier. Two kinds of organic fertilizer treatments were assayed; on 4 March 1993, each plot of the field was treated either with cow manure (50 tons ha^{-l}) or with pig slurry (50 tons ha-l). Moreover, there were control plots that were not treated with organic fertilizers. The field was tilled to a 20-25 cm depth. Sugar beet (cv. Victoria G) was sown on 1 April 1993. Imidacloprid was applied in the seed dressing at the rate of 90 g ha^{-l}. (Kleinwanzlebener Saatzucht, Einbeck, Germany). There were 4 replicate plots for each organic fertilizer treatment and a control plot. At intervals during the trial (Table 1), samples were taken separately (and analyzed separately) in the 8-cm diameter half-sphere of soil around the sugar beet root or, after 24 May 1993, in the 4 cm-thick and at 0-10 cm depth soil envelope around the cone of the root of the sugar beet. This was done in each of the four replicate plots, of each of the two organic fertilizer treatments and control. In addition, on 24 May 1993 samples were taken separately in each of the 4 replicates (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 5 July 1993 in the 4 cm-thick soil envelope around the cone of the root of the sugar beet 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 composited and then stored at -25°C until analyzed. The same was done with the foliage and roots of the sugar beets.

In the present work, we also analyzed for the imidacloprid soil metabolites in the sugar beet trial made at Remicourt in 1992; we previously analyzed for imidacloprid (Rouchaud et al. 1994). This sugar beet trial at Remicourt (clay 12%, silt 79%, sand 9%, silt) had been made in the same way as at Lubbeek, except the following. The field was divided in two parts. In the first one, the soil concentration of organic matter was of a usual level (2.4%); for 18 years, cow manure (40 tons ha-l) 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(KC1) was 6.9. Sugar beet with imidacloprid in pelleted seed dressing was sown on 11 April 1992 (Table 2). Additional soils samples in the 10-20 cm soil layer were taken on 31 May and 13 July 1992.

Table 1. Imidacloprid (1) and its metabolites 2 to 5 soil concentrations in the sugar beet trial made in 1993 at Lubbeek.

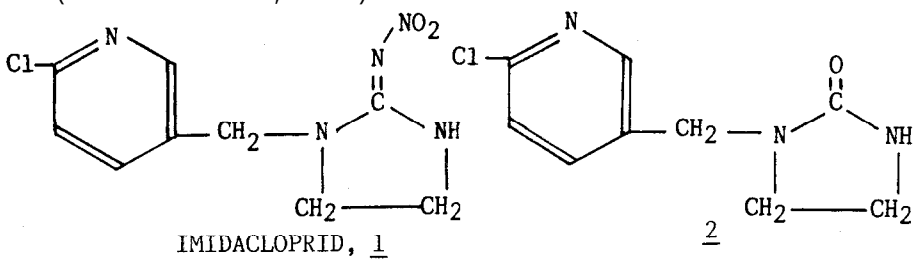
Days after sowing[a]	Imidacloprid (1) and its metabolites (as equivalents of imidacloprid) concentrations (mg kg^{-1} dry soil)[b]				
	1	2	3	4	5
1. Control (no organic fertilizer treatment):					
0	7.2±0.4	nd	nd	nd	nd
14	6.3±0.3	0.1	0.1	0.1	nd
27	4.9±0.3	0.4	0.2	0.4	0.1
39	3.7±0.2	0.5	0.5	0.6	0.1
54	2.7±0.1	0.7	0.4	0.8	0.1
68	1.7±0.1	0.2	0.3	0.3	0.1
81	0.1±0.1	0.1	0.1	0.2	nd
96	nd	nd	nd	0.1	nd
Corr. coeff.c −0.9893; 1 soil half-life: 42±2 days					
2. Cow manure (50 tons ha^{-1}):					
0	7.2±0.4	nd	nd	nd	nd
14	6.7±0.3	0.1	0.1	0.1	nd
27	6.5±0.3	0.1	0.1	0.1	nd
39	6.2±0.3	0.1	0.1	0.2	0.1
54	5.6±0.3	0.2	0.2	0.3	0.1
68	1.1±0.1	0.5	0.8	0.9	0.1
81	0.2±0.1	0.1	0.2	0.3	nd
96	nd	nd	nd	0.1	nd
Corr. coeff.c −0.9090; 1 soil half-life: 129±6 days					
3. Pig slurry (50 tons ha^{-1}):					
0	7.2±0.4	nd	nd	nd	nd
14	6.9±0.3	nd	nd	nd	nd
27	6.1±0.3	0.2	0.1	0.2	nd
39	5.3±0.3	0.2	0.2	0.6	0.1
54	5.1±0.3	0.2	0.1	0.5	0.1
68	1.4±0.1	0.4	0.5	0.9	0.1
81	0.2±0.1	0.1	0.1	0.3	nd
96	nd	nd	nd	0.1	nd
Corr. coeff.c −0.9294; 1 soil half-life: 87±4 days					

