tural soils, especially tobacco, vegetable, and orchard soils. Work in the United States, particularly by Lichtenstein and his coworkers, has shown that certain crops will absorb residues of the cyclodiene insecticides from soils in amounts dependent on both climate and soil type. To establish the significance of these residues in soil, a logical extension of this program would be to investigate, under practical agricultural conditions, the absorption of pesticides from different soil types by root crops. In addition, because of the rotation pattern utilized in tobacco- and turnip-growing areas, which involves both forage crops and cereals, studies on the amount of absorption should also be initiated in this area. The former study was initiated in 1965; the latter is being undertaken in 1966.

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RESIDUE DETERMINATION

Residues in Tissues of Fish Killed by Antimycin

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Highly radioactive antimycin was obtained by reformylation of deformylantimycin with H^3-formic acid, and was used to kill trout and carp at 5 and 10 p.p.b. water concentrations. Antimycin levels in the fish tissues were estimated from their H^3 content. This method probably gives high results because of degradation and protein binding of the toxicant. Tissue concentrations so observed ranged from 30 to 950 μg. per kg. of fresh weight. Edible portions averaged 76 to 201 μg. per kg., while heart, liver, and kidney averaged 736, 683, and 388 μg. per kg., respectively. Whole body levels averaged 203 μg. per kg. Concentrations in carp were 2 to 3 times higher than in trout, probably because of greater resistance and longer survival times. The levels found are so low that no harmful effects would be anticipated from use of antimycin-poisoned fish in animal feeds. Preliminary evidence suggests that such fish probably are also safe for human consumption.

ANTIMYCIN (*14*) is a potent fish poison (*2*) and has other properties which give it promise as a fish control agent (*17*). The possibility of its widespread use for this purpose raised the question of whether fish killed in this manner are safe for animal or human consumption. A determination of the residual antimycin levels in the tissues of such fish was therefore undertaken.

Since the antimycin concentrations to be anticipated were so low (*2*) that they probably could not be estimated by available assay procedures (*12, 13*), it was decided as a first attempt to rely on radioactively labeled antimycin, provided a product with sufficiently high specific activity could be obtained. This objective was accomplished by reformylation of deformylated antimycin with H^3-formic acid. The preparation obtained was used for the studies reported in this paper.

Experimental

Materials and Methods. DETERMINATION OF RADIOACTIVITY. All counting was done in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003, equipped with a Packard automatic standardizer, Model 3951. The phosphor solution was made from 15.0 grams of 2,5-diphenyloxazole (PPO), 0.19 gram of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), 3000 ml. of toluene, and 750 ml. of absolute ethanol. All scintillation vials were filled with 18 ml. of the solution to be counted.

DEFORMYLANTIMYCIN A_3 HYDROCHLORIDE. This compound was obtained by acid hydrolysis of blastmycin [antimycin A_3 containing a small proportion of A_4 (*7*)] as described previously (*16*). After recrystallization from ethanol and concentrated hydrochloric acid, it gave an infrared spectrum identical with that of an authentic specimen (*11*). Further confirmation of the identity of the deformylated compound was obtained by reformylation (as described below) with nonradioactive formic acid. The product, obtained in 75% yield, showed melting point of 168–172° C.;

mixed melting point with blastmycin (melting point 166–168° C.), 165–168° C. The infrared spectrum and enzyme inhibition activity (*3*) were indistinguishable from those of an authentic sample of blastmycin.

H^3-Formic Acid. Tritiated formic acid (Nuclear Research Chemicals, Orlando, Fla.) was received in 0.1-ml. sealed glass ampoules containing 0.25 mmole each. The contents of one ampoule were dissolved in absolute ethanol, and several aliquots were removed for radioactivity measurements. Another aliquot was added to a slight excess of aqueous sodium hydroxide solution, the mixture evaporated to dryness, and the radioactivity of the residue determined after taking up in dilute aqueous hydrochloric acid. Specific activities found were 74 mc. per mmole before and 10 mc. per mmole after the sodium hydroxide treatment. The latter value was assumed to represent the carbon-bound, nonexchangeable H^3 content.

