12. WAHAB, A. H., P. AITKEN and I. E. JOHNSON. Paper presented at Seminar on Rural Development held under the aegis of Iowa State University and IICA/OAS; Ames, Iowa, U.S.A. Sept. 29 – Oct. 3, 1980. IICA/Jamaica Publication No. IV – 16.

13. WAHAB, A. H., P. AITKEN–SOUX, I. E. JOHNSON and H. MURRAY. Paper presented at Seminar on Cropping Systems, Ja. School of Agriculture, Twickenham Park, Jamaica, Dec. 4, 1980. pp. 11-39. IICA/ Jamaica Publication No. IV – 21.

Behaviour of ^{14}C-metalaxyl in Brazilian soils.

Resumo. Estudou-se em laboratório o comportamento do ^{14}C-metalaxyl em três tipos de solos brasileiros (Gley Húmico, Latossolo Roxo e Latossolo Vermelho Escuro). O ^{14}C-metalaxyl apresenta alta mobilidade nas três cromatografias de solo e baixos coeficientes de sorção, mesmo em solos com alto teor de matéria orgânica (Gley Húmico e Latossolo Roxo) e foi persistente nos três solos pelo período estudado de 8 meses. A degradação do metalaxyl no solo Latossolo Roxo correspondeu após esse tempo à 29%, representada por um metabolito nao identificado detectado pela cromatografia em camada delgada. A população bacteriana foi maior no Latossolo Roxo e provavelmente associada à esse processo de degradação

Metalaxyl is a new systemic fungicide (CGA 48988) (DL-methyl-N (2, 6-dimethyl-phenyl) -N-(2-methoxyacetyl) alaninate) with specific activity against pathogens belonging to the order Peronosporales (1, 2, 6, 7). The systemic activity of metalaxyl in azalea by applying it to the soil has been demonstrated (1). The structure of systemic fungicides applied to the soil can be altered by chemical breakdown process and by action of the microorganisms. Also uptake of fungicides by plants from the soil can be very inefficient if there is a tight adsorption of the fungicide to the soil.

The purpose of this investigation was to obtain information on the behaviour of this new compound when applied to samples of Brazilian soils.

Materials and methods

Chemicals

Metalaxyl WP 25 was provided by Ciba Geigy, Brazil and ^{14}C-metalaxyl by Ciba Geigy, Basel, Switzerland. The ^{14}C-metalaxyl uniformly ring labelled had a specific activity of 46.5 μCi/mg.

Soils

The three soil types used, consisted of Humic Gley, Red Latosol and Dark Red Latosol, differing in their properties (Table 1). Ten gram samples of each dried soil were added to wide necked screw capped jars, followed by addition of water to 2/3 field capacity. A week after, 1.0 ml of a mixture of ^{14}C-ring labelled metalaxyl (140.000 dpm/ml) and unlabelled metalaxyl (10 ppm) in acetone, was added on each soil sample. During the test, water was added periodically to maintain the moisture content of the soil. This was achieved by monthly quantity of distilled water to attain the desired soil water content. Duplicate samples of each soil were incubated at 20 – 25°C and the entire flasks content were extracted at monthly intervals over an 8-month period.

Extraction of ^{14}C-metalaxyl

Each sample was extracted with 50 ml of methanol, by shaking the mixture for 4 hr. When necessary a sequence of two extractions with methanol was realized. The soil was separated by

Table 1. Properties of the soils

Soil Type	Organic matter (%)	Clay (%)	Sand (%)	pH	Microbial population after 8 months (g of soil) 10^3 Bacteria	Fungi
Humic Gley	4.3	32	57	5.7	34	4.7
Red Latosol	3.8	7.72	62	5.3	136.7	6.7
Dark Red Latosol	2.0	63	24	4.8	11.4	7.7

allowing to stand overnight. The extracted products in methanol were quantified by scintillation counting and thin-layer chromatography.

Scintillation counting

A Nuclear Chicago Liquid Scintillation Spectrometer, model Mark 1, was used for counting. Aliquots of each sample were transferred to scintillator vials, and after evaporation of methanol the scintillation counting solution was added (5). Recovery of ^{14}C-metalaxyl is presented as percent ^{14}C representing the ^{14}C recovered on each extraction over the ^{14}C-metalaxyl added.

Thin-layer chromatography (tlc)

Silica gel thin-layer plates (Merck F 256) were used for separation of extracted samples: 10 ml samples of soil extracts were concentrated and spotted on the plates, developed in the solvent systems either benzene:ether (BE) (50:50) or acetone: ether: acetic acid (Ace Eth AcoH) (79:20:1). ^{14}C-metalaxyl was chromatographed at the same time as reference.

After drying, the plates were exposed to X-ray films. The amount of degradation was determined by dividing the plates in 1 cm zones, scraping the silica into scintillation vials and assaying by scintillation counting (4).

Values were calculated as percent degradation representing the dpm per spot over the sum of dpm's in the plates.