a. Sampling dates (day-month, 1993), number of days after sowing, and cumulative rainfall (mm) respectively were: l-4, 0, 0; 14-4, 14, 33; 27-4, 27, 52; 9-5, 39, 54; 24-5, 54, 79; 7-6, 68, 120; 20-6, 81, 169; 5-7, 96, 173. b. Soil concentrations of compounds 1 to 5 in the soil half-sphere 8 cm diameter around the sugar beet root or, after 24-5-1993, in the 4 cm-thick and at O-10 cm depth soil envelope around the cone of the root of the sugar beet. Means of 4 replicates; ± s.d. for imidacloprid; s.d. for metabolites 2 to 5 were ± 0.1, nd=Not detected. c. For the first 54 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 around the sugar beet root, against time t (days) following sowing: correlation coefficient. Imidacloprid soil half-lives with their 95% confidence intervals obtained using the SAS logical CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC 27512).

Table 2. Imidacloprid (1) and its metabolites 2 to 5 soil concentrations in the sugar beet crop made in 1992 at Remicourt.

Days after sowing[a]	Imidacloprid (1) and its metabolites (as equivalents of imidacloprid) concentrations (mg kg-1 dry soil)[b]				
	1	2	3	4	5
1. No organic fertilizer treatment: high old soil organic matter concentration:					
0	7.2	nd	nd	nd	nd
13	5.8	0.2	0.4	0.3	nd
21	5.4	0.1	0.4	0.4	0.1
37	4.3	0.3	0.3	0.7	0.1
50	2.9	0.5	0.6	0.9	0.1
64	0.2	0.9	0.9	0.9	0.1
78	0.1	0.3	0.1	0.4	nd
93	nd	nd	nd	0.1	nd
1 Soil half-life: 44±2 days					
2. Cow manure each 3 year; low recent soil organic matter concentration:					
0	7.2	nd	nd	nd	nd
13	7.0	nd	nd	nd	nd
21	6.4	0.1	0.1	0.2	0.1
37	5.7	0.2	0.2	0.4	0.1
50	5.2	0.3	0.3	0.3	0.1
64	0.4	1.0	1.0	1.2	0.1
78	0.1	0.4	0.2	0.4	0.1
93	nd	nd	nd	0.1	nd
1. Soil half-life: 85±4 days					

a,b: As in Table 1, except the following. Sampling dates (day-month, 1992), number of days after sowing, and cumulative rainfall (mm) respectively were: 11-4, 0, 0; 24-4, 13, 25; 2-5, 21, 59; 18-5, 37, 82; 31-5, 50, 98; 14-6, 64, 184; 28-6, 78, 251; 13-7, 93, 301. The imidacloprid soil concentrations were already reported (Rouchaud et al., 1994).



