The filtrate from acetone treatments was evaporated to a dark-brown oil which was triturated with water (50 mL) to yield a tan residue which was filtered off (5.4 g) and found by mixed melting point to be identical with coumarin starting material.

The monoacetate III of the methyl ester of the acid I was prepared by heating the acid I with acetic anhydride and quenching the reaction with excess methanol to yield a precipitate which melted at 146–148 °C after recrystallization from hot water.

Anal. Calcd for $C_{12}H_{15}O_4N$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.9; H, 6.36; N, 5.88.

The dibenzoate of the methyl ester of β-aminohydrocoumaric acid was prepared from the acid I by the Schotten-Baumann reaction with benzoyl chloride. The oily product mixture was taken up in methanol, and water was added to induce crystallization. The product, on recrystallization from aqueous methanol, melted at 146–147 °C.

Anal. Calcd for $C_{24}H_{21}O_5N$: C, 71.45; H, 5.25; N, 3.47. Found: C, 71.5; H, 5.18; N, 3.47.

The monoacetate and dibenzoate of methyl β-aminohydrocoumarate were prepared from β-aminohydrocoumaric acid which in turn was prepared from coumarin with hydroxylamine by the method of Posner (1909) and found to be identical with those from the reaction with ammonia.

β-**Aminohydrocoumaramide (II).** Finely ground coumarin (10 g) was suspended in 28% aqueous ammonia (250 mL) and allowed to stand for 20 days at room temperature in subdued light with occasional stirring. The deep-green reaction mixture was evaporated down to small volume with a rotary vacuum evaporator under water aspirator vacuum and at a bath temperature of 40 °C. The brown syrupy residue was extracted with four 250-mL portions of chloroform, and the combined extracts were evaporated on the steam bath to 100 mL at which point crystals began to appear. The mixture was cooled and 3.3 g of white crystalline material was recovered. Recrystallization from a large volume of chloroform yielded clusters of blades of β-aminohydrocoumaramide, mp 125–126 °C.

Anal. Calcd for $C_9H_{12}O_2N_2$: C, 59.98; H, 6.71; N, 15.55. Found: C, 59.7; H, 6.58; N, 15.3.

The diacetate IV of the amide II was prepared by heating on the steam bath for 3 min 0.2 g of II in 5 mL of acetic anhydride containing 0.2 g of freshly fused sodium acetate. The reaction mixture was poured into water and a white solid was collected, yielding felted needles from acetone–methanol, mp 228–230 °C.

Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 59.2; H, 6.14; N, 10.6.

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B. J. Bergot
William L. Stanley
M. Sid Masri*

Western Regional Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Albany, California 94710

Microbial Transformation of S-Methyl N-[(Methylcarbamoyl)oxy]thioacetimidate (Methomyl) in Soils

Microbial transformations of methomyl in two tobacco-growing soils were determined in a perfusion study where the soils (10 g) were perfused with aqueous solutions (230 cm³) containing 6 ppm of methomyl with and without sodium azide. Adsorption of methomyl was indirectly assessed. The contribution of adsorption to the loss of methomyl from solutions was small (approximately 5%) when compared with that of microbial transformation. Microbial transformation of methomyl in both soils occurred after a lag phase of about 7 to 14 days. However, in enriched soils, transformation occurred with virtually no lag phase.

The application of methomyl to soil in transplant water to control tobacco yellow dwarf in Australia necessitates the investigation of the behavior of methomyl in soil. It has been shown that methomyl decomposes in soil (Harvey and Pease, 1973); however, individual processes influencing the behavior of methomyl in soil have not been investigated. Microbial transformation and adsorption would appear to be the most important processes affecting the

behavior of methomyl in soil. The combined effects of microbial transformation and adsorption of methomyl in two soils were determined (experiment 1) by perfusing the soils with solutions of methomyl and by measuring the changes in concentration of methomyl in the perfusing solutions at regular intervals. To assess indirectly the effect of adsorption (experiment 2) sodium azide was added to the solutions to prevent any microbial trans-