Combustion technique

Residues of ^{14}C-metalaxyl, together with any ^{14}C-labelled degradation products in the soils not recovered after a sequence of two extractions by methanol, were determined by soil combustion (8).

Adsorption experiment

^{14}C-metalaxyl solution (10 ml = 2.5 μg) in 0.01 M calcium chloride was added to air dried soils (1.0 g) in wide necked screw capped jars. After shaking for 4 hr the soil was allowed to stand, and 1.0 ml aliquot of the supernatant solution taken for ^{14}C assay. The soil/water distribution coefficient (K) was calculated as concentration on soil (dpm/g) over the equilibrium solution concentration (dpm/ml).

Soil thin-layer chromatography

Mobility of the fungicide in the soils was determined by soil tlc techniques, and ^{14}C-metalaxyl in the plates was located by autoradiography (3, 4).

Soil microbial population

Soil microorganisms were enumerated at the end of the study by soil dilution. Soil dilutions were plated in triplicate on Czapek-Dox medium agar, and the plates incubated at 28°C for one week. The Czapek-Dox medium consisted of (g/l): saccharose: 30, $NaNo_3$: 3, K_2HPO_4: 1, $MgSO_4$. $7H_2O$: 0.5, KCl 0.5, $FeSO_4$ $7H_2O$:0.1 and agar 15. Bacteria

Table 2. Recovery of radiocarbon (%) from soils treated with ^{14}C-metalaxyl

Soil Type	Methods for determining radioactivity	Time (days)							
		0	30	60	90	120	150	210	240
Humid Gley	1st Extraction	95	92	71	72	47	62	75	66
	2nd Extraction	–	–	27	25	12	15	10	13
	Wet Combustion	–	–	–	–	21	17	14	11
	Total	92	92	98	97	80	94	99	90
Red Latosol	1st Extraction	92	82	56	62	47	51	55	55
	2nd Extraction	–	–	30	33	13	11	15	14
	Wet Combustion	–	–	–	–	35	26	25	18
	Total	92	82	86	85	95	88	95	87
Dark Red Latosol	1st Extraction	95	95	69	70	51	66	73	67
	2nd Extraction	–	–	25	19	11	10	13	9
	Wet Combustion	–	–	–	–	18	16	13	10
	Total	95	95	94	89	80	92	99	86

were counted after 3 days and fungi colonies after 7 days. Populations are expressed as colonies per gram of soil.

Results and discussion

Recovery of metalaxyl from soils as a function of incubation time is presented on Table 2. After a 30 days incubation of ^{14}C-metalaxyl in soils, one solvent extraction was sufficient to recover most of the radiocarbon added to the three soils. After 60 days a second extraction of the samples with methanol was necessary to recover the same amounts of radiocarbon. As ^{14}C-metalaxyl aged in the soils, it was necessary to combust the samples to recover the amount of radiocarbon not extracted by methanol (Table 2). During the period of time from 120 to 240 days, values of radiocarbon adsorbed to the soils and detected by wet combustion were in the range of 10 to 30%. Red Latosol was the soil that showed the higher percentage of radiocarbon not recovered by the solvent and these amounts can be considered as bound residues or degradation of metalaxyl to more polar compounds, not extracted by methanol (Table 2). Comparing with results obtained in the same soils with a benzimidazole fungicide like carbendazim (5) metalaxyl showed to be less adsorbed to the soils and easily extracted by the organic solvent.

Thin-layer chromatography of soil extracts showed that most of the recovered radiocarbon chromatographed was ^{14}C-metalaxyl; however a metabolite was detected in higher amounts in Red Latosol extracts. The percentage of this metabolite (Rf 0, 10 in BE), varied from 14% in the 60 days to 29% in 240 days extracts (Table 3).

When this compound was eluted from plates developed in BE, followed by further chromatography in Ace Eth AcoH the activity was located in a zone of tlc at Rf 0.3 to 0.4; ^{14}C-metalaxyl had an Rf 0.67 in this solvent. The second methanolic extraction from soils incubated over an 8 month period with ^{14}C-metalaxyl showed after chromatography the same pattern of degradation, as assessed by tlc.

Red Latosol supported a steady increase in the degradation. This observation may be related with the greater bacterial population noted in the Red Latosol and therefore the reduced degradation in Dark Red Latosol, a consequence of a much less active microbial population (Table 1). Though in less amount this metabolite is also detected in Humic Gley soil; however the variation at each sampling time is probably a consequence of the fluctuating microbial population with time.

Sorption coefficients of ^{14}C-metalaxyl in Humic Gley, Red Latosol and Dark Red Latosol were respectively of 0.80; 0.56 and 0.36. Sorption results are in agreement with the high mobility of ^{14}C-metalaxyl on soil thin-layers of Dark Red and Red Latosol soils and to an intermediate mobility of the fungicide on Humic Gley soils (Figure 1).

