HPLC Determination of the New Insecticide Imidacloprid† and Its Behavior in Rice and Cucumber

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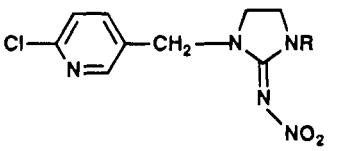
A rapid, sensitive, and reliable HPLC method has been developed for the determination of imidacloprid residue in nine kinds of crops and soil. The developed method consisted of extracting with acetonitrile/water (80:20 v/v), prewashing of the concentrated extracts with cyclohexane and alkaline solution, silica gel column chromatography, and finally reversed-phase HPLC. The recoveries of imidacloprid were 75–109%. The limits of determination of the method were 0.005, 0.01, and 0.02 mg/kg for crops, rice straw, and soil, respectively. The method was applied to determine residues and rate of disappearance of imidacloprid from rice (nursery box treatment, 1 g of ai/1800 cm^2) and cucumber plants (stem injection, 20 μg of ai/plant). The insecticide incorporated into the plants decreased rapidly with a half-life of less than 3 days. In the rice, however, it controlled the brown planthopper even at 60 days after application, with the marginal concentration of 0.01 mg/kg for practical control.

Keywords: *Imidacloprid; residue analysis; chloronicotinyl insecticide; rice; cucumber; soil; brown planthopper*

INTRODUCTION

Imidacloprid (**1**), 1-(6-chloro-3-pyridinylmethyl)-*N*-nitroimidazolidin-2-ylideneamine (commercial names Admire, Gaucho, Confidor) (Figure 1), is a new type of insecticide possessing potential activity and novel modes of action (Kagabu et al., 1992; Moriya et al., 1992; Shiokawa et al., 1992). It is effective for controlling aphids, whiteflies, thrips, scales, psyllids, plant bugs, leafhoppers, planthoppers, and other various harmful pest species including resistant strains. Owing to its insecticidal effectiveness and its safety for humans and the environment, imidacloprid has been drawing attention as a promising newcomer (Moffat, 1993). The physiological study started quite recently; it interferes with neural functions as do organophosphate, carbamate, and pyrethroid insecticides. Different from the latter pesticides, it acts as an agonist of acetylcholine by binding to nicotinergic acetylcholine receptors on the postsynaptic membrane (Bai et al., 1991; Tomizawa and Yamamoto, 1993; Liu and Casida, 1993).

Imidacloprid has been introduced to French and Japanese markets and will be widely used in other areas of the world in the following years. We now present its residue analysis method, studies of its behavior in soils and plants, and its biological activity in response to the residue on plants. This work deals with a rapid, sensitive, and quantitative method using HPLC for the determination of the insecticide (Kobori et al., 1990, 1991). Herein are also described recovery experiments for Japanese pears, apples, peaches (pulp and peel), grapes, radishes (root and leaf), cucumbers, eggplants, rice (grain, green, and straw), potatoes, and soils. The extraction efficiencies were tested with ^{14}C-labeled imidacloprid incorporated in rice.



1 : R=H, **2** : R=CH_3

Figure 1. Structures of imidacloprid (**1**) and the methyl derivative **2**.

The study has been further extended to establish the marginal concentration of the residue for practical application. The relationship between the concentration in rice and the insecticidal activity against the brown planthopper was surveyed over 90 days. The fate of imidacloprid after stem injection in the cucumber plant was also surveyed.

MATERIALS AND METHODS

Reagents. All reagents were of analytical quality. HPLC grade acetonitrile and silica gel for column chromatography (Wakogel C-200) are available from Wako Pure Chemicals. Analytical standard of imidacloprid was prepared according to the procedure described in the literature (Moriya et al., 1992) and recrystallized from ethanol (98.1% purity on HPLC). Imidacloprid standard solutions were prepared by dissolving the accurately weighed sample in a water/acetonitrile (80:20 v/v) mixture. ^{14}C-Labeled imidacloprid, radiochemical purity: > 99%, specific activity: 1.42 GBq/mmol (38.4 mCi/mmol), was prepared by Bayer A. G. (internal report: Marsmann, Körmeling, Brauner and Morishima, 1987).