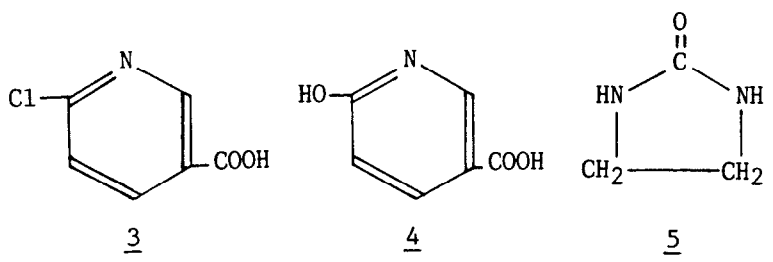


Figure 1. Imidacloprid (1) and its soil metabolites 2 to 5.

Thin-layer chromatography (TLC) was done using silicagel plates 20x20 cm, 0.2-mm thick. The sample solution was applied as a band, and the standard was applied next to this band, on another part of the TLC plate. Imidacloprid (1) and compounds 2 and 5 were analyzed by gas-liquid chromatography (GLC) after trifluoroacetylation; compounds 3 and 4, after methylation with diazomethane. GLC conditions for compounds 1 to 5: inlet and detector at 225°C, 1.80 m x 2 mm i.d. glass column packed with 80-100 mesh Gas-Chrom Q, nitrogen as carrier gas at 40 mL min^{-1}. For imidacloprid (1) and compounds 2, 3 and 5, detection by electron capture; for compound 4, detection by flame ionization. Column oven temperature and retention times were: trifluoroacetylated imidacloprid, 225° C, 2.1 and 5.3 min (two isomers); trifluoroacetylated compound 2, 225°C, 2.6 min; methylated compound 3, 120°C, 2.6 min; methylated compound 4, 120°C, 2.2 min; trifluoroacetylated compound 5, 12O°C, 1.5 min. Infra-red (IR) spectra: KBr disks, cm^{-1}. Proton nuclear magnetic resonance (1H-NMR) spectra: 250 MHz, tetramethylsilane as internal standard, δppm. Mass (MS) and GC-MS spectra: 70 eV, electron impact, m/z, relative abundance, %. Frequently, underivatized imidacloprid (1) and its metabolites 2 to 5 extracted from soil were analyzed by MS by direct introduction after several TLC purifications, or by GC-MS after derivatization.

For the standards of analysis, the standard of imidacloprid (1) was prepared by a described procedure (Rouchaud et al. 1994). l-[(6-Chloro-3-pyridinyl)methyl]-2-imidazolidone (2). The mixture of imidacloprid (2 g, 7.8 mmole) in water (100 ml) containing potassium hydroxide (4 g, 0.7 N) was heated to reflux (stirring, 1 hr). The cooled mixture was brought to pH 8.3 with conc. HCl, extracted with ethyl acetate, the ethyl acetate solution was dried (Na_2SO_4), concentrated to dryness under vacuum, and the residue was recrystallized in hexane+dichloromethane, giving compound 2 (1.56 g, 7.4 mmole, 95%). Spectra of compound 2: IR: 3235 (NH), 3081, 1690(CO), 1572, 1499, 1462, 1441, 1393, 1354, 1264, 1132, 1100, 1026, 847, 758, 741. lH-NMR ($CDC1_3$): 3.39(m, 4H, NCH_2CH_2N); 4.39(s, 2H, CH_2-pyridine); 5.72(br, lH, NH); 7.32, 7.67, 8.32(m, 3H, pyridine H). MS: 211(M+, 100); 213(211+2, 32); 194(M-OH, 2); 196(194+2, 0.6); 182(M-HCO, 12); 184(182+2, 3.6); 176(M-C1, 8); 169(M-NCO, 7); 171(169+2, 2.1); 148(176-CO, 14).

