Relative Sensitivity of Dormant and Germinating Seeds to 2,4-D

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It has been reported that seeds or seedlings can be killed by adding 2,4-D to soil or manure in which they occur (*1–4*). Later experiments at the U. S. Plant Industry Station have shown, however, that some seeds will eventually germinate, although the manure or soil in which they occur is treated with sufficient 2,4-D to be lethal for a period of several weeks. These results suggest that seeds of some species may be relatively insensitive to 2,4-D while dormant, and that those remaining dormant in soil or manure until the chemical is inactivated, may not be affected. Experiments described here were made to study the effect of treating dormant and germinating seeds with 2,4-D.

In the first experiment mustard seeds (variety Southern Giant Curled) were incubated on moist blotting paper in Petri dishes at 25°–27°C. until they had reached the desired stages of germination. Treatments were applied after the seeds had reached the following stages: I, unswollen seeds immediately after wetting; II, swollen seeds; III, seeds that had swollen and ruptured the coats; IV, seeds that had developed radicles 5 mm. in length. In treating, selected seeds or seedlings were immersed in the desired concentrations of aqueous solutions of the ammonium salt of 2,4-D (ammonium 2,4-dichlorophenoxyacetate) for definite periods of time. They were immediately washed for 15 seconds with running distilled water, blotted, and incubated. Untreated seeds were immersed for comparable periods in distilled water and designated controls. After both the controls and those treated with the salt had reached stage III, elongation of the radicles was recorded for the following 24-hour period. In the case of stage IV, elongation of the radicles was measured for a 24-hour period immediately after they had reached a length of 5 mm.

TABLE 1

RELATIVE ROOT ELONGATION OF MUSTARD SEEDLINGS TREATED FOR 30 SECONDS WITH CONCENTRATIONS OF THE AMMONIUM SALT OF 2,4-D AT 4 STAGES OF DEVELOPMENT*

Concentrations (ppm)	Stage of development at treatment			
	I	II	III	IV
0	100	100	100	100
10				76
100	120	72	46	35
500	87	25	0	0
1,000	31	9	0	
2,000	12	7	0	

* Values represent percentages of elongation of radicles, the respective controls rated as 100 per cent.

Root growth of mustard seeds treated at stage I for *10 minutes* in a solution containing 500 ppm of the ammonium salt of 2,4-D was reduced 70 per cent. A *1-second* treatment of seeds in stage IV completely checked radicle elongation.

The sensitivity of mustard seeds was also determined by treating selected seeds in different stages of germination with a solution containing 500 ppm of the salt for a period of 30 seconds. On the basis of subsequent root growth, resting seeds were relatively insensitive to the salt. The seeds became more sensitive as they absorbed water and ruptured their coats. Seeds from which the radicles protruded were extremely sensitive to the salt solution (Table 1, Fig. 1).

Seeds of subterranean clover (*5*) (*Trifolium subterraneum*) germinate readily when subjected to a temperature of 20°C., on the other hand, they absorb water, yet remain dormant, when subjected to a temperature of 30°C. It is thus possible to plant the seeds in treated soil and hold them dormant for the period during which the acid remains active in soil (*4*). Once the 2,4-D has become inactivated, the seeds and soil can then be transferred to a temperature favorable for germination and the effect of the previous chemical treatment observed.

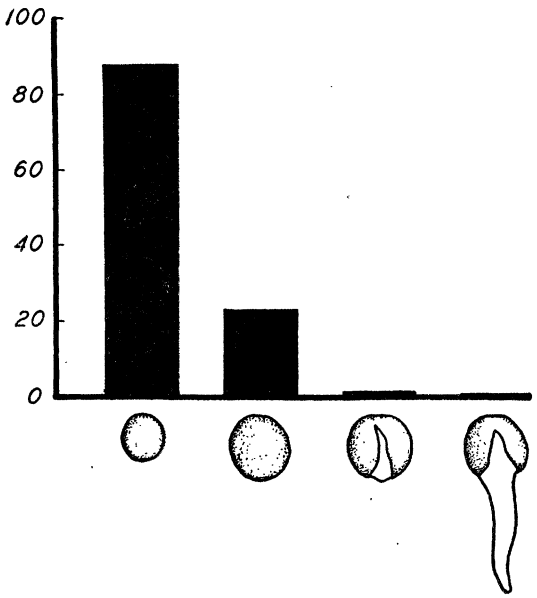

FIG. 1. Effect of ammonium 2,4-dichlorophenoxyacetate on root growth of mustard seedlings treated at the four stages of germination indicated by the drawings. The bars represent percentages of root elongation for the four treated lots, the respective controls being considered as 100 per cent.

The clover seeds were first placed on moist blotters at controlled temperature of 30°C., a condition under which they would absorb water but remain dormant with the seed coats intact. At intervals, samples of the seeds were weighed to

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determine when the maximum amount of water had been absorbed. They were then divided into 20 groups of 25 selected seeds each. Five groups were planted individually in 0.45 pound of moist, composted soil contained in individual waxed-paper boxes. Five other groups were planted in individual boxes containing an equal amount of composted soil with which 2,4-D had been mixed at the rate of 4 pounds/acre. All 10 boxes were placed at a controlled temperature of 20°C. The same two procedures were then repeated using the remaining groups of seed, but these were maintained in the moist soil at a constant temperature of 30°C.

