Pirimicarb and Its Metabolite Residues in Lettuce. Influence of Cultural Environment

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The fate of Primicarb (I) residue on field and greenhouse lettuce was studied. The parent compound was quickly transformed into its *N*-methyl-*N*-formyl (II) and *N*-demethyl (III) analogues. Five days after treatment, another metabolite, *N*-demethylphenol (VI), was determined, though in low amounts. These metabolites were rapidly degraded, and 10 days after application of Pirimicarb, only III was detected, showing a very low residue. The very rapid decay rate of Pirimicarb into its metabolites should indicate that volatilization does not represent the primary means of loss of I in plants and that the aphicide action could also be ascribed to its metabolic products. Though the microclimatic conditions were different in the two experiments, the degradative kinetics were similar in both cases, because in the field relative humidity and wind speed, compensating for each other, determined a tendency to evaporation analogous to that occurring in the greenhouse.

Aphids are among the most important pests in the cultivation of lettuce. Of the numerous insecticides on sale, Pirimicarb, 2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (I) (Figure 1), is one of the few to possess selective action against aphids but not against their natural predators.

Pirimicarb is rapidly lost in plants after spraying, mainly by volatilization but also by photochemical and metabolic degradation. The major degradative products are the carbamate-containing metabolites II (2-[(methylformyl)amino]-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) and III (2-methylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate), while the minor products are the carbamate-containing metabolite IV (2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) and the hydroxypyrimidines V (2-(dimethylamino)-5,6-dimethyl-4-hydroxypyrimidine), VI (2-(methylamino)-5,6-dimethyl-4-hydroxypyrimidine), and VII (2-amino-5,6-dimethyl-4-hydroxypyrimidine) (Figure 1) (FAO/WHO, 1977). The rate of degradation of Pirimicarb, varying with crop type and weather conditions, has been studied by numerous authors. As regards lettuce, Wuest and Meier (1983) and Cabras et al. (1988) have only carried out investigations limited to the active ingredient (AI), whereas Braun et al. (1980) and Szeto et al. (1984) have also determined metabolite III, which included metabolite II (previously converted to III); as a matter of fact, by the analytical method employed (Bullock, 1973) it was not possible to determine the two metabolites separately. All these trials were carried out on lettuce cultivated in the field.

Lettuce is also cultivated in the greenhouse where volatilization is different from that occurring in the field. Since volatilization is of great importance, it being the primary means of loss for Pirimicarb depending on the ambient temperature (FAO/WHO, 1977), we carried out an investigation in order to evaluate degradation of this AI in lettuce cultivated both in the greenhouse and in the field. Another goal of our work was to verify presence of metabolites of Pirimicarb in lettuce by an analytical method (Cabras et al., 1989) that allows determination of AI and its major metabolites (II–VII).

EXPERIMENTAL SECTION

Materials and Methods. The trial was carried out in alluvial soil in a 4000-m^2 iron-glass greenhouse and in a contiguous field usually employed for horticultural cultivation. In both cases irrigation was carried out by a drop system. The lettuce employed was cv. Odessa (Sluis & Groot) type cos.

Seeding was carried out on March 9, 1989, and transplantation on April 13, 1989, on double rows, 70 × 30 cm apart, resulting in a plant density of 67 000/ha. A random-block scheme was used, with four replications, and each block measured 18 m × 1 m and contained 120 plants.

The environment conditions in the greenhouse (temperature and relative humidity) were continuously recorded with an MT 1100 (SIAP, Bologna, Italy) thermohygrograph. In the field, an S 2000 (SIAP) automatic weather station was employed, which continuously recorded wind speed and rainfall, in addition to the above cited parameters.

Treatments were made with 1200 L/ha of a liquid formulation of Pirimor, containing 25% of AI, applied with an F 320 (Fox Motori, Bologna, Italy) portable motorized sprayer in the dose recommended by the manufacturer (150 g/hL, single dose (SD)) and double-strengthened (DD). Treatments were carried out on May 15 and May 23, 1989, in the greenhouse and field, respectively. Samplings started about 1 h after spraying and were repeated 1, 3, 5, 7, and 10 days and 1, 3, 6, and 10 days after treatment, in the greenhouse and field, respectively.