H^3-Antimycin. An ampoule containing 0.25 mmole of the above described H^3-formic acid was cooled in a dry ice–acetone bath and opened by snapping off a small portion of the neck. Acetic anhydride (30 μl., 0.32 mmole) was added and the flask was resealed rapidly, heated in a water bath at 60° C. for 2 hours, and then allowed to stand at room temperature for 18 hours. After this time, the ampoule was reopened, and the contents were transferred to a 2-ml. vial containing 26 mg. (0.049 mmole) of deformylantimycin A_3 hydrochloride dissolved in 0.2 ml. of chloroform. The vial was capped, allowed to stand in the dark at room temperature for 18 hours, and the volatile components then were removed in a stream of nitrogen. The residue was dissolved in chloroform, the solution extracted with water, and the chloroform layer again evaporated to dryness under nitrogen. The residue, after recrystallization from ethyl acetate–pentane, keeping the temperature at 27° C. or below at all times, melted at 166–168° C. and was obtained in a yield of 18.3 mg. (72%).

The recrystallized product was as active in inhibiting reduced coenzyme Q-cytochrome c reductase (*3*) as was a control sample of antimycin. The deformylated starting material, however, showed only trace activity in this test. For determination of radioactivity of the product, 2.15 mg. were dissolved in acetone, the solution was diluted with acetone to 25 ml., and six 25-μl. aliquots were placed in separate scintillation vials. Counts ranging from 10,960 to 10,060 c.p.m. were observed, with counting efficiencies of 18 to 21%. The results corresponded to a specific activity of 5.9 ± 0.3 mc. per mmole. This product then was used without further purification for the fish experiments detailed below.

To determine whether tritium in the *N*-formyl group of antimycin was readily exchangeable, 100 μl. of a chloroform solution (unknown concentration) of the labeled product were placed in a small vial and two 10-μl. samples were removed for counting. The observed counts were: sample 1, 17,496 c.p.m.; sample 2, 19,200 c.p.m. The solvent was removed from the remainder of the chloroform solution, the residue dissolved in 150 μl. of approximately 75% ethanol, and the resultant solution was allowed to stand in the dark at room temperature for 15 hours. The solvents were then removed in a stream of nitrogen. 80 μl. of chloroform were added, and two 10-μl. samples were removed and counted. The results were: sample 1, 17,459 c.p.m.; sample 2, 15,640 c.p.m. Thus, no significant exchange was observed under these conditions.

Fish Experiments. The experimental treatments were carried out at the Fish Control Laboratory, U.S. Department of Interior, Bureau of Sport Fisheries and Wildlife, LaCrosse, Wis., on fish provided by the laboratory, in equipment and under the conditions routinely used in the evaluation of fish toxicants (*6*). The species used were carp, rainbow trout, and brook trout. The trout designated as "small" were brook trout, and those designated as "larger" were rainbow.

Five-gallon glass jars containing 15 liters of water were used for tests with small trout and carp (40 to 115 grams of body weight). As soon as each fish that was exposed to the toxin had died, it was removed from the jar, and the remaining antimycin solution was discarded.

Two metal tanks, each containing 43 liters of water, were used to hold the larger trout (230 to 320 grams of body weight). Initially, one trout was exposed to antimycin in each tank. As soon as this trout died, it was removed and replaced by a small carp (approximately 100 grams of body weight), which in turn was replaced, as soon as it had died, by another trout. Thus, three fish were exposed successively to the same antimycin solution in each of the metal tanks. This type of experiment was carried out only in the tanks and not in any of the glass jars.

The time of death of each fish was noted, and they were frozen immediately after death. Controls were maintained for each species and size of fish. None of the control fish died during the experimental periods.

Determination of Tissue Radioactivity. Each small trout and carp was divided into three fractions—viscera plus head, gills, and remainder of the body. In each case the first and last fractions were homogenized separately in water in a Waring Blendor, and duplicate aliquots (corresponding to about 1 gram of fresh tissue) were taken for radioactivity determinations. The gills were not homogenized but were divided as equally as possible into left and right halves which served as replicate samples.

The radioactivity in each sample was determined by combusting the lyophilized sample in oxygen and collecting and counting the water formed (*5*, *8*). The samples were ignited with a Thomas-Ogg oxygen flask infrared igniter (Model 6472-B), and the water was collected quantitatively on a dry ice–cooled condenser and dissolved subsequently in the phosphor solution. Samples were counted for at least 10 minutes. All gave at least 100 and most gave 300 to 2000 c.p.m. The background was approximately 30 c.p.m., and the counting efficiency varied from 13 to 22%. The combustion procedure was tested by combusting known amounts of H^3-antimycin, and in every case over 96% of the added radioactivity was recovered. This result agrees well with the results reported by Kelly *et al.* (*5*).

