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

inches high, the growing medium (coarse quartz sand and vermiculite) was supplied for 7 days with Hoagland's solution containing 3000 p.p.m. MH. Ten days later the leaves and stems were harvested, freeze-dried immediately, and ground to a fine powder (60 to 100 mesh). The bean powder was incorporated into a standard cornmeal-agar *Drosophila*-rearing medium (*16*). Flies could tolerate the incorporation of dry bean powder up to 5% of the wet weight of the media, although the equivalent amount of fresh bean material caused high mortality of adults.

The introduction of freeze-dried material resulted in sporadic heavy growth of molds in the incubating cultures which caused fly mortality. Methods of mold control that were attempted included boiling and autoclaving media and containers; addition of antibiotics and chemical mold inhibitors to the media; and gamma irradiation of freshly prepared media in their containers. None of these techniques gave consistent mold control. The system finally adopted was to add 0.5% propionic acid plus 0.05% methyl *p*-hydroxybenzoate to boiled media. This treatment appeared to have no harmful effects on the breeding of flies.

Ten female and 10 male 1-day-old flies were transferred from the breeding stock into a 12-ounce glass jar containing approximately 100 grams of media. The top of the jar was closed with a sheet of tissue paper, and breeding proceeded for 10 days at 25° C. and 60 to 70% R.H. before the parent flies were removed and counted. Thereafter, newly emerged flies were removed from the culture every 48 hours for a period of 10 to 12 days; total number of flies emerging from each treatment was compared when production of the first generation was completed. Succeeding generations of flies were inbred from the F_1 mixture in each treatment.

Four treatments in triplicate were included in each experiment: standard bean-free medium (std); std plus 5% dry untreated beans (CB); std plus 5% dry untreated beans plus 2 ml. of an aqueous solution containing either 1500 or 2000 p.p.m. MH added immediately before inoculation with parent flies (CB + MH); and std plus 5% dry MH-treated beans (MHB). From chemical analysis (*6*) of the bean powder, the concentration of MH in the MHB medium was calculated to be 863 p.p.m.

Additional experiments were conducted with NaCl extracts of MH-treated beans in an attempt to make MH or its metabolites more readily available to *Drosophila* larvae and to overcome mold difficulties. Chemical analysis showed that 83% of the MH was removed in the first extraction. The extract was added to the standard rearing medium at the rate of 2 grams of freeze-dried beans in 10 ml. of 10% NaCl per 100 grams of medium giving a final MH concentration of 286 p.p.m. Three replicates were made, and numbers of flies were compared with those produced from the extract of untreated beans with 3 mg. of MH added immediately before testing. The bean material remaining after extraction was bioassayed in the same way.

Housefly Tests. The response of another insect species, *M. domestica*, to MH was tested using a modified form of an insect-chemosterilant technique (*9*, *13*). A laboratory strain of insecticide-susceptible housefly was used in these tests.

Broad beans (*Vicia faba* L.) were grown in pots and supplied for 2 days with Hoagland's solution containing 3000 p.p.m. of MH, following the technique of Robinson (*11*). Thereafter, these plants were watered daily with Hoagland's solution only. Since this level of MH treatment caused the cessation of growth, a second set of plants was watered daily for 10 days with a reduced concentration of MH (100 p.p.m.) in Hoagland's solution. It was expected that the continued growth of these plants would facilitate metabolism of MH.

Standard CSMA (Chemical Specialties Manufacturers Association) medium was modified by substituting 20 grams of freeze-dried bean powder for 7 grams of bran and 13 grams of alfalfa meal per 100 grams of solids. Water, yeast, and malt were added to 15 grams of the mixed solids to give a total weight of 50 grams, and this loose mixture was placed in 4-ounce jars. From chemical analysis (*6*) of the bean powders, final concentrations in the rearing media were calculated to be 183 p.p.m. for the heavier treatment and 12 p.p.m. for the 100-p.p.m. treatment.

Newly laid eggs were obtained from a stock culture of flies bred on normal CSMA medium, incubated overnight on moist filter paper, and 100 eggs selected at random were transferred in water to each jar. The culture jars were closed with tissue paper and incubated at 25° C. and 60 to 70% R.H. for 6 days; the surface of the medium was covered to a depth of 1.5 cm. with vermiculite, and the jars were incubated for 2 days more. Pupae were separated from the vermiculite by sieving, total numbers and weight were recorded, and the pupae from the three replicates of each treatment were mixed and placed in a screen cage. After 5 days, flies which had emerged were counted and sexed; 50 females and 25 males were returned to a clean cage and fed daily with a dilute solution of sugar and milk. After 2 days, oviposition dishes of cellucotton containing milk and yeast (*4*) were placed in the cage and renewed every 24 hours; eggs were washed off, and the volumes of sunken and floating eggs were recorded (*12*). Hatchability tests were conducted by incubating four replicates of 25 eggs from each treatment for 24 hours at 25° C. and counting the proportion of empty shells left on the surface of moist filter paper, using a microscope. The experiment was continued for three successive generations for each treatment.