Preparation of Rice Straw Samples Treated with [^{14}C]-Imidacloprid and Evaluation of Extraction Efficiency. [^{14}C]Imidacloprid was applied to rice seedlings planted in 500 cm^2 plastic pots at a rate of 0.5 kg of ai/ha (planting hole application). The plants were cultivated for 145 days in a greenhouse. After the sampling, the rice straw was stored in a −20 °C freezer until analysis. The straws were extracted with acetonitrile/water (80:20 v/v) and methanol/water (80:20 v/v) using a high-speed homogenizer (Polytron, Kinematica, Central Science, Tokyo) or a hand-held shaker for 30 min, and the extracted amount of the total radioactivity was measured by a liquid scintillation counter (Beckman, LS-3801). The

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† Chloronicotinyl Insecticides, see Moriya et al. (1993).

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Table 1. Extraction Efficiency of Imidacloprid from Rice Straws Treated with ^{14}C-Labeled Imidacloprid and Cultivated in a Greenhouse for 145 Days

solvent	^{14}C extracted[a] (%)	
	Polytron	shaker
acetonitrile/water (80:20 v/v)	75.8	69.4
methanol/water (80:20 v/v)	73.1	69.0

[a] Recoveries for the total ^{14}C, the average of three replicates.

mean extraction efficiencies of the triplicate operations are shown in Table 1.

Field Trials. Field trials were conducted at the field station of Yuki Research Center, Ibaraki, Japan, in 1990.

Preparation of Cucumber Samples. A solution of imidacloprid in water/dimethyl sulfoxide (80:20 v/v) of a concentration of 2 mg/mL was prepared; ten μL of the solution, i.e. 20 μg of ai/plant, was applied into 2–3-leaf-stage cucumber seedlings with a microsyringe at a position 2–3 cm above the ground. The aerial parts of the plants were sampled 1, 3, and 7 days after the treatment and frozen at −20 °C until analysis.

Preparation of Rice Samples and Insecticidal Test Method. *Preparation of Rice Samples.* Rice seedlings were raised in nursery boxes (30 × 60 × 3 cm) until the 2–3-leaf stage. Treatment was made with 50 g/box of Admire 2 GR (2% imidacloprid), i.e., 1 g of ai/box (1800 cm^2). The seedlings were transplanted to the paddy field 3 days after the treatment. Samplings were started just before the transplanting and then were repeated at 10, 17, 24, 31, 38, 45, 53, 66, 80, and 94 days. Until 24 days, the samples drawn from the field were divided into aerial parts and roots. After 31 days, the aerial parts were subdivided into leaves and sheaths. These samples were stored at −20 °C in a freezer until analysis.

Insecticidal Test Method. Artificial inoculation of 18 heads (12 females, 6 males) of adult brown planthopper (*Nilaparvata lugens*) was made for a plot composed of six hills of rice covered with mesh cages, and conducted eight times in a 1-week interval, starting at 36 days after transplanting and continuing for as long as 49 days after that. The number of insects living on each hill and in the untreated hill was counted 51, 58, 65, 72, 80, 86, and 93 days after transplanting.

Preparation of Analytical Samples. The scheme for the sample preparation for the HPLC analysis is depicted in Figure 2. The frozen samples were subjected to the analysis before melting. During storage of the plant samples, 2 years under frozen conditions (−20 °C), no degradations of imidacloprid (recovery 95–105%) were observed. This validates the residue values analyzed in this study.

Extraction and Cleanup. *Crops.* Ten grams of homogenized rice straw and 20 g of each of the other crop samples were weighed into 1 L and 500 mL beakers, respectively, containing 400 and 200 mL of acetonitrile/water mixture (80:20 v/v), respectively. The sample was extracted with a Polytron for 2 min and filtered by suction through a filter aid (Celite 545, Kanto Kagaku, Tokyo) on a Büchner funnel fitted with a sintered-glass disk. The beaker was rinsed with about 50 mL of the mixed solvent, and the filter cake was washed with the solvent. The combined filtrate was transferred to a 500 mL round-bottom flask, and most of the acetonitrile was distilled off using a rotary evaporator at 40 °C. The aqueous remainder was diluted with 150 mL of 10% brine and transferred into a 500 mL separatory funnel. The content was washed twice with 100 mL of cyclohexane, and the cyclohexane layer was discarded. The aqueous phase was partitioned three times with 70 mL of dichloromethane. The combined dichloromethane layer was washed with 70 mL of 0.05 M potassium carbonate solution and dried over anhydrous sodium sulfate. The solution was concentrated to about 2 mL on a rotary evaporator at ambient temperature and evaporated to dryness under a gentle nitrogen gas stream. The residue was dissolved in 4 mL of *n*-hexane/ethyl acetate mixture (1:1 v/v) and subjected to column chromatography.