The heart, liver, kidney, muscle, gills, and skin of each of the larger trout were sampled separately. The remainder of the carcass was divided into two fractions consisting of the head plus remainder of the viscera and the balance of the body. These two fractions were homogenized, and aliquots were taken for assay. The radioactivity in each sample was determined as described above.

Extraction Studies. Small carp (90 to 200 grams of body weight) which had been poisoned by 10 p.p.b. of nonradioactive antimycin were frozen immediately after death and kept frozen until used. These fish were obtained from a field trial carried out at West Salem, Wis., during October 1965. For extraction, the carp were partially thawed and homogenized in a Waring Blendor with water. A portion of the homogenate corresponding to about 100 grams of fresh tissue then was ground in a mortar with an approximately equal weight of sea sand and 60 ml. of chloroform. The solvent was decanted and the extraction repeated six times in the same manner. Several other portions of the homogenate were extracted by grinding with six 50- to 100-ml. portions of acetone to dehydrate the tissue followed by three 50-ml. portions of chloroform. The extracts were combined, filtered, the solvents removed at room temperature on a rotary vacuum evaporator, and the residue which consisted mainly of lipid was re-extracted with 95% ethanol. The ethanol solutions were evaporated to dryness, and the residues (about 0.6 to 2.1 grams) were taken up in acetone or in an ethanol-acetone mixture.

The antimycin content of the above solutions was estimated roughly by determining their toxicity to goldfish. For this purpose, several 3-liter beakers were prepared, each containing two goldfish (1 to 3 grams of body weight) in 1.5 liters of distilled water, and the solution to be analyzed was added. The total volume of solvent (alcohol or acetone) introduced with the test solution was not over 2 ml. in any case. The beakers were maintained at laboratory temperature without aeration. Other beakers were prepared with known concentrations of antimycin and controls were also set up which contained 2.5 ml. of acetone or ethanol, respectively, but without antimycin. The time of death of each fish was noted.

As a control on the extraction procedure, a carp (body weight 430 grams) which had not been exposed to antimycin was killed by a blow on the head and the entire carcass homogenized in water. The homogenate was divided into four approximately equal fractions, one of

which was extracted directly and the others after known amounts of antimycin were added. The latter were rehomogenized before being extracted. The ethanol-soluble oil from each extract was tested for antimycin by the use of goldfish as described above. The ethanol-insoluble oil from the portion containing no added antimycin, and the same fraction from one of the portions to which antimycin had been added, were also tested against goldfish. None of the control fish in jars without added antimycin died during the experimental periods, whether or not the ethanol-insoluble oil was present. Likewise, neither the alcohol-soluble nor the alcohol-insoluble oil from the carp not exposed to antimycin had any observable effect on the goldfish even after 5 days' exposure.

Results and Discussion

The antimycin levels given in Tables I through IV have been calculated from radioactivity of the tissue samples on the assumption that all of the radioactivity was contributed by intact H^3-antimycin. This assumption is of questionable validity. The H^3 was located exclusively in the formyl group of the antimycin molecule, a group which is rather easily split off. If such splitting had occurred in the body of the fish, the resulting formic acid might have remained in the tissue and contributed to the apparent antimycin content. However, deformylated antimycin does not possess the characteristic biological activity (and presumably, therefore, not the toxicity) of the intact compound (*3*).

A second consideration is that antimycin is bound quite firmly by serum albumin and is inactive as an electron transport inhibitor when in this bound condition (*9*, *10*). Whether release of protein-bound antimycin occurs during digestion is not known, but such release, even if it occurs, may very well be far from complete. Thus, the tissue levels based on radioactivity must be regarded as maximum values.

Within these limitations, the data in Table I show a total body antimycin concentration averaging 203 μg. per kg. of fresh weight (range, 59 to 450) in 18 fish killed by 5 to 10 p.p.b. of the antibiotic. Concentrations in the carp were several-fold higher than in the trout, no doubt because of the greater resistance of the former to antimycin (*17*) and their longer survival times (Table I). The levels in fish killed at 10 p.p.b. were higher, but not twice as high, as in those killed by 5 p.p.b. The concentrations were roughly constant for a given species and dosage level.

Some notion of the internal distribution of the compound was derived from the results shown in Table II. Lowest antimycin concentrations occurred in the body (the portion most nearly corresponding to a fish dressed for home use)—viz. (in μg. per kg., fresh weight), trout 75 to 141, average 113; carp 139 to 290, average 201. Gills averaged 204 and 306, and the viscera plus head (except gills) 225 and 565 in the trout and carp, respectively (Table II).