Table 3. Percent of matalaxyl and a metabolite detected in extracts from three soils incubated with ^{14}C-metalaxyl.

Soil Type	Compounds	Days after treatment					
		30	60	90	120	210	240
Humic Gley	Metalaxyl	82	80	78	79	72	72
	Metabolite	4.0	5.0	6.3	4.6	2.0	10
Red Latosol	Metalaxyl	83	80	63	74	64	68
	Metabolite	6.7	14	17.4	12.6	13	29
Dark Red Latosol	Metalaxyl	85	82	89	77	72	75
	Metabolite	2.0	5.5	5.1	5.3	3.4	4.2

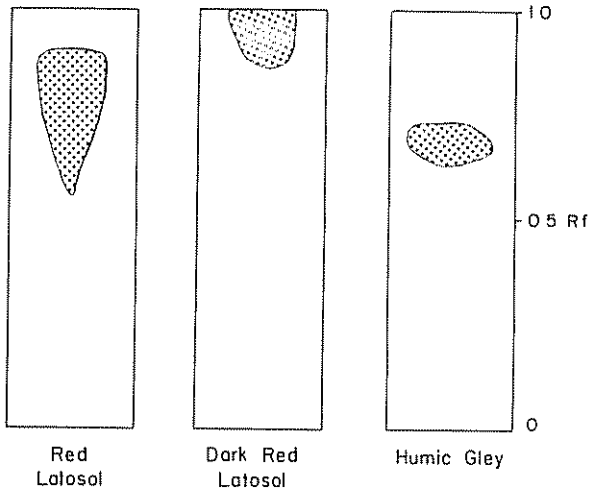

Fig. 1. Movement of ^{14}C-metalaxyl on soil thin-layer plates.

The data obtained in laboratory tests indicate a persistence of ^{14}C-metalaxyl in soils and therefore its low sorption coefficients associated with its systemic activity (2, 6) indicate a long term availability for the control of soil plant pathogens. However, its low sorption coefficient and the high mobility also indicated a facility for leaching, and this must be considered in field applications

Over an 8-month period, only one metabolite was detected from ring – labelled metalaxyl, indicating a high stability of the benzene ring to microbial attack and that degradation in these soils proceeded probably by hydrolization in the side chain of the molecule.

Persistence of metalaxyl showed to be dependent on soil type and the degradation process probably associated with a bacterial population on soils.

Summary

Behaviour of ^{14}C-metalaxyl in three types of Brazilian soils (Humic Gley, Red Latosol and Dark Red Latosol) was studied in the laboratory. Metalaxyl had high mobility on the three soils thin-layers and low sorption coefficients, even in soils with high organic matter content (Humic Gley and Red Latosol). Metalaxyl was persistent on the three soils over an 8-month period. Degradation of metalaxyl in Red Latosol was 29 percent after this time, represented by a non identified metabolite detected by tlc analysis. Bacterial population was higher in Red Latosol and probably associated with this degradation process.

September 15, 1981

M. RAPHAELA MUSUMECI*
ELZA F. RUEGG*
C. A. CAMPACCI**

* Scientific Researchers, Radioisotope Centre, Biological Institute, S. Paulo, Brazil.

** Scientific Researcher, Fungicide Dept., Biological Institute, S. Paulo, Brazil.

Literature cited

1. BENSON, D. M. Efficacy and **in vitro** activity of two systemic acylalanines and ethazole for control of *Phytophthora cinnamomi* root rot disease of azalea. Phytopathology 69:174-178. 1979.

2. COHEN, Y., M. REUVENI and H. EYAL. The systemic activity of Ridomil against *Phytophtohora infestans* on tomato plants. Phytopathology 69:645-649. 1979.

3. HELLING, C. S. and B. C. TURNER. Pesticide mobility. Determination by soil thin-layer chromatography. Science 162:562-563. 1968.

4. HELLING, C. S., D. GAYLE-DENNISON and D. D. KAUFMAN. Fungicide movement in soils. Phytopathology 64:1091-1100. 1974.

5. MUSUMECI, M. R., K. A. LORD and E. F. RUEGG. Adsorption movement and persistence of carbendazim in Brazilian soils. Arq. Inst. Biol. 47(1/2):9-14. 1980.

6. STAUB, T., H. DAHEMN and F. J. SCHWINN. Biological characterization of uptake and translocation of fungicidal acylalanines in grape and tomato plants. Plant Disease and Protection 85:162-168. 1978.

7. URECH, P. A., F. J. SCHWINN and T. STAUB. CGA 48988, a novel fungicide for the control of late blight, downy mildew and related soilborne diseases. Proc. British Crop Protection Conference 623-631. 1977.

8. SMITH, G. H., P. D. LUDWIG, K. C. WRIGHT and W. R. BAURIEDEL. Simple apparatus for combustion of samples containing ^{14}C-labelled pesticides for residue analysis. Journal Agriculture and Food Chemistry 12:172-175. 1964.