Soils. Twenty grams of dry weight of soil was placed into a 500 mL Erlenmeyer flask, into which 200 mL of acetonitrile/water (80:20 v/v) was added. The mixture was extracted in an ultrasonic bath at 40 °C for 30 min. The flask was rinsed with 50 mL of the solvent mixture, and the filter cake was washed with the solvent. The solid on the glass filter including the Celite was transferred to the flask containing 200 mL of the mixed solvent, and the operation of sonication and filtration was repeated. The combined extract was transferred to a 1 L round-bottom flask and concentrated using a rotary evaporator at 40 °C. The subsequent procedures from the brine dilution before the column chromatography were done as described above for crops.

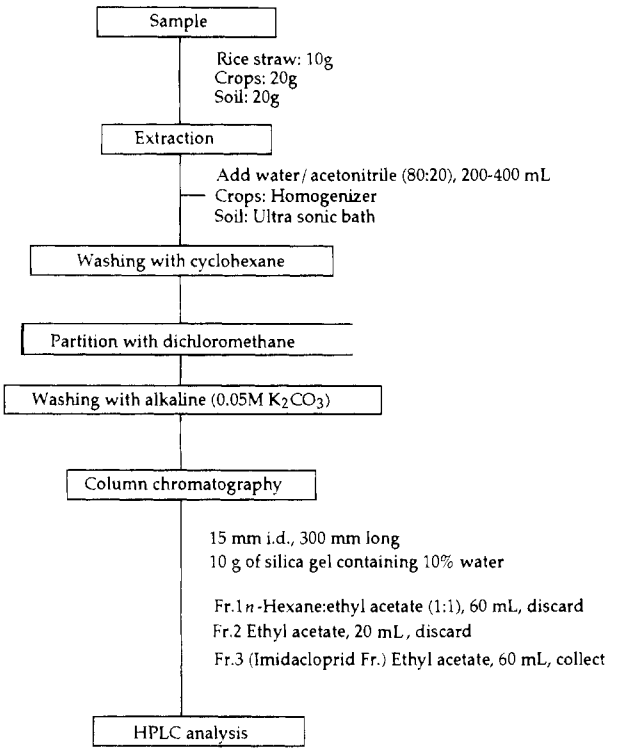

Figure 2. Analytical procedure of imidacloprid for crops and soil.

Column Chromatography. The sample solution was subjected to a column (250 × 15 mm i.d.) packed with 10 g of silica gel (containing 10% weight of water) and eluted first with a total volume of 60 mL of a mixed solvent of *n*-hexane/ethyl acetate (1:1 v/v) and subsequently with 20 mL of ethyl acetate. The fractions eluted with these solvents were discarded. The imidacloprid residue was collected from the following fractions eluted with another 60 mL of ethyl acetate. The eluted fraction was concentrated to about 2 mL with a rotary evaporator at a bath temperature of 40 °C and evaporated to dryness under a gentle stream of nitrogen gas. The residue was dissolved in a minimum of 2 mL of a water/acetonitrile (80:20 v/v) mixture. Then 20 μL of the solution was subjected to HPLC analysis.

HPLC Analysis. The HPLC system used in these studies was a Jasco 800 (Nihon Bunko, Tokyo) consisting of an 801-SC system controller, an 880-PU pump, an 850-AS autoinjector, and an 870-UV ultraviolet detector. The chromatograph was connected to an HP 3390A integrator (Hewlett-Packard, Tokyo). Columns of LiChrospher RP-8(e) (250 × 4 mm i.d., 5 μm; Merck, Tokyo) were used for the separation of imidacloprid with an 80:20 mixture of water and acetonitrile at a flow rate of 1 mL/min. The wavelength was 270 nm according to its UV spectrum. The amount of imidacloprid in the sample was calculated according to the equation based on the ratio of the peak areas of the external standard and the sample

$$R = A_p W_s V_e / (A_s V_i G_p)$$

where R is the residue of imidacloprid (mg/kg), A_p is the peak area of the sample solution, W_s is the amount of imidacloprid injected in the standard solution (ng), V_e is the final volume of the sample solution (mL), A_s is the peak area counts of the standard solution, V_i is the volume injected (μL), and G_p is the weight of analytical sample (g).