The levels found in the four larger trout are given in Table III. Since so few individual fish were examined, and because replicate values on the unhomogenized samples often differed quite widely, all determinations are included.

Table I. Whole Body Antimycin Levels in Fish Killed with Radioactive Antimycin

Fish			Antimycin Dosage Level, P.P.B.	Antimycin Levels in Individual Fish Based on Radioactivity Present, μg./Kg. Fresh Weight
No. used	Species	Survival time, hours		
4	Carp	15–22	5	219, 238, 247, 286[a]
4	Carp	12–16	10	323, 334, 355, 450[a]
3	Small trout	2.5–6	5	109, 135, 137
3	Small trout	2–4	10	152, 160, 172
2	Larger trout	2–5	5	59.2, 92.3[b]
2	Larger trout	2–3	10	109, 79.3[b]

[a] These carp were treated in metal tanks; see text.
[b] These fish were the second trout placed in the tanks after the first had died; see text.

Table II. Distribution of Antimycin in Brook Trout and Carp Killed with Radioactive Antimycin

Fish			Antimycin Dosage Level, P.P.B.	Antimycin Equivalent to Radioactivity Present, μg./Kg. Fresh Weight		
No.	Body weight, g.	Survival time, hours		Gills	Head and viscera	Remainder of body
			Brook Trout			
1	70.0	2.5–4.3	5	158, 166	217, ...	75.2, 74.8
2	43.5	6.2	5	179, 203	239, 258	98.7, 101
3	52.0	5.2	5	176, 177	232, 228	103, 105
4	47.4	2.2–4.0	10	224, 236	204, 190	132, 141
5	66.2	2.2–4.0	10	282, ...	244, ...	138, 123
6	44.3	2.2–4.0	10	223, 222	178, 263	133, 130
			Av.	204	225	113
			Carp			
1	106	22.2	5	299, ...	460, 480	139, 146
2	112	20.3	5	279, ...	473, ...	167, 164
3	107	20.8	5	238, 242	362, 417	218, 203
4	98.5	15.3	10	331, ...	700, 780	261, 248
5	100	14.3	10	386, 406	413, ...	290, ...
6	90.2	16.7	10	298, 275	751, 811	190, 181
			Av.	306	565	201

Table III. Antimycin Distribution in Rainbow Trout Killed with Radioactive Antimycin

Fish No., Body Wt., Survival Time, and Original Antimycin Dosage Level		Antimycin Equivalent to Radioactivity Present,[a] μg./Kg. Fresh Weight						
		Liver	Kidney	Gills	Muscle	Skin	Head plus rest of viscera	Remainder of body
No. 1	319 g.	470	334	153	66.6	95.3	80.6	41.6
	2.7 hours	475	300	143	74.4	38.1	74.6	40.8
	5 p.p.b.	563	446	168	22.6			
		502						
No. 2	263 g.	954	304	159	170	100	127	83.3
	2.3 hours	790	598	163	96.7	68.4	127	82.9
	10 p.p.b.	762	461	151	48.5			
		855						
No. 3[b]	230 g.	702	496	134	150	30.4	101	69.4
	5.0 hours	795	406	178	40.0		42.4	65.7
	5 p.p.b.	797	210	132	44.2			
No. 4[b]	271 g.	670	397	121	110	145	77.7	145
	2.7 hours	538	319	149	46.6		87.9	
	10 p.p.b.			148				
	Av.	683	388	150	83	70	97	76

[a] Single values were obtained on heart tissue as follows: Fish No. 1, 797; No. 3, 667; No. 4, 745 μg. per kg. fresh weight.
[b] Second trout placed in tanks, see text.

The observed variations probably reflect real differences—i.e., uneven distribution in the tissues studied since replicates on homogenized samples generally showed good agreement (Table II, and last two columns in Table III).

In these fish, the lowest antimycin levels again occurred in the edible portions, averaging 83 μg. per kg. in the muscle samples and 76 in the bodies after heads and viscera were removed. On the other hand, in such metabolically active tissues as the heart, kidney, and liver, the levels were much higher—e.g., liver 470 to 954, average 683, and kidney 210 to 598, average 388. Only three values were obtained on heart tissue, but these also were relatively high, 667 to 797 μg. per kg. (Table III).

