index to indicate the susceptibility of plants to weed-killers, particularly 2-4-D.

On the south coast of Puerto Rico, the "Santa María" or "emajagüilla," *Thespesia populnea,* a malvaceous tree commonly grown along the roadsides, serves as an index of susceptibility in this area of Puerto Rico. The distorted leaves of this tree (Fig. 1) show symptoms of 2-4-D toxicity at all times of year, but most noticeably during the spring, when "primavera" cane as well as ratoon cane are being sprayed with weed-killers. Even more conspicuous is the injury to another common roadside tree which grows more generally in the northern part of the Island: the "almendro," *Terminalia catappa* (Fig. 2). In the Naguabo-Humacao area many mature trees, 30 to 50 ft in height, have been observed that were killed by the effects of 2-4-D used in adjacent cane fields (Fig. 3).

FIG. 1. Foliage of *Thespesia,* showing effect of 2-4-D toxicity.

FIG. 2. Terminal twig of "almendro," *Terminalia,* showing effect of weed-killer.

FIG. 3. Dead and dying roadside trees of "almendro," *Terminalia catappa,* on the Naguabo-Humacao road, Puerto Rico. (Photos courtesy of the Photographic Department, Agricultural Experiment Station, Río Piedras, Puerto Rico.)

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Effect of Irradiation with Cobalt-60 on Trichina Larvae

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A previous study of the effect of 200 kv x-rays on trichina larvae (*1*) showed that a dose of 750,000 r kills trichina larvae *in vitro,* whereas a dose of approximately 5000 r inhibits maturation of larvae to adult forms, and a dose of approximately 3500 r produces sexual sterility of the adult forms that mature from irradiated larvae. The present study was undertaken to determine the radiation dose from a cobalt-60 source that would produce the same effects.

Alicata and Burr (*2*) found that exposure of trichinous meat to 12,000 r of cobalt irradiation produced sterility in 60–100% of the adult female worms recovered from the intestinal tract of experimental rats 6 days after the latter were fed the irradiated larvae.

Trichinous rat muscle and isolated trichina larvae obtained by the digestion of trichinous rat muscle

TABLE 1

NUMBER AND AVERAGE PERCENTAGES OF FEMALE ADULT TRICHINAE HAVING EMBRYOS, AMONG DESIGNATED NUMBER OF FEMALE ADULTS EXAMINED, AS RECOVERED FROM GUT OF RATS 6 DAYS AFTER FEEDING ANIMALS 5000 LARVAE EXPOSED TO Co^{60} RADIATION

Radiation dose applied to larvae fed to rats r	Source of larvae fed to rats	Number of gravid female adult trichinae among adult females examined			
		Rat number in group			Average %
		1	2	3	
0		50 of 50	50 of 50	50 of 50	100
12,800	Irradiated trichinous muscle	0 of 50	1 of 50		1
15,360		0 of 50	0 of 50		0
0		50 of 50	50 of 50	50 of 50	100
10,240	Irradiated isolated trichina larvae	8 of 25	5 of 25	7 of 25	26.7
10,240		1 of 50	0 of 50	0 of 50	
		0 of 50	1 of 50	6 of 50	2.7
12,800		4 of 25	6 of 25		20
15,360		0 of 4	0 of 7		0
15,360		0 of 1	0 of 1	0 of 3	0
		0 of 4			

were placed in lusteroid tubes for irradiation, as in our previous study (*1*). The filled lusteroid tubes were then placed inside a cylindrical cobalt-60 irradiation unit for exposure at a rate of 1280 r/min (*3*). The radiation source at the University of Michigan has been described in detail elsewhere (*4*).[1]

Measurements were made to determine the maturation-inhibiting dose and sexual-sterilizing dose for both isolated larvae and larvae in trichinous rat muscle. Measurements of the lethal dose were made only on the isolated larvae.

In determining the maturation-inhibiting and sterilizing levels of radiation, doses ranging from 5120 r to 20,480 r were applied. Larvae, irradiated in muscle, were subsequently isolated by artificial digestion of the muscle with 1% pepsin and 1% hydrochloric acid. The radiation effect was measured by reduction in the ability of the irradiated larvae to mature in 6 days in the intestinal tract, and by the absence of encysting trichina larvae in the muscle tissue of test rats 30 days after ingestion of trichina larvae. Each rat was tube-fed with 5000 larvae. For each radiation dose, whether applied to isolated trichinae or trichinous rat muscle, at least 6 test animals were used. Three of the 6 rats were sacrificed after 6 days and the contents of the small intestines examined for adult worms. The number of adult worms was calculated (*6*). When adult worms were present, 50 female worms were examined for the presence within them of embryos. The other 3 rats were sacrificed at the end of 30 days and examined for larvae in the muscles. When larvae were found, the number present in the entire carcass was calculated (*6*). In all, 160 rats were used to obtain the data on sexual sterilization and maturation inhibition.

[1] The kilocurie cobalt source at the University is located in the Waste Fission Products Laboratory, an activity devoted to research on use of radioactive fission by-products and supported by the U. S. Atomic Energy Commission and the Michigan Memorial-Phoenix Project (*5*).

The lethal dose for isolated larvae was determined from motility measurements on larvae irradiated at 269,000, 512,000, 768,000, and 1,024,000 r. At the end of each period of irradiation and at 2 hr and 24 hr following irradiation, 200 of the irradiated larvae were examined on a warm stage (43.3° C) for evidence of motility; in each case the percentage of motile forms was recorded. (Nonmotile [dead] larvae, when killed by irradiation, often appear tightly coiled under the microscope, so that the mere examination for coiled and uncoiled states does not determine viability. Death due to irradiation does not usually cause the larvae to uncurl as do heat and other killing methods.) In addition to microscopic examination, 5000 irradiated larvae were fed to 6 test rats, as in the experiments to determine the sterilizing and maturation-inhibiting doses.