Table 2. Recovery Tests of Imidacloprid Fortified to Crops and Soil

sample	fortified amt (mg/kg)	recovered (%)			av (%)	coeff of variation (%)
Japanese pear	0.1	101	101	102	101	0.6
	0.01	94	96	99	97	2.6
	0.005	94	97	100	97	3.1
apple	0.1	93	93	95	94	1.2
	0.01	90	90	94	93	2.5
	0.005	92	95	101	96	4.8
peach/pulp	0.1	95	95	97	96	1.2
	0.01	101	103	109	104	4.0
	0.005	92	92	96	93	2.5
peach/peel	0.1	87	88	94	90	4.2
	0.01	94	94	96	95	1.2
	0.005	85	89	96	90	6.2
grape	0.1	95	95	98	97	1.8
	0.01	94	100	107	100	6.5
	0.005	94	101	102	99	4.4
radish/root	0.1	105	105	108	106	1.6
radish/leaf	0.1	95	95	96	95	0.6
cucumber	0.1	100	100	102	101	1.1
	0.01	103	103	104	103	0.6
	0.005	99	99	100	99	0.6
eggplant	0.1	98	99	99	99	0.6
	0.01	103	103	104	104	0.6
	0.005	97	97	99	98	1.2
rice grain	0.1	95	95	95	95	0
	0.01	98	101	103	101	2.5
	0.005	92	97	97	95	3.0
rice green	0.1	94	100	102	99	4.2
	0.005	91	91	95	92	2.5
rice straw	0.2	87	89	92	89	2.8
	0.02	75	81	83	80	5.2
	0.01	77	82	91	83	8.5
potato	0.1	94	94	95	94	0.6
	0.01	101	102	103	102	1.0
	0.005	95	96	99	97	2.2
soil	0.1	86	88	94	89	4.7
	0.05	88	92	101	94	7.1
	0.02	80	85	91	85	6.5

Recovery Assays. Untreated plant samples (20 g) were spiked with 0.005, 0.01, and 0.1 mg/kg pesticide by adding 1 mL of 0.1, 0.2, and 2 mg/L of standard solution, respectively. In the case of rice straw, the concentrations of the pesticide were 0.01, 0.02, and 0.2 mg/kg, and for soil they were 0.02, 0.05, and 0.5 mg/kg. Samples were equilibrated for 1 h prior to extraction and subsequently taken through the extraction procedure described above. The recovery assays were replicated three times (Table 2).

RESULTS AND DISCUSSION

Imidacloprid has a unique structure comprised of two nitrogen heterocyclic moieties. The insecticidal potential of imidacloprid is so high as to be effective at a dose as low as 0.3 ppm to control pests (Moriya et al., 1992). The analytical method is therefore required to be capable of quantifying the pesticide in trace amounts.

Vapor-phase chromatography has been a conventional tool for the determination of pesticide residues in foods and plants, and countless examples have been reported thus far (Ogden, 1989; Zweig and Sherma, 1978). However, the injection of imidacloprid under normal GC conditions gave a chromatogram of complex fragment peaks. This is probably attributed to the thermolabile and polar *N*-nitroguanidinyl moiety. Substitution of the acidic hydrogen of the NH at the 3-position of the imidazolidine ring is expected to render the molecule more volatile and thereby shorten the retention time. The methylation with diazomethane or dimethyl sulfate/KOH, the acylation with acetic anhydride or trifluoroacetanhydride, or the trimethylsilylation with bis(trimethylsilyl)trifluoroacetamide did not give the N-substituted derivative in any case. *N*-Methyl derivative **2** could be isolated in 10% yield by treatment with NaH/Me_2SO_4 in DMF. However, even **2** was not sufficiently stable under the GC analytical conditions, giving no adequate chromatogram either.