Each sample, drawn from each block, consisted of two to three tufts, depending on plant development; it was triturated with a mincing knife, homogenized, and analyzed immediately after sampling. The extraction procedure was carried out as follows. A 25-g portion of the homogenized sample was accurately weighed in a 250-mL screw-capped flask; 50 mL of the extraction solvent (chloroform for I–IV or methanol for V–VII) was added and the flask shaken in a flask-shaker (Stuart Scientific) for 15 min. The organic layer was dehydrated with anhydrous sodium sulfate (0.1 g/mL of chloroform or 1 g/mL of methanol) and

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Table I. Residues (ppm ± SD) of Pirimicarb and Some of Its Metabolites (II, III, VI) on Lettuce in the Greenhouse after Treatments in Single and Double Doses

days after treatment	tuft wt, g	Pirimicarb	II	III	VI	total carbamate as Pirimicarb
			Single Dose			
0	325 ± 34	7.25 ± 1.52	nd[a]	nd	nd	7.25
1	319 ± 37	0.08 ± 0.02	2.12 ± 0.48	1.67 ± 0.16	nd	3.85
3	383 ± 31	0.04 ± 0.01	0.75 ± 0.21	1.43 ± 0.19	nd	2.26
5	385 ± 61	nd	0.25 ± 0.08	0.89 ± 0.23	0.04 ± 0.00	1.18
7	477 ± 41	nd	nd	0.26 ± 0.10	0.03 ± 0.01	0.28
10	545 ± 42	nd	nd	0.09 ± 0.04	nd	0.09
			Double Dose			
0	333 ± 34	15.65 ± 2.15	nd	nd	nd	15.65
1	321 ± 16	0.38 ± 0.08	6.68 ± 1.72	2.23 ± 0.46	nd	9.06
3	369 ± 16	0.13 ± 0.01	1.46 ± 0.33	2.18 ± 0.60	nd	3.82
5	422 ± 29	nd	0.40 ± 0.11	1.24 ± 0.36	0.08 ± 0.02	1.69
7	447 ± 49	nd	0.09 ± 0.05	0.34 ± 0.13	0.04 ± 0.01	0.45
10	518 ± 43	nd	nd	0.15 ± 0.06	nd	0.16

[a] nd = not detectable.