Table I. Some Chemical and Physical Characteristics of Soils I and II

Characteristics	Soil I	Soil II
Field texture	Fine sandy loam	Fine sandy clay loam
Color	Greyish brown	Greyish yellow
Munsell notations (dry)	10 YR 5/3	7.5 YR 5/6
pH (1:5 H_2O)	6.1	5.8
Organic matter, %	2.1	2.3
Exchangeable cations, m.e.%[a]		
Ca^{2+}	1.2	0.9
Mg^{2+}	0.3	0.3
K^+	0.7	0.7
Na^+	<0.1	<0.1
H^+	8	10.2
C.E.C., m.e.%	10.2	12.1
Clay, %	10.1	19.3
Composition of clay, %		
Kaolinite	35	20
Chlorite	10	5
Mica	40	50
Vermiculite	15	25

[a] m.e.% = milliequivalents per 100 g of dry soil.

formation from taking place. The phenomenon of enrichment of the soils (Audus, 1960) was investigated (experiment 3) by perfusing the soils which had been exposed to methomyl in experiment 1 with fresh solutions of methomyl.

EXPERIMENTAL SECTION

Origin and Preparation of Soils. Two tobacco-growing soils collected from the northeast of Victoria (Australia) were used: Myrtleford fine sandy loam (soil I) and Ovens fine sandy clay loam (soil II). Some chemical and physical characteristics of these soils are listed in Table I. The top 15 cm of the soils was sampled from areas where methomyl had been applied previously, air-dried at room temperature, ground, and passed through a 2-mm sieve. The soils were then treated with polyvinyl alcohol to give a final concentration of 0.5% (w/v). The soil conditioner was added to maintain the soils in a flocculated condition which was required for adequate drainage in the perfusion experiments. The soils were analyzed for methomyl residues using the GLC method described by Fung (1976).

Perfusion Apparatus. The apparatus employed (Figure 1) was a modification of the one used by Kearney et al. (1965). Twelve identical units of the apparatus were connected in series and a common source of air pressure for the units was provided by a cylinder of commercial compressed air. In order to maintain an equal pressure head of the solution in each unit, the distance (X) between the Y piece (j) and the top of the leak tube (m) was kept constant (Figure 1). The incorporation of a stainless steel hypodermic needle (Luer lock 26G × 2.5 cm) into each air line ensured an equal flow of air through each unit.

Perfusion Experiments. Four 10-g portions of each soil were placed in eight of the chromatography columns and the remaining four columns were left empty as blanks. The soils were equilibrated by perfusing the columns with a saturated aqueous solution of calcium sulfate (230 cm^3) at a rate of 8 cm^3 min^{-1}. The saturated solutions were used to prevent dispersion of soil colloids. After 2 days, the solution in each unit was replaced with an equal volume of a saturated aqueous solution of calcium sulfate containing 6 ppm of methomyl in experiment 1 and, in experiment 2, with an equal volume of a similar solution containing both the methomyl and 10^{-3} M sodium azide (15 mg). The concentration of methomyl used was calculated as being the approximate concentration which can be expected in the soil solution after the transplantation
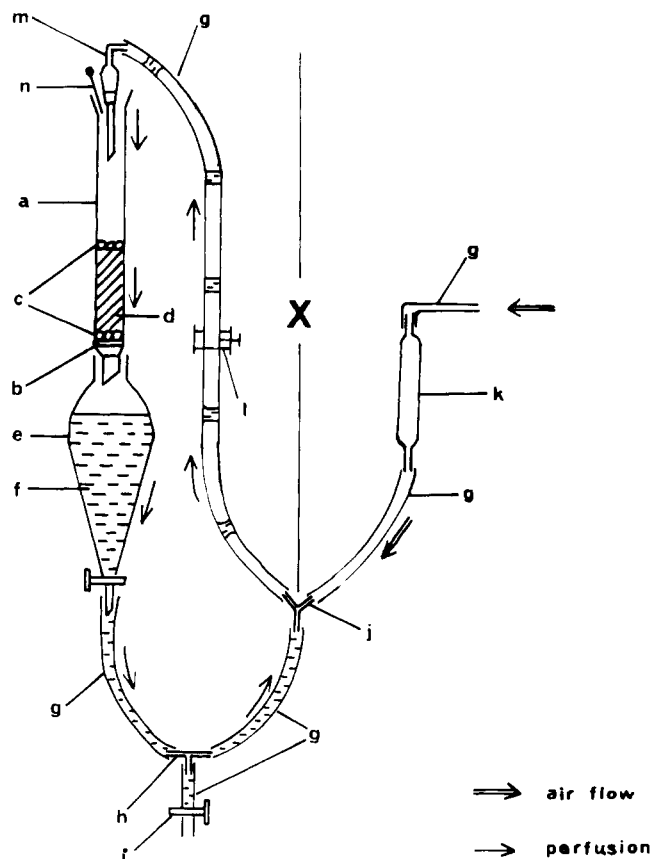