Compounds 3, 4 and 5 were obtained from Janssen Chimica, Belgium. Their spectra were the following:
6-Chloronicotinic acid (3): IR: 3200-3061, 2893, 1688(CO), 1582, 1420, 1375, 1300, 1144, 1107, 1019, 918, 831, 772. lH-NMR (DMSO--d6): 7.66(d, 2H); 8.32(d, 1H); 8.93(s, 1H). MS: 157(M+, 100); 159(M+2, 32); 139(M-H_2O, 65); 140(M-OH, 62); 141(139+2, 21); 142 (140+2, 20); 122(M-C1, 6); 112(M-CO_2H, 34); 114(112+2, 11); 94 (122-CO, 7).
6-Hydroxynicotinic acid (4): IR: 3150(OH), 2924, 2490, 1709(CO), 1638, 1609, 1416, 1283, 1235, 1130, 916, 781. lH-NMR (DMSO-d6): 6.41(m, 1H); 7.72(br, 1H); 7.83(m, 1H); 8.08(m, 1H); 12.40 (br, 1H). MS: 139(M+, 100); 122(M-OH, 12); lll(M-CO, 35); 94(M-CO_2H, 47); 67(94-$CHCH_2$, 28).
2-Imidazolidone (5): IR: 3408(NH), 3291, 2959, 1687(CO), 1503,

1449, 1275, 1105, 1042, 934. lH-NMR(DMSO-d6): 3.31(s, 4H, CH_2CH_2); 6.23(br, 2H, NH). MS: 86(M+, 100); 58(M-CO, 8); 42(NCO, 12); 30(CH_3CH_3, 79).

For the soil analysis, in the air-dried soil, whole or fragments of seed pellets were removed and analyzed separately in the same way as soil; they were present during the 50-day period following sowing, and generally corresponded to less than 20% of the total imidacloprid soil residue; their imidacloprid contents were added to the amount found in the 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) for extraction of imidacloprid (1) and of its metabolites 2 and 5. 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 ethyl acetate gave one TLC band containing imidacloprid at Rf=O.42, and another one at Rf=0.05-0.22 containing the mixture of metabolites 2 and 5. The TLC bands were scraped off separately, extracted with ethy1 acetate (40 mL) in a small glass column giving extract 1 containing imidacloprid (1), and extract 2 containing the mixture of metabolites 2+5. Extracts 1 and 2 were concentrated to 1 mL under a slow stream of nitrogen (2O°C). When the clean-up was insufficient, the TLC was repeated one or two times. To the final extract in ethyl acetate (1 mL) trifluoroacetic anhydride was added (2 mL; Janssen Chimica, Belgium), the mixture was carefully heated to boiling until concentrated to 0.1 mL (~L 5 min), ethyl acetate was added, and each extract was analyzed by GLC for imidacloprid (1), or for the metabolites 2 and 5. In several cases, the final trifluoroacetylated extracts from soil were further analyzed by GC-MS for 1, 2 and 5 or, before trifluoroacetylation, by MS (direct introduction).

The soil already extracted with acetone+water, was again extracted with water containing 1 g% KOH (150 mL, 20°C, 15 min, stirring) for the analyses of metabolites 3 and 4. The mixture was filtered, the KOH solution was brought to pH 2.5 with hydrochloric acid, the mixture was extracted with ethyl acetate (200+150 mL), the ethyl acetate solution was dried (Na_2SO_4), concentrated successively to 40 and 0.5 mL. The concentrate was applied to a TLC plate. Elution with ethyl acetate gave the mixture of compounds 3+4 in the band at Rf=0-0.25. This band was separated, extracted with ethyl acetate, the ethyl acetate solution was concentrated and applied onto a second TLC plate. Elution with dichloromethane+acetic acid, l0+l, mL/mL, gave compound 3 at Rf=0.75 and compound 4 at Rf=0.15. The bands were scraped off separately, extracted with ethyl acetate, the extracts were concentrated to 1 mL, an ethereal diazo-

methane solution was added until persistence of the yellow colour, and the extracts were concentrated and analyzed by GLC for metabolites 3 and 4. In several cases, the final unmethylated extracts from soil were analyzed by MS (direct introduction) or, after methylation, by GC-MS. At the 0.1 mg kg^{-1} level in soil, the recoveries of imidacloprid (1), and of compounds 2, 3, 4 and 5 were 83-96, 87-93, 78-89, 75-91 and 73-87%, respectively. The analytical limit of sensitivity for imidacloprid (1) and its metabolites 2 to 5 was 0.01 mg kg^{-1} dry soil. At harvest, the 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 and of its metabolites 2 to 5 were similar to the ones in the soil. The limit of detection of imidacloprid (1) and of its metabolites 2 to 5 in the sugar beet leaves and roots was 0.01 mg kg^{-1} fresh weight.