High-performance liquid chromatography (HPLC) generally has an advantage over GC with respect to the mild conditions for the separation and detection of analytes. HPLC with a UV detector would be suitable for the detection of imidacloprid having a strong absorption at 270 nm (ϵ 21 700) due to the $\pi-\pi^*$ of the nitroguanidine chromophore. We attempted analysis with reversed-phase columns (RP-8, RP-18) and different mobile phases (water, methanol, acetonitrile). Finally, satisfactory elution of the pesticide could be achieved using an end-capped reversed-phase column (RP-8e) with a water/acetonitrile (80:20 v/v) solution as eluent. Under optimum conditions at 270 nm, a calibration curve was constructed by plotting concentration vs peak area. Good linearity was achieved in the 1–20 ng range with a correlation coefficient of 0.9999.

Table 1 shows that the pesticide can be effectively extracted from crops with an acetonitrile/water (80:20 v/v) mixture using a Polytron. The extract follows a sequence of cleanup procedures to remove substances in the extracted samples interfering with the residue analysis. The cleanup solvents should dissolve the obstacle substances originated in the plants but not the test pesticide. Cyclohexane was the best among the solvents tried for that purpose. The aqueous extracts obtained from the treated plants were washed first with cyclohexane. The chlorophyllous materials and oily constituents could be eliminated, which hampered a quick quantitation of the pesticide owing to their HPLC peaks with retention times greater than 1 h (Figure 3 B).

The obstacles still remaining, which are presumably composed mostly of acidic substances (carboxylic acid or phenolic components of the plants?), could be effectively removed by treatment of the dichloromethane solution of the residues with 0.05 M potassium carbonate. Finally, the resinous tissue of the plants and the contaminating materials during the cleanup operation were separated by column chromatography with silica gel. The scheme of the cleanup procedures is depicted in Figure 2. The cleaned up final extracts did not give any interfering peaks around the retention time of imidacloprid of 11.2 min on the HPLC chromatograms when the water/acetonitrile (80:20 v/v) mobile phase was used (Figure 3C).

The limit of determination of imidacloprid for rice straw was 0.01 mg/kg, and that for the other crops was 0.005 mg/kg; the limit for the soils was 0.02 mg/kg. The recovery range was 75–109%, with less than 9% relative standard deviation (Table 2). On the basis of these results, this residue analysis method provides precision and reliability. Figure 4 shows typical chromatograms.

An experiment to evaluate the behavior of residues in the rice plants cultivated in the field was performed. The mean residue values are determined in the leaves, sheaths, and roots over 3 months (Table 3). Three days after the nursery box treatment, imidacloprid was found in the roots at a concentration of 65.7 mg/kg. Interestingly, leaves also contained residues as high as 93.9 mg/

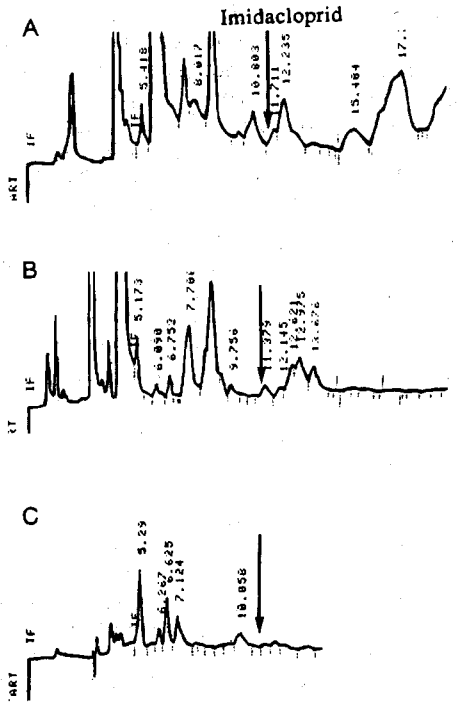

Figure 3. HPLC profiles of untreated rice straw samples according to the cleanup procedures: (A) without washing; (B) washing with cyclohexane; (C) washing with cyclohexane and alkaline.