Figure 1. Perfusion apparatus: (a) chromatography column with integral sinter; (b) sinter (porisity 0); (c) glasswool; (d) soil; (e) 250-cm^3 separating funnel; (f) perfusing methomyl solution; (g) polyvinyl chloride tubing; (h) T-piece Kartell connector; (i) tap; (j) Y-piece Kartell connector; (k) trap; (l) Hoffman screw clip; (m) leak tube; (n) air-leak.

of tobacco plants in the field. In the three experiments which were carried out, the rate of perfusion was 8 cm^3 min^{-1} and the ambient temperature was 25 °C.

(a) *Experiment 1: Investigation of Microbial Transformation and Adsorption.* Duplicate samples of each soil and two blanks were perfused with the solutions containing methomyl. On the third day and at weekly intervals for 6 weeks after the perfusion had commenced, a sample (10 cm^3) of solution was taken from each unit to be analyzed for methomyl using the GLC method for the determination

of methomyl in water described by Fung (1976).

(b) *Experiment 2: Investigation of Adsorption.* This experiment was the same as experiment 1 except that the perfusing solutions used contained both methomyl and sodium azide; the experiment was run at the same time as experiment 1. After 21 days, it was suspected that the azide may have become ineffective and so an additional 10 mg of sodium azide was added to the solutions (170 cm³) at the end of the experiment. Further samples of solution were taken for analysis at weekly intervals for another 2 weeks.

(c) *Experiment 3: Investigation of Enrichment.* At the end of experiment 1, the soils which had been exposed to methomyl together with the two blanks were perfused further with fresh solutions of methomyl for another 6 weeks. Sampling and analyses were carried out in the same way as described in experiment 1.

RESULTS AND DISCUSSION

Methomyl residues detected in soils I and II prior to the perfusion experiments were 0.004 and 0.01 ppm, respectively; these small levels of methomyl were unlikely to affect the magnitude of the results of these experiments.

(a) **Experiment 1.** The concentration (6 ppm) of methomyl present in the solutions perfusing the blanks remained unaltered for 28 days. From 35 days onward, the concentration of methomyl was found to be 5.6 ppm. This decrease in concentration was also detected in experiments 2 and 3 and although it may be attributable to some experimental error, it could also be due to some absorption of methomyl by the polyvinyl chloride tubings and some chemical transformations of methomyl which were not expected to occur.

Initially (at 3 days) the loss of methomyl (5%) from solutions perfusing both soils was small (Figure 2). However, at 42 days, 58% of the methomyl had disappeared from the solution perfusing soil I while only 38% of methomyl had disappeared from the solution perfusing soil II. Some of these results are comparable to those reported by Harvey and Pease (1973) in their laboratory experiments where [¹⁴C]methomyl was added to three different soils. They found that 52 to 69% of the [¹⁴C]-methomyl was lost after 42 days, while in field studies in summer and still in the absence of plants, more rapid decomposition of methomyl occurred.

(b) **Experiment 2.** Initially (up to 21 days) the loss of methomyl from the azide-treated solutions perfusing the soils was only 3 to 5%. However, there was a substantial increase in loss after 21 days (Figure 2) which presumably resulted from the azide becoming partially ineffective. Evidence for this explanation was obtained when no further loss of methomyl was observed following the further addition of sodium azide to the solutions. Normally, the reactions of adsorption and desorption are expected to reach equilibrium within a few days. Therefore, if the azide had been fully effective, the loss of methomyl, e.g., after 7 days may have been attributable to chemical transformation. However, since it appears that the azide lost some of its effectiveness, then even if chemical transformation contributed to the loss of methomyl observed after 7 days, the loss would be small.