RESULTS AND DISCUSSION

No imidacloprid 1 nor its metabolites 2 to 5 were detected in the 10-20 cm soil layer under and around the sugar beet in all the organic fertilizers, treated or untreated plots of the trials at Lubbeek and Remicourt. There was thus no leaching of these compounds in soil. No residues of imidacloprid nor of its metabolites 2 to 5 were detected in the roots and the leaves of sugar beet at harvest. Compound 2 (1-[(6-chloro-3-pyridinyl)methyl]-2--imidazolidone), 6-chloronicotinic acid (3) and 6-hydroxynicotinic acid (4) were the main metabolites of imidacloprid (1) in soil (Figure 1). Two months after sowing, in the control plots of the trial at Lubbeek, their soil concentrations attained their maximum; these were 26, 15 and 30%, respectively, of the imidacloprid soil concentration at that moment (Table 1). Only traces of compound 5 (2-imidazolidone) were observed. Because ^{14}C-imidacloprid was not used, other imidacloprid soil metabolites could have been formed and were not detected. 39 Days after sowing, the sum of the imidacloprid (1) plus its metabolites 2 to 5 soil concentrations corresponded to 75% of the applied dose in the organic fertilizers untreated control plots at Lubbeek; in the cow manure and pig slurry treated plots, that sum corresponded to 93 and 89%, respectively. This indicated that metabolites 2, 3 and 4 indeed were the main imidacloprid soil metabolites. Similar observations were made in the trial at Remicourt (Table 2). In both trials at Lubbeek and Remicourt, the imidacloprid (1) metabolites soil concentrations aenerallv were in the following decreasing order: metabolite 4⟩ 2⟩ 3⟩⟩ 5. Compound 2 corresponds to the biochemical hvdrolysis of the nitro-imine chemical function of imidacloprid (1) into the keto function. Compound 3 corresponds to the separation of the imidazolidone ring from the benzylpyridyl group, by an amide dealkylation biochemical procedure, and the subsequent oxidation of the benzylpyridyl methylene. Compound 4 corresponds to substitution of the chlorine atom by an hydroxyl. This imidacloprid soil metabolism pathway indicates that imidacloprid was progressively transformed in the soil of sugar beet crop into non-toxic and common compounds.

During the first three crop months, the concentration of metabolites 2 to 5 first increased, attained a maximum, and then decreased. At sugar beet harvest, they were no longer detected in soil, as for imidacloprid (1). The recent organic fertilizer treatments slowed down imidacloprid (1) disappearance in soil, and its metabolites 2 to 5 formation in both trials at Lubbeek and Remicourt (Tables 1 and 2). At Lubbeek, this effect occurred in spite of the fact that prior to the organic fertilizers treatments, the concentration of organic matter in soil was already high (4.4%). This was a result of the use of a meadow which had been ploughed 4 years previously. This indicated, as at Remicourt, that the old soil organic matter has no more influence on the rate of the insecticide soil biodegradation. The effect of the organic fertilizers treatments was in addition to the one given by imidacloprid encapsulation in the pelleted seed, which also delayed imidacloprid soil biodegradation. Forty days after sowing, some whole pelleted seed were indeed still found in soil. Analysis indicated that their imidacloprid content was the same as in the freshly pelleted seed. As we previously suggested, the increase of the reversible adsorption of imidacloprid 1 onto the soil organic matter, whose concentration was increased by the recent organic fertilizers treatments, protected imidacloprid in some way against the soil microbial activity which metabolizes its (Honnay 1992). This should account for the lower rate of formation of metabolites 2-5 in soil caused by the recent treatments with organic fertilizer. The results obtained in the trial at Remicourt indicate that the very old soil organic matter no longer has this property. This should be due to the progressive long term transformation of the soil organic matter, especially the decrease of the density of the oxygenating chelating functions, i.e., the coalification of the soil organic matter.

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