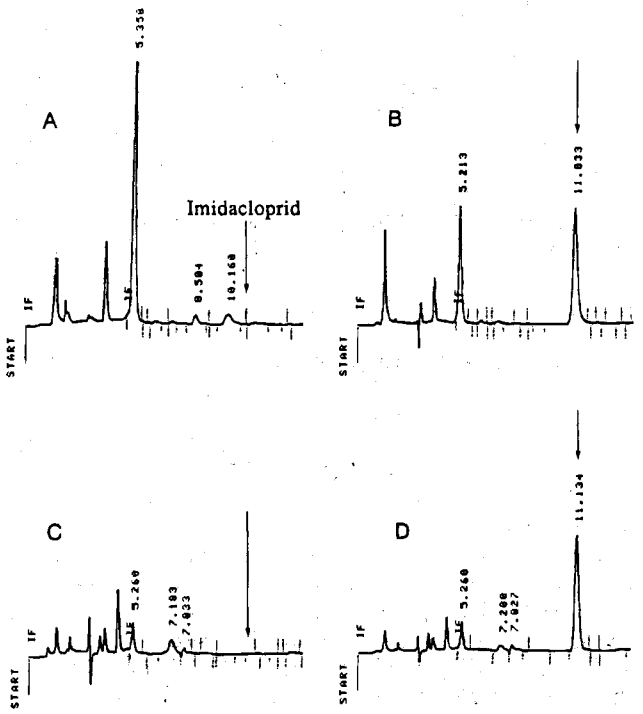

Figure 4. Chromatograms of (A) untreated rice grain, (B) rice grain fortified with 0.1 mg/kg of imidacloprid (95% is recovered), (C) untreated cucumber, and (D) cucumber fortified with 0.1 mg/kg of imidacloprid (100% is recovered).

kg. This indicates that the pesticide was rapidly incorporated into the rice plants from the roots in 3 days. After seedlings were transplanted to the paddy field, the residues showed a tendency to progressively decrease, and 24 days later they averaged 0.326 and 0.152 mg/kg for leaves and roots, respectively.

To better understand the disappearance of the pesticide residue in rice, residue concentrations were transformed to natural logarithms for regression analysis (Timme et al., 1986). Linearity of the relationship between concentration (y) and time (x) was evaluated as follows: $\ln y = \ln 187.8019 - 0.2655x$ and $\ln y = \ln 144.9773 - 0.2897x$ for aerial parts and roots, respectively. There were no statistically significant deviations from linearity for the pseudo-first-order reactions with $r^2 = 0.9984$ and 0.9997, respectively. Another remark is that the slopes are comparable in both relations. This means that the residues are degraded in the aerial and hypogeal parts with similar rate constants. After day 31, the aerial part of the plants was divided into leaf and sheath; 0.364 mg/kg of the pesticide was found in leaves and 0.044 mg/kg in sheaths. The residues decreased progressively, and they were 0.017 mg/kg in leaves and 0.007 mg/kg in sheaths on day 53. Following day 53, however, gentle degradation slopes were observed in both leaves and sheaths. This shows a two-phase profile of the degradation behavior of imidacloprid in rice plant. The residues at day 94 were 0.007 and 0.002 mg/kg in leaves and sheaths, respectively. Since the residue concentrations were 2–8 times higher in the leaves than in the sheaths during the testing period, the pesticide moved to the leaves acropetally.

Table 3. Residue Behavior of Imidacloprid in Rice Plant after Treatment with 1 g of ai/Box and Transplanted in the Field

	residues of imidacloprid (avd mg/kg)			
days after treatment	aerial part[a]	leaf	sheath	root
3	93.9			65.7
10	10.9			7.51
17	2.22			0.950
24	0.326			0.152
31		0.364	0.044	
38		0.126	0.025	
45		0.054	0.013	
53		0.017	0.007	
66		0.018	0.008	
80		0.015	0.004	
94		0.007	0.002	

[a] After 31 days, aerial parts were divided into leaf and sheath.

The relationship of the residues in rice to biological activity was examined next. The insecticidal experiment was carried out in the field against the brown planthopper (*N. lugens*). This insect is one of the most injurious planthoppers to rice and severely damages rice growth by so-called "hopper-burn". The migration of adult brown planthopper from southeast Asia starts in June and often lasts for about 2 months. Admire 2 GR was applied to the nursery box at 50 g/box (1 g of ai/box; 0.2 kg of ai/ha) 3 days before transplanting. The artificial inoculation of 18 adults per six hills was made for the plot eight times at 1-week intervals, starting at 36 days after transplanting and continuing for as long as 49 days after that. Head counts of the insects living on the plants were continued after the inoculation. The rice plants of untreated plots were totally killed by hopper-burn already at the heading crop stage. As seen in Figure 5, imidacloprid depresses the multiplication of the insects effectively for more than 60 days when the residue concentration is at level of 0.01 mg/kg, and prevention of hopper-burn for the crops lasts as long as 80 days after transplanting. This experiment shows that a one-shot application of imidacloprid to the nursery box can control the brown planthopper practically for the entire season at an application rate of 1 g of ai/box.