Results

The results of repeated tests covering a number of generations of *Drosophila* (Table I) were variable over-all, but showed no large or consistent effect on numbers of progeny due to added MH or to bean-metabolized MH. In the second series of experiments (Table I), the F_2 and F_3 generations were split, some of the F_1 produced in a bean medium continued breeding in the same type medium, while the remainder continued breeding in the standard medium. No evidence of a continued or magnified effect on fecundity caused

Table I. Number of *Drosophila* Adults Produced from Different Larval Media

	Number of Flies per Generation (Average of 3 Reps. of Progeny of 10 ♀ Flies)						
	Series I						
Generation	Std[a]		CB[b]		CB + MH		MHB[d]
Parent stock	539		515		495		580
F_1	399		541		476		456
F_2	653		506		498		593
F_3	601		719		658		569
	Series II						
	Std[a]	Std[a]	CB[b]	Std[a]	CB + MH[e]	Std[a]	MHB[d]
Parent stock	428	...	523	...	320	...	388
F_2[f]	503	597	1002	509	588	545	582
F_3	473	494	604	537	591	456	392

[a] Cornmeal/agar.
[b] Untreated beans + *a*.
[c] MH (3 mg.) added to *b*.
[d] MH-treated beans + *a*.
[e] MH (4 mg.) added to *b*.
[f] Mold difficulties; enough F_1 produced to breed F_2.

Table II. Number of *Drosophila* Adults Produced from Media Containing NaCl Extracts of Beans

Treatment	Number of F_1 Adults Produced (Average of 3 Reps. of Progeny of 10 ♀ Flies)			
	Std[a]	CB[b]	CB + MH[c]	MHB[d]
NaCl extract of beans	567	514	500	605
Residue from extract	401	404	460	499

[a] Cornmeal/agar. [b] Untreated beans + *a*. [c] MH (3 mg.) added to *b*. [d] MH-treated beans + *a*.

Table III. Effects on Reproduction of Houseflies from Incorporating MH-Treated Broad Beans in Larval Medium

(100 eggs for each starting sample)

Generation	Larval Medium	Pupae[a]		Adults[a]		Eggs	
		No. formed	Av. wt., mg.	No. emerged	♀, %	Vol. from 50 ♀, ml.	Hatch in 24 hr., %
Parent stock	CB[b]	50	25.0	43	42		
	CB + MH[c]	65	23.3	60	54		
	MHB 100[d]	56	20.3	51	61		
	MHB 3000[e]	56	20.7	52	57		
F_1	CB	75	19.3	70	51	1.7	84
	CB + MH	79	19.9	77	49	2.2	..
	MHB 100	76	20.4	71	55	1.8	70
	MHB 3000	74	20.0	70	48	1.9	85
F_2	CB	53	22.5	52	51	2.4	86
	CB + MH	73	22.9	73	51	2.0	75
	MHB 100	71	18.6	70	53	2.4	69
	MHB 3000	32	22.0	32	47	2.3	79

[a] Average of 3 reps. [b] CSMA medium + untreated beans. [c] MH (3 mg.) added to *b*. [d] CSMA + beans grown with 100 p.p.m. MH. [e] CSMA + beans grown with 3000 p.p.m. MH.

by MH treatment of the original parent population was found in this test. Neither was any MH effect apparent in the numbers of flies produced in the experiments using NaCl extracts of beans (Table II).

In the housefly tests, no fecundity effects of either added MH or bean-metabolized MH was evident over three successive generations (Table III). Egg hatchability, larval survival and pupation, pupal emergence, sex ratio, fertility, and longevity were all checked, and no MH effect was found at any of these stages of development.

Discussion

The fecundity of two species of insects was not altered by rearing in media containing large amounts of MH or MH-treated plants. Extreme conditions of dosage were chosen for this work in order to assure maximum likelihood of response occurring both in plants and insects. These amounts are far in excess of residue levels that could be expected from normal agricultural usage.

Kanehisa (*8*) has investigated the effects of MH on tumor incidence in *D. melanogaster*. Although he found that MH had no effect on tumor incidence, he observed that it caused prolongation of the period of larval development. Nasrat (*10*) has recently found genetical evidence for mutagenic activity of MH in the same species. However, neither of these authors specified the actual amounts of MH to which the insects were exposed, but presumably very large amounts were used. At the concentrations used in the present work (176 to 300 p.p.m. dry weight of medium), no retardation of larval development occurred either in the same species or in *Musca* when compared with standard rearing conditions (*14*, *16*). Similarly, there was no morphological (phenotypic—e.g., size, shape, weight, sex ratio, and fecundity) evidence of mutagenic activity of MH on these two insect species at the above dosages.

Robinson (*11*) found a marked reduction in the fecundity of an asexual form of pea aphid reared on broad bean grown in MH-treated soil. We repeated a part of this experiment and confirmed the result. Three replicates of broad beans were grown in pots treated by keeping them in a solution of 3000 p.p.m. of pure MH for 48 hours after five virginopara of a particular biotype of *Acyrthosiphon pisum* Harris were placed on each plant. When nymphs were counted 5 days later, less than half as many were produced on the MH-treated beans as on the untreated beans. Since it was observed that growth of the MH-treated bean plants was greatly retarded, it seems possible that the reduction in aphid fecundity was due indirectly to nutritional deficiencies in the less vigorous MH-treated host plant rather than directly to MH or its metabolites. Van Emden (*3*) has proposed such an explanation for reduction in fecundity of another aphid species on Brussels sprouts treated with Cycocel [(2-chloroethyl) trimethylammonium chloride] plant growth retardant.

Conclusions

On the basis of the work reported herein, the authors conclude that MH translocated in bean plants and fed to two species of insect had no effect on their reproduction. On the other hand, the results of Fischnich's work on feeding potatoes to rats appear to demonstrate an antifertility effect of MH when translocated. Further studies are being made on specificities toward MH of these plants and animals.

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