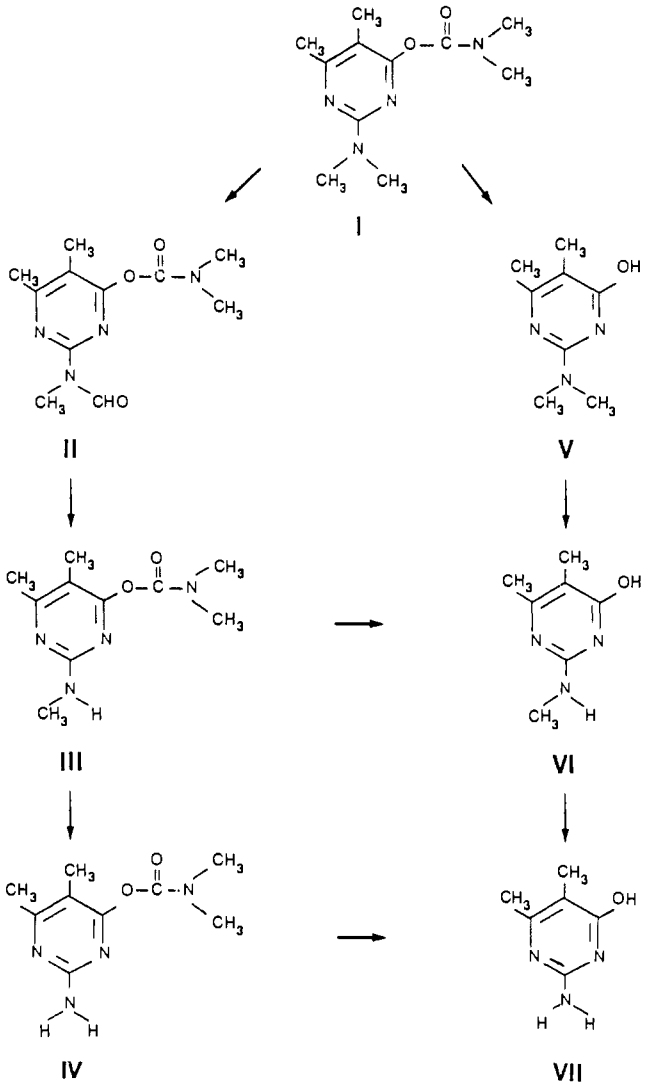

Figure 1. Pirimicarb (I) and six of its metabolites (II–VII).

then filtered with a PTFE 0.45-μm syringe filter (Alltech, Deerfield, IL). After filtration, 2 mL of the organic layer was evaporated nearly to dryness by cold ventilation, with an electric fan, in a 10-mL beaker (heated at 35 °C on a heating plate when methanol was used) and then let to evaporate completely in the air. The residue was then recovered with an appropriate volume of the eluting mixture (0.5–10 mL), in order to bring the concentrations of the compounds to be determined into the calibration ranges, and then injected for HPLC analysis.

Recovery assays, at 0.02 ppm, 1.00 ppm, and at the maximum concentration found in the samples tested, showed values, obtained from four replicates, ranging between 89.2% and 101.0% for carbamates I–IV and between 70.0% and 76.7% for hydroxypyrimidines V–VII. Different extraction solvents were tested for these compounds, but only methanol allowed acceptable recovery percentages, even though not completely satisfactory.

Apparatus and Chromatography. Liquid chromatographs Varian 5020 and Spectra Physics SP 8700 were employed, equipped respectively with a variable-wavelength UV/vis UV 100 detector and a Rehodyne injector (loop 50 μL), and with an SP 770 spectrophotometric detector and a Valco AH 20 injector (loop 50 μL). The chromatographs were connected to HP 3390 A reporting integrators. Columns of Hibar RP-8 and NH_2 respectively (250 × 4.0 mm (i.d.), 10 μm; Merck, Darmstadt, West Germany) were used for the determination of I–IV with a 70:30 mixture of buffer solution (10^{-2} M monobasic potassium phosphate with 5 mL/L of glacial acetic acid) and acetonitrile and for the determination of V–VII with a 93:7 mixture of acetonitrile and water. In both cases the flow of the mobile phase was 1 mL/min. The wavelength was 245 nm for Pirimicarb and its metabolite II, 230 nm for III and IV, and 295 nm for V–VII, according to their UV spectra. Under these conditions and with the above-described extraction procedure, the detection limit was 0.02 ppm for each compound. Standard curves for Pirimicarb and its metabolites were constructed by plotting peak heights vs concentrations; a good linearity was achieved in the range 0–1.5 ppm with correlation coefficients between 0.9992 and 0.9997.

Chemicals. Acetonitrile, chloroform, and methanol were HPLC grade solvents (Carlo Erba, Milano, Italy); water was distilled twice and filtered through a Milli-Q apparatus (Millipore, Molsheim, France) before use. Monobasic potassium phosphate, glacial acetic acid, and sodium sulfate were of analytical grade (Carlo Erba). Pirimicarb and its metabolic products were supplied by ICI Solplant (Milano, Italy).

RESULTS AND DISCUSSION

Though the analytical method employed allows the determination of Pirimicarb and six of its metabolites (II–VII), only three (II, III, VI) were detected during our experiments.

The tuft weights and the concentrations of each single compound and of total carbamate (I + II + III) expressed as Pirimicarb are reported in Tables I and II for the trials carried out in the greenhouse and field, respectively.

The degradation of total carbamate and that of metabolite II were considered as functions of time under the assumption that they were pseudo-first-order kinetics. Data were processed by a statistical package for the analysis of the two-variable model (STATGRAPHICS, 1986) running on a P.C. Olivetti M 24.