Another experiment on the residue behavior was carried out in the field for cucumbers. Imidacloprid residues and mean weight were determined on vegetable samples to evaluate the diluting effect on the residue caused by plant growth during the experiment.

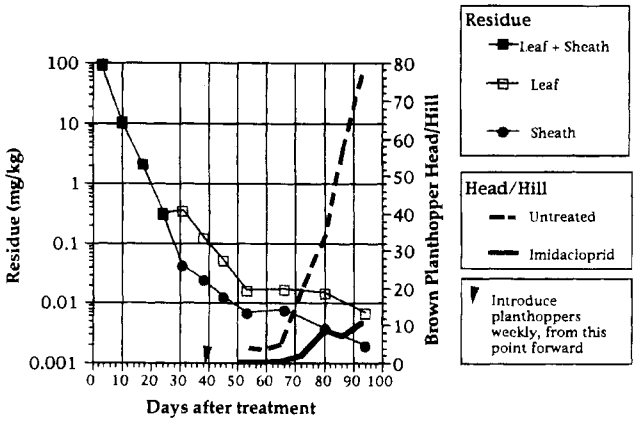

Figure 5. Chemical concentration and effect of imidacloprid on brown planthopper (*N. lugens*) in rice field.

Table 4. Residue of Imidacloprid in Cucumber after Stem Injection with 20 μg of ai/Plant

days after injection	plant wt (g)	residue amt (μg/plant)	residue concn (mg/kg)
1	12.1	11.88	0.982
3	17.7	3.06	0.173
7	22.0	0.14	0.006

A solution of 20 μg of ai/plant was injected into the stem of cucumber, and the aerial part of the cucumber plants was analyzed 1, 3, and 7 days after treatment (Table 4). The residue amount at day 1 was 11.88 μg (0.982 mg/kg), corresponding to 59% recovery from the initial dose. The residues declined rapidly, and 7 days later they decreased to 0.14 μg by more than a factor of 140. If we evaluate the increase in the weight of the vegetable only by a factor of 2, the decrease of the residues was not significantly ascribed to the growth-diluting effect but should be caused by degradation of the pesticide itself. The degradation rate of imidacloprid in the cucumber was expressed as a pseudo-first-order reaction: $\ln y = \ln 22.8876 - 0.7182x$ ($r^2 = 0.9876$). The theoretical half-life in cucumbers, 0.97 day, was shorter than that in the rice plant. This would be attributed to the fact that the rice plant could absorb imidacloprid from the treated root zone continuously, whereas the cucumber was injected directly to the stem only once.

CONCLUSIONS

Reversed-phase HPLC employing a UV detector allows the rapid and highly sensitive determination of imidacloprid for crops and soil. Samples could be effectively cleaned up by a simple liquid–liquid partition with cyclohexane followed by alkaline treatment. This method needs no structural derivatization for the analysis. The residue detection could be achieved in the minimum amount of 1 ng at the level of 0.005 mg/kg for most crops and 0.02 mg/kg for soil with high accuracy. Recoveries were satisfactory and ranged from 75 to 109% for crops and from 80 to 101% for soil. The method was applied for the investigation of the residue behavior of imidacloprid in rice and cucumber. The experiment shows that the pesticide possesses systemic properties and is translocated to the aerial parts quickly but has quite low persistency in the plants. The residue behavior of imidacloprid in the rice plants shows two phases and follows a pseudo-first-order reaction with a rapid decay rate of about 2.6 day^{-1} in the first phase. The residues decreased progressively to a level of less than 0.02 mg/kg about 2 months after the treatment, but in the second phase the rate shows sagging curves in a semilogarithmic system. Since imidacloprid has good biological activity with low concentration, it controls the brown planthopper in rice for 60–80 days. The marginal residue concentration was 0.01 mg/kg.

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