Seeds in the untreated soil at 20°C. germinated and grew vigorously within a few days after they were planted. Emergence and growth of those in the treated soil were greatly inhibited by the acid (Table 2). After 11 days at 20°C., some plants in the treated soil had grown above the surface, but they were deformed and stunted.

The seeds at 30 C. remained dormant. Inactivation of 2,4-D in the treated soil was determined by repeatedly planting mustard seeds in it. After 19 days, the 2,4-D was completely inactivated, as indicated by the fact that mustard seeds germinated, and the plants grew as vigorously in the treated as in the untreated soil. Boxes containing the treated and those containing the untreated soil were then transferred to 20°C. The clover seeds in both germinated and grew vigorously, none being deformed (Table 2).

It is evident from results with this species of clover that the seeds were relatively resistant to 2,4-D while dormant, but

TABLE 2

PERCENTAGES OF EMERGENCE OF SUBTERRANEAN CLOVER (*Trifolium subterraneum*) IN UNTREATED SOIL (U) AND IN SOIL TO WHICH 2,4-D WAS ADDED IN AMOUNTS EQUIVALENT TO 4 POUNDS/ACRE (T)

Replications	7 days at 20°C.		11 days at 20°C.		19 days at 30°C.; 3 days at 20°C.		19 days at 30°C.; 9 days at 20°C.	
	U	T	U	T	U	T	U	T
1	76	0	100	48	88	68	88	80
2	72	0	100	32	44	76	80	88
3	80	0	92	48	48	68	88	80
4	64	0	92	48	44	24	96	96
5	64	0	100	32	92	84	96	80
Average	71	0	97	42	63	64	90	85

became extremely sensitive to the acid after the seed coats were broken and the seeds began to germinate. Mustard seeds likewise proved to be relatively insensitive to 2,4-D when the compound was applied to the resting seed. Mustard seeds in later stages of germination were extremely sensitive to the compound. It is indicated on the basis of these results that 2,4-D is likely to be most efficient as a preplanting soil treatment for killing seeds of obnoxious plants if applied at a time when the greatest number of such seeds are germinating.

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Inactivation of Certain Neurotropic Viruses *in Vitro* by Serum Lipids

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The inactivation of viruses by some lipids, particularly fatty acids and their soaps, is well known (*1*) and has been attributed generally to the surface action of the chemicals. While investigating various aspects of the neutralization test with neurotropic viruses, a slight but definite and reproducible reduction of the titer of the viruses was noted when the latter were incubated with clear sera deriving from several normal animal species, as compared with that shown by viruses in buffered saline solution controls. Moreover, the lipid fraction obtained from such sera either by acetone-ether or by hot alcohol extractions, emulsified in buffered saline solution and added to a virus suspension, induced rapid and pronounced reduction in titer—a drop of .01 to .0001 within 5 minutes.

The test was carried out by mixing equal volumes of a 1:500 suspension of infected mouse-brain in buffered saline solution and of the emulsion of lipid; as a control, a similar mixture was used in which buffered saline solution substituted for the emulsion of lipid. The pH was maintained at 7.0–7.2. The mixtures were then placed in a water bath at 37°C., portions being removed at intervals from 5 minutes to 24 hours and titrated in mice by means of intracerebral injection. Serum lipids from

TABLE 1

INTRACEREBRAL TITER OF JAPANESE B ENCEPHALITIS VIRUS FOLLOWING INCUBATION AT 37°C. IN THE PRESENCE OF LIPIDS

Test	Virus suspended in:	Length of incubation			
		5 min.	4 hr.	8 hr.	24 hr.
1	Buffered saline (control)	$10^{-3.3}$	$10^{-3.3}$	$10^{-3.5}$	$10^{-3.5}$
	Horse serum lipids	$10^{-5.8}$	$10^{-3.3}$	$<10^{-2.5}$	$<10^{-2.5}$
2	Buffered saline (control)	$10^{-3.7}$		$10^{-3.5}$	$10^{-7.2}$
	Egg-yolk lipids	$10^{-3.3}$		$10^{-3.3}$	$10^{-6.3}$
	Human brain lipids	$10^{-7.6}$		$10^{-6.0}$	$10^{-5.0}$

mice, hamsters, rabbits, and horses, have been found active; for practical reasons horse serum has been used extensively. The viruses thus far tested with similar results were those of St. Louis, Russian Far East, and Japanese B encephalitis. Test 1 (Table 1) shows a typical experiment.

The viral inactivation took place in the presence of serum protein at 2–4°C., although more slowly, and the inactivating agent was not dialyzable and withstood heating at 99° C. for 1 hour. There was no correlation between the change in surface or interfacial tension brought about by the lipid and the degree of viral inactivation.

Fractionation of serum lipids by using several solvents showed that when serum was extracted in succession with acetone, ether, and hot ethyl alcohol-ether, each extract was effective in inactivating virus. Lipids from other animal sources have been tested. Whole lipids obtained from egg yolk were not effective even after 24-hour incubation; those deriving from brain showed only a moderate activity (Test 2). On frac-