The tuft growth and its relative diluting effect have been considered since the reduction of the residue (mg/

Table II. Residues (ppm ± SD) of Pirimicarb and Some of Its Metabolites (II, III, VI) on Lettuce in the Field after Treatments in Single and Double Doses

days after treatment	tuft wt, g	Pirimicarb	II	III	VI	total carbamate as Pirimicarb
			Single Dose			
0	205 ± 16	0.18 ± 0.04	4.27 ± 1.41	0.29 ± 0.10	nd[a]	4.52
1	235 ± 20	0.12 ± 0.03	1.07 ± 0.23	0.72 ± 0.23	nd	1.89
3	288 ± 30	0.06 ± 0.02	0.28 ± 0.14	0.62 ± 0.08	nd	0.98
6	312 ± 8	nd	0.06 ± 0.02	0.23 ± 0.05	0.04 ± 0.01	0.30
10	415 ± 10	nd	nd	0.03 ± 0.01	nd	0.03
			Double Dose			
0	196 ± 21	0.48 ± 0.10	9.79 ± 2.38	0.44 ± 0.06	nd	10.19
1	215 ● 7	0.36 ± 0.10	2.15 ± 0.74	2.22 ± 0.64	0.04 ± 0.01	4.63
3	245 ± 12	0.13 ± 0.04	0.39 ± 0.07	1.14 ± 0.08	0.09 ± 0.01	1.70
6	300 ± 26	0.06 ± 0.02	0.09 ± 0.03	0.44 ± 0.11	0.06 ± 0.02	0.61
10	388 ± 26	nd	nd	0.07 ± 0.03	nd	0.07

[a] nd = not detectable.

kg), caused by the increase in weight of the samples, was not to be considered as a degradation.

The best bivariate fitting of experimental data was the linear model

$$Y = a + bX$$

where Y = time in days and X = ln (molal concentration). In all the samples tested, the analysis of variance for the significance of the regression was $\ll 0.0001$.

With this model the theoretical half-life ($t_{1/2}$) of the residue was obtained by means of eqs 1 and 2, where K is a rate constant.

$$\ln (X/X_0) = -K(t - t_0) \quad (1)$$

$$t_{1/2} = (\ln 2)/K \quad (2)$$

The values of a, b (and the relative standard error, SE), and $t_{1/2}$ are reported in Table III, in which the correlation coefficients (r) and the determination coefficients (R^2) are also given.

In the trial carried out in the greenhouse (Table I), 1 day after the treatment, Pirimicarb, which was initially present in consistent amounts (7.25 ppm in SD and 15.65 ppm in DD), disappeared almost completely. The simultaneous presence of considerable residues of metabolites II and III, though not in stoichiometric quantities, should show that transformation of Pirimicarb into metabolites II and III does not necessarily represent the only means of AI loss. The major product appears to be metabolite II, which changes into metabolite III, so as to become undetectable 10 days after initiation of the experiment. Hydroxypyrimidine VI, derived from metabolite III by hydrolysis of the carbamate moiety, becomes detectable (in low amounts) 5 days after treatment, showing a considerable reduction on day 7 and absence on day 10.

Degradation of total carbamate (I + II + III) shows pseudo-first-order kinetics and a half-life a little greater than 1.5 days (1.67 days in SD and 1.54 days in DD). Taking into account that the half-life of metabolite II is 1.29 days in SD and 0.97 day in DD, this compound appears to affect the decay rate of total carbamate to a greater degree.

In the trial carried out in the field (Table II) the AI had almost completely been transformed into metabolites II and III from the very first sampling. The residue, considered total carbamate, showed lower initial values with respect to the corresponding values found in the greenhouse, but proportional when considering the reduced weight of the tufts.

When the two experiments are compared at first sampling, it can be seen that the residual data in the greenhouse are only due to the AI, which in the field is almost completely transformed into its metabolites II and III in 1 h. The only difference between the two samplings was that tufts in the field were perfectly dry while they were still wet in the greenhouse. Therefore, it could be supposed that, in the former case, the AI had already penetrated the leaves and that it had been quickly transformed biologically, while in the latter case the AI was still on the leaves and not yet degraded.