After about 21 days, the rate of loss of methomyl from solution in the presence of azide was much less than the rate of loss in experiment 1 even though the azide had lost some of its effectiveness (Figure 2). This suggests that microbial transformations were responsible for the greater part of the loss in experiment 1. The changes in concentration in and the losses of methomyl from solution resulting from microbial transformation were calculated
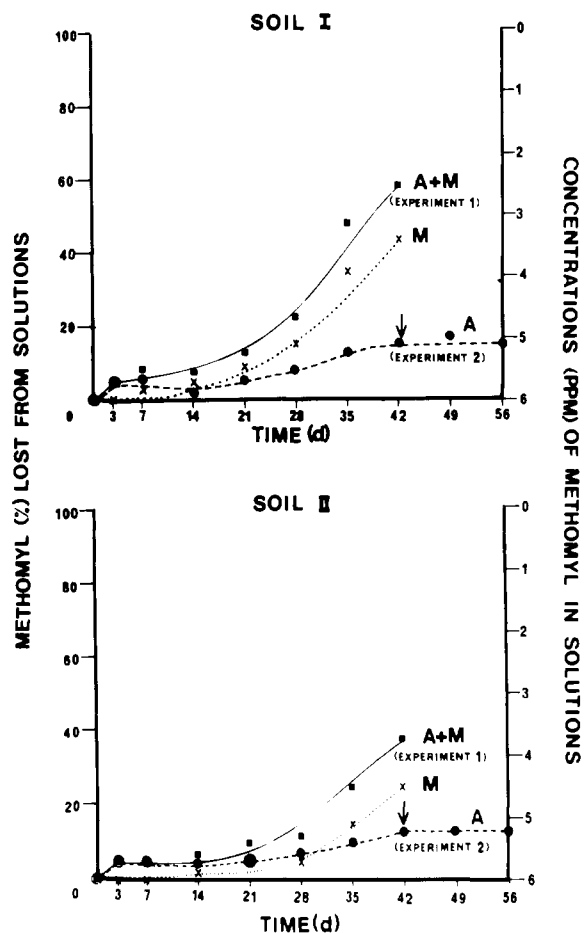

Figure 2. Effects of microbial transformation and adsorption on rate of loss of methomyl and on changes in concentration of methomyl in solutions: (A + M) presumably adsorption + microbial transformation (measured in experiment 1); (A) adsorption (measured in experiment 2); (M) microbial transformation (calculated from the results of experiments 1 and 2); (↓) additional sodium azide introduced into the perfusing solution.

from the results obtained from experiments 1 and 2. Microbial transformations in both soils began after a lag phase of 7 to 14 days (Figure 2). The time taken for half of the methomyl to disappear (half-life) in soil I was 5 to 6 weeks while that in soil II was longer. The half-life of a structurally similar carbamate pesticide, namely aldicarb, was found to be 9 to 12 days in three different soils (Coppedge et al., 1967).

The polyvinyl alcohol used to condition the soils could provide an excellent source of carbon to the microorganisms, and it is possible that the soil conditioner was being metabolized together with the methomyl during the perfusion experiments.