This pattern of distribution is consistent with the well known affinity of antimycin for the electron-transport system, and supports the idea that the fish die because their energy-transforming apparatus is inhibited. The ability of the fish to absorb and accumulate antimycin against a steep concentration gradient—e.g., 5 p.p.b. in the water *vs.* 700 to 900 p.p.b. in certain tissues—also points to a firm binding of the toxicant with some body constituent, which might very well be the antimycin-sensitive site of the electron-transport system (*15*).

The extraction studies were carried out in an attempt to determine whether any detectable amount of biologically active antimycin could be recovered from the poisoned fish. It was thought that sufficient quantities might be extracted by solvents in which antimycin is readily soluble (*7*) to permit detection by the most sensitive available method—namely, its toxicity to fish. The survival time of goldfish exposed to antimycin tends to decrease with increasing concentrations. This relationship might, therefore, provide a basis for estimating unknown concentrations in the p.p.b. range. In the current study, the results were essentially only qualitative, even when control fish exposed to known concentrations of standard antimycin were included with each test.

The results of one test series are shown in Table IV. At least some antimycin was recovered from the poisoned carp. While the amount cannot be estimated closely from these results, it tends to be lower than the concentrations indicated from the work with H^3-antimycin. This is, no doubt, in part attributable to incomplete extraction, since recovery of added antimycin was poor (Table IV, jars 6 and 7). This extraction was made with acetone followed by chloroform. Similar tests (not included in Table IV) on another portion of the poisoned carp extracted with chloroform only showed that more than 53 but less than 107 μg. per kg. were extracted. Thus, some part of the radioactivity in the fish killed with H^3-antimycin in the laboratory experiments must have been contained in the intact antibiotic, but the results do not permit a reliable estimate of how large a part of the total this may have been.

Table IV. Goldfish Assay of Antimycin Extracted from Homogenized Carp Tissue

Jar No.	Sample Tested	Survival Time, Hours	Conclusions from Test
1	Standard A.A.,[a] 2 p.p.b.	9.5, 9.5	...
2	Standard A.A., 5 p.p.b.	2.7, 6.8	...
3	Standard A.A., 10 p.p.b.	4.2, 4.9	...
4	Alc.-sol. extract from 100-g. poisoned carp	8.1, 9.7	ca. 30 μg. A.A. extracted per kg.
5	Alc.-sol. extract from 200-g. poisoned carp	13.7, 14.7	<15 μg. A.A. extracted per kg.
6	Alc.-sol. extract from 93-g. control carp plus 35.5 μg. of added A.A.	8.8, 10.1	ca. 3 μg. A.A. recovered (8.5%)
7	Alc.-sol. extract from 95-g. control carp plus 35.5 μg. of added A.A.	6.5, 8.5	ca. 7.5 μg. A.A. recovered (21%)

[a] A.A. = antimycin A (*14*).

Table V. Fraction of Total Available Antimycin Absorbed by Test Fish

Fish	Body Weight, G.	Total Antimycin, μg. In water used	Total Antimycin, μg. In body of fish	Fraction of Available Antimycin Absorbed by Fish, %
Trout No. 1	70.0	75	7.63	10.2
2	43.5	75	5.96	8.0
3	52.0	75	7.02	9.4
4	47.4	150	7.20	4.8
5	66.2	150	11.4	7.6
6	44.3	150	7.09	4.8
Carp No. 1	106	75	23.2	32
2	112	75	26.7	36
3	107	75	26.4	35
4	98.5	150	35.0	23
5	100	150	32.3	22
6	90.2	150	30.1	20
Large Trout No. 1	319	215	18.9	8.9
2	263	430	28.7	6.7

Acute toxicity studies in which antimycin in the form of an aqueous suspension was administered orally gave LD_{50} values, in mg. per kg. of body weight, as follows: mouse 55 ± 7, rat 28 ± 6, rabbit 10, dog > 5, guinea pig 1.8 ± 0.28, quail 39 ± 11, and lamb 1 to 5 (*4*). Another similar study showed values of 2.9 for ducks, and >160 for chickens (*18*). A much earlier report (*7*) indicated that rats tolerated higher levels of antimycin when it was mixed with the ration (weight loss but no deaths among six animals that ingested 24 to 27 mg. per kg. each day for 7 days) than when it was administered by stomach tube as a solution in methyl laurate (single doses of 12 mg. per kg. tolerated; 30 mg. per kg. fatal).