Braun et al. (1980) also reported a similar behavior for Pirimicarb, which was rapidly transformed into metabolites II and III immediately after treatment.

The quick degradation of Pirimicarb may mean that the decrease of the residue is not exclusively due to volatilization of the AI and that the aphicide action could also be ascribed to its metabolites.

In the field the degradative behavior of total carbamate ($t_{1/2}$ = 1.71 days in SD and $t_{1/2}$ = 1.61 days in DD) and metabolite II ($t_{1/2}$ = 1.06 days in SD and $t_{1/2}$ = 0.94 day in DD), as well as that of the other metabolites, present a course similar to that found in the greenhouse. The analogous kinetic behavior could be ascribed to similar microclimatic conditions. From the data reported in Table IV it can be seen that the mean temperature values in the greenhouse and in the field are similar, while the relative humidity in the greenhouse is much lower than in the field. These data should lead to greater tendency to evaporation in the greenhouse than in the field.

The wind speed, which averaged 1.3 m/s during the residue evaluation, could be supposed to have compensated for the lower tendency to evaporation due to the greater relative humidity. It is to be noted, however, that on the day of treatment the wind speed was 2.2 m/s, which accounts for the fact that the treated lettuce dried rapidly. Temperature and evaporation, which are the microclimatic parameters that most affect the decrease of Pirimicarb on plants, are to be considered similar both in the greenhouse and in the field. For this reason the analogous kinetic behavior in different operating conditions is justified. The moderate rainfall after the penultimate sampling could not have had any significant washing-away effect on the residues.

CONCLUSIONS

Though the microclimatic conditions were different in the two experiments, the degradative kinetics were similar in both cases, because in the field relative humidity and wind speed compensated for each other by their opposite effect on evaporation. In this way they must have determined a tendency to evaporation similar to that occurring in the greenhouse, where evaporation is only due to relative humidity.

The investigation carried out allowed verification of

Table III. Statistical Parameters of Degradation and Half-Lives of Total Carbamate (as Pirimicarb) and Metabolite II[a]

	applicn rate	a	b	SE	r	R^2	$t_{1/2}$, days
			Total Carbamate				
greenhouse	SD	−24.845	−2.409	0.119	−0.974	0.949	1.67
	DD	−21.349	−2.223	0.103	−0.978	0.957	1.54
field	SD	−26.915	−2.460	0.098	−0.986	0.972	1.71
	DD	−23.637	−2.325	0.108	−0.981	0.963	1.61
			Metabolite II				
greenhouse	SD	−20.517	−1.858	0.181	−0.956	0.914	1.29
	DD	−13.661	−1.403	0.082	−0.977	0.955	0.97
field	SD	−17.230	−1.530	0.115	−0.963	0.927	1.06
	DD	−14.288	−1.359	0.105	−0.961	0.923	0.94

[a] The parameters indicated refer to the linear model $Y = a + bX$ in which Y = time after spraying (days) and x = ln (molal concentration).

Table IV. Microclimatic Conditions[a] in the Greenhouse (G) and Field (F) during the Period of Residue Evaluation

days after treatment	rel humidity, %		temp, °C		wind speed, m/s:	rainfall, mm:
	G	F	G	F	F	F
0	71	94	22.2	23.0	2.2	
1	71	97	20.5	22.0	2.3	
2	69	96	20.5	23.0	2.2	
3	69	96	21.5	19.5	1.1	
4	67	97	22.5	19.0	1.0	
5	66	91	23.5	19.5	1.0	
6	65	87	23.0	20.0	1.0	1
7	68	97	22.2	22.0	1.3	7
8	68	97	24.0	19.0	1.2	
9	73	96	23.2	20.5	1.0	
10	72	87	23.5	20.3	1.0	

[a] Daily averages.

the presence of Pirimicarb metabolites II and III (the major degradative products) and VI (detected at a very low level).

Furthermore, the rapid degradation of Pirimicarb also raises the problem of establishing different maximum residue limits and preharvest intervals according to whether this pesticide is considered only as AI or as total carbamate.

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