The relative contributions of microbial transformation and adsorption to the losses of methomyl from solution are illustrated in Figure 2. The contribution of adsorption was small (approximately 5%) when compared with that of microbial transformation. Assuming that the adsorption isotherm of methomyl is linear, i.e., $n = 1$ in the Freundlich equation (Hamaker and Thompson, 1972), and that after 28 days equilibrium was reached between the soil (10 g) and the perfusing solution (190 cm³), an approximate distribution adsorption coefficient (K_d) of about 2 was calculated. It would be unwise to speculate as to the degree of adsorption of methomyl because the K_d of methomyl could be changed substantially by altering the soil to solution ratio (Grover and Hance, 1970), and because no precise data exist on the adsorption behavior of methomyl
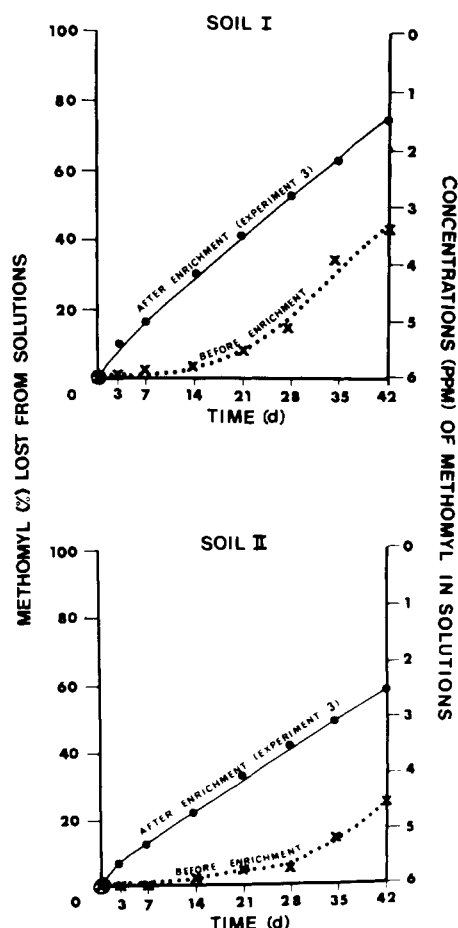
Figure 3. Effects of microbial transformation on rate of loss of methomyl and on changes in concentration of methomyl in solutions before and after enrichment of soils I and II.

(e.g., value of n). Cognizance should also be taken of the fact that the polyvinyl alcohol in the soils could affect the degree of adsorption of methomyl.

(c) **Experiment 3.** Since this experiment was run after experiment 1, most sites of adsorption for methomyl in the soils were probably occupied and the contribution of adsorption to the loss of methomyl from solution was expected to be small (certainly less than 5%). Therefore, the rapid loss of methomyl in experiment 3 (Figure 3) can be attributed largely to microbial transformation. Since virtually no lag phase was detected it would appear that the treatment of the soils with methomyl in experiment 1 resulted in enrichment. However, after about 3 days the rate of loss of methomyl was constant, and after about 21

days the rate of loss was similar to that before enrichment (Figure 3). Whereas in another situation (Kearney et al., 1965) where enrichment has been described, not only has the duration of the lag phase decreased but also the rate of transformation has increased. Therefore, in the absence of control soils (i.e., soils untreated with methomyl) in experiment 3, it is not absolutely certain if the soils used in the experiment were enriched. The important issue here, with reference to the field situation, is that the time required for the concentration of methomyl to be reduced to half of the initial concentration will probably be less in soils recently treated with methomyl (Figure 3).

CONCLUSION

The contribution of adsorption to the dissipation of methomyl from solutions was small when compared with that of microbial transformation. Therefore the results of the perfusion studies, although of limited applicability to the field situation, show that microbial transformation is likely to be of major importance in determining the behavior of methomyl in soils.

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Kenneth K. H. Fung *
Nicholas C. Uren [1]

Division of Agricultural Chemistry
Department of Agriculture
Melbourne, Victoria, 3002, Australia
[1]Present address: School of Agriculture
La Trobe University
Bundoora, Victoria, 3083, Australia

A Clean-Up Procedure for HPLC Analysis of Aflatoxins in Agricultural Commodities

A minicolumn method has been modified as a simple, efficient clean-up procedure for use of high-pressure liquid chromatography for determination of aflatoxins in peanuts, rice, and corn. The procedure, which utilizes a heavy metal salt precipitation combined with a short alumina filtration under vacuum, can be easily adapted to various sample sizes and provides adequate clean-up for use with both microparticulate and pellicular solid support columns.

The analysis of aflatoxins by high-pressure liquid chromatography has been reported by Rao and Anders (1973), Seiber and Hsieh (1973), Seitz (1975), and Pons

(1976). None of these authors reported a clean-up procedure to remove interferences. A procedure, which Holaday and Lansden (1975) used to qualitatively de-