In the light of these data, rats would be expected to suffer no ill effects from consuming fish killed with antimycin. A feeding trial in which whole ground carp, bass, and pike poisoned by 5 to 10 p.p.b. of antimycin in a field trial at Delafield, Wis., provided 50% of the total diet of 10 weanling rats for 25 days fully substantiated this expectation. The test animals grew equally as well as the controls and appeared entirely normal (*18*).

The toxicity of antimycin for the human is not known, but in comparison with the above animal data it seems most unlikely that fish killed with antimycin would carry harmful amounts. Thus, a normal 4-ounce serving of fish containing 201 μg. per kg.—the highest average level for the edible portion found in the present study—would provide 23 μg. of antimycin, or 0.33 μg. per kg. for a 70-kg. man. A dose of even 1 mg. per kg. would require the consumption of 3000 such servings.

The authors have personally eaten rainbow and brook trout killed with antimycin and know of 19 other persons who also have consumed antimycin-poisoned fish. Nine of these people had one meal each, eight had two, one had four, and three had five. The amounts consumed were in the range of normal 4-ounce servings. The earliest date was September 30, 1964, and the most recent October 24, 1965. While no formal medical examinations or reports have been made, none of the individuals involved has experienced any ill effects to date (May 1966). These informal observations therefore, provide some confirmation of the safety of antimycin-poisoned fish as a human food.

Another item of interest relating to the present work concerns the fraction of the available antimycin extracted from the water by the test fish. These values ranged from 4.8 to 36% (Table V). The magnitude of this percentage undoubtedly depends on the minimum tissue levels lethal for the species involved, the weight of fish per unit volume of water, and the amount of antimycin available. The fish evidently continue to extract the compound from the water and concentrate it in their bodies as long as they are alive and pumping water through their gills. After death, this accumulation presumably stops as the tissue concentration is then so much higher than in the surrounding water. It is probable, therefore, that fish removed from antimycin-treated water several hours after death would contain no more of the antibiotic than those collected immediately.

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TOXICOLOGY USING INSECTS

Toxicology of Plant-Translocated Maleic Hydrazide. Lack of Effects on Insect Reproduction

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The effect of plant-translocated maleic hydrazide (MH) on animal reproduction was investigated using two species of insects. Bean plants grown in MH-treated soil were freeze-dried and were incorporated in standard diets for rearing *Drosophila melanogaster* Meig. and *Musca domestica* L. Untreated beans with and without the addition of MH were used for comparison. Continuous rearing of several generations showed no reduction in the fecundity of either species owing to plant-translocated MH.

MALEIC HYDRAZIDE (MH), 1,2-dihydro-3,6-pyridazinedione, has become widely established as an herbicide, fungicide, plant growth inhibitor, and growth regulator (*17*). Although MH is reported to have pronounced effects on the biochemistry, physiology, and developmental morphology of plants (*2*), its relative toxicity is low (*7*).

In studies on animals, Barnes *et al.* (*1*) found that the sodium salt of MH injected and fed to rats and mice had no effect on their growth and general health—that is, it appeared to be relatively nontoxic and noncarcinogenic. However, Fischnich *et al.* (*5*) found a significant reduction in fertility of rats fed with potato tubers from plants sprayed with MH before harvest compared with rats fed a diet with tubers treated with MH during storage. Furthermore, Robinson (*11*) found a large reduction in the fecundity of pea aphids reared on broad beans grown in soil treated with MH compared with those reared on plants freshly dipped or sprayed with MH. Both Fischnich and Robinson used the diethanolamine salt of MH in their experiments. Tate (*15*) found that only the MH fraction of this formulation was translocated into potato tubers. We used pure MH throughout the present work.

From the reports cited above it would appear that MH per se has no significant effect on certain small mammals and asexual stages of aphids at comparatively large doses. However, it might also be concluded that MH when translocated in certain plants is converted to a form, or produces changes in the plant, which can inhibit reproduction in some animals.

The experiments reported in this paper were made to test the hypothesis that plant metabolites resulting from treatment with MH can interfere with animal reproduction. It was considered that fecundity, and subsequent mode of action studies if warranted, could be made conveniently using sexual insects.

Experimental

Bean plants were grown in soil treated with MH, the leaves and stems were harvested and freeze-dried, and the powder was incorporated in standard diets used in this department for rearing the fruitfly (*Drosophila melanogaster* Meig.) and the housefly (*Musca domestica* L.).

Drosophila Tests. Soybeans (*Glycine max*) were grown in pots in the greenhouse. When the plants were about 3