The examination for embryos, within adult female worms recovered from the gut 6 days after irradiation (Table 1), shows that a small number of females may develop embryos after a dose of 12,800 r, but that no embryos develop after a dose of 15,000 r. The dose of radiation with Co^{60} necessary to cause sterilization of trichina while they are still encysted in rat muscle

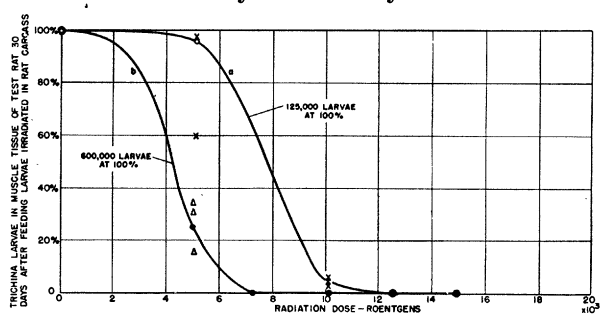

FIG. 1. Sterilization of isolated trichina larvae and of trichina larvae in rat carcass by Co^{60} gamma irradiation. △ = isolated trichina larvae. × = larvae irradiated in rat carcass.

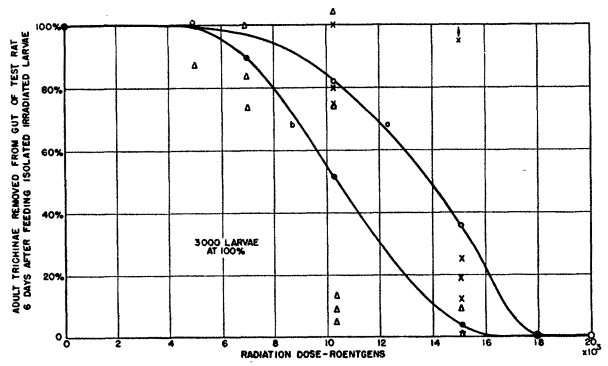

FIG. 2. Inhibition of maturation by Co^{60} gamma irradiation of isolated trichina larvae and of trichina larvae in rat carcass. △ = isolated trichina larvae. × = larvae irradiated in rat carcass.

is indicated in Fig. 1. With a dose of 12,800 r over 99% of adult females were rendered sterile. Complete sterilization was reached by a radiation dose of 15,000 r. To achieve complete sterilization of isolated larvae irradiated *in vitro,* about 12,800 r were required (Fig. 1). These curves are based on the degree of infection found in muscle 30 days after feeding rats 5000 irradiated larvae.

By irradiation, it was possible to prevent larvae from maturing, even though the larvae showed normal motility in the warm-stage test. The amount of irradiation required to do this was somewhat higher than that for sterilization. When trichinous muscle was irradiated, 18,000 r reduced maturation to less than 1%. The variation in inhibition with dosage is shown in Fig. 2. The complete maturation-inhibiting dose for trichina larvae *in vitro* was 18,000 r (Fig. 2).

Radiation dosages required to kill were measured only on isolated larvae. Complete kill, as determined by the motility test, required 750,000 r. When the larvae were examined 2 hr after completion of irradiation, the killing dose had dropped to 700,000 r, and when examined 20 hr after irradiation the killing dose was 400,000 r. From previous results, there is good reason to believe that the killing dose for irradiation of trichinous muscle would be substantially higher than the 700,000 r observed for irradiation *in vitro.*

Work is continuing on the irradiation of pork as a possible method of controlling trichinosis. Undesirable flavor changes occur in many foods when preserving doses of radiation (about 2,000,000 r) (*5*) are applied. However, one-hundredth of that dose is more than adequate to prevent maturation of encysted trichinae. Preliminary tests of pork irradiated with doses up to 38,400 r have shown negligible flavor change (*7*). A detailed report on this work will be published soon.

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Some Applications and Limitations of Tetrazolium Chloride

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The use of 2,3,5-triphenyltetrazolium chloride (TTC) and its various derivatives in seed testing, in viability determinations, and in various other branches of research has become widely known. The reaction involving reduction of TTC seems to be essentially the same as that with methylene blue. However, TTC becomes colored when reduced, instead of colorless, and the oxidation reaction which reverses the color change reaction in methylene blue does not seem to occur or only occurs slowly under certain circumstances in TTC.

Characteristics of the stain. Young, rapidly growing tissues of such plants as corn and onion will show the red color in about 20 min when soaked in a 1% TTC solution. The carmine red color, which may alter to a cherry color in 24 hr, will also appear in solutions without the presence of living cells, e.g., when TTC is heated together with a reducing sugar to 50° C or subjected to strong light. With higher pH the light reaction will occur at lower light intensities for a given length of time. A higher pH also results in a more rapid reduction of TTC in living cells of a uniform kind (Table 1).

TTC-stained cells which appear red macroscopically may not show any red color under the microscope with an incandescent or daylight light source. It may, however, bring out the contour of mitochondria more clearly in such cells as those of corn coleoptiles. Other derivatives of TTC sometimes produce more striking effects.

The change in appearance of mitochondria was first demonstrated by H. Ziegler when the writer worked with him in B. Huber's laboratories in Munich. We have tried to reproduce his results in this country and also have observed the smaller mitochondria in corn coleoptile cells to be red after soaking for 2 hr in a 1% TTC solution. The color is variable depending on the depth of focus so that there may be some doubt as to whether the bodies are refracting light from in back of them or are giving off a color peculiar to the bodies themselves.

Chloroplasts may also become red in such cells as

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