is added slowly with stirring, care being taken to maintain the temperature at −5° C. The mixture is allowed to stand at −5° C.; the precipitate is removed in a refrigerated centrifuge at −5° C. and is freed of as much of the supernatant as possible by drainage. The precipitate is dissolved to 1/10 the original SA volume with ice-cold M/15 phosphate buffer of pH 7.4 and clarified by centrifugation at 4,000 r. p. m. for 15 minutes at 0° C. The purified SA samples were maintained at −30° C. until assayed.

Table 1 summarizes the results obtained with SA fractions separated between pH 2.0 and 7.0 in 40 per cent methanol at

TABLE 1

Conditions		Mg. N*/ ml.	PD_{50} (ml.)	PD_{50}'s/ mg. N	PD_{50} yield (%)†
pH	Methanol (%)				
2.0	40	0.31	0.6	5	3
3.1	40	0.47	0.06	35	27
4.1	40	0.45	0.017	131	94
4.6	40	0.36	0.025	111	64
5.1	40	0.30	0.042	79	38
6.0	40	0.21	0.041	116	39
7.0	40	0.17	0.10	59	16
4.1	25	0.35	0.16	18	10
4.1	10	0.27	0.11	34	15
Parent antigens		4.55	0.16	1.4	—

* Ten times the concentration of parent antigens.

† $\frac{\text{Total } PD_{50} \text{ precipitated}}{\text{Total } PD_{50} \text{ in parent antigens}} \times 100$.

−5° C. These data indicate that the substances responsible for the protection of mice against *H. pertussis* are quantitatively precipitated (within the limits of accuracy of the test) at pH 4.1 in 40 per cent methanol. Hydrogen-ion concentrations greater than pH 4.1 lead to an increased solubility of protective substances; those less than pH 4.1 also result in a progressive

TABLE 2

Antigen	PD_{50} (ml.)	No. of Phase I organisms/ PD_{50} (billions)
Purified SA, alum precipitated	0.004	—
Purified SA, alum precipitated + 5,000,000,000 Phase I Vaccine*	0.0026	0.013
Purified fluid SA	0.017	—
10,000,000,000/ml. Phase I Vaccine*	0.015	0.150

* Lederle # 2074-21.

loss of antigen into the supernatant fluid. On a nitrogen basis, the PD_{50} of the fraction separated at pH 4.1 has been purified about 95-fold over parent antigens. Further fractionation of this fraction has resulted in products of over 200-fold purity (*5*).

In other experiments it was noted that methanol concentrations under 40 per cent resulted in the incomplete precipitation of the antigenic factors. These experiments, as well as others to be reported later, suggest that two or more soluble antigens are responsible for the full protection of mice against *H. pertussis*. The above results have been duplicated with different lots of parent SA varying in PD_{50} from 0.09 to 0.29 ml.

Table 2 summarizes the results of a comparative study of the antigenic potencies of concentrated SA fractions separated at pH 4.1 in both the fluid and alum-precipitated state and of Phase I vaccine. The alum-precipitated sample is over 3 times more antigenic than the plain bacterial vaccine. The antigenicity of a mixture of alum-precipitated SA and 5,000,000,000 Phase I organisms was 5 times as great as that of the vaccine. Concentrated fluid SA compares favorably with a 10,000,000,000 Phase I vaccine.

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Some Effects of Ultraviolet Light on 2, 4-D and Related Compounds[1]

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Studies of the action of ultraviolet light on plant growth-regulating compounds have been limited. In 1938 Gilman, in studying the effect of ultraviolet light on unsaturated compounds (*3*), reported that ultraviolet light changed trans-cinnamic acid to a mixture of cis-cinnamic, truxillic and truxinic acids. A year later, Zimmerman and Hitchcock (*5*) showed that the relatively inactive trans-cinnamic acid could be changed to the active cis-cinnamic acid by ultraviolet light. Other workers have noted the effect of light on naturally occurring growth substances. It was shown by du Buy in 1933 (*2*) that white light (not specified) plus heat decreased the growth substance supply in the *Avena* coleoptile. The work of Boysen (*1*) suggested that auxin-a is inactivated, at least in part, by white light but that heteroauxin (indole-3-acetic acid) is not.

The objective of the study reported here was to determine the effect of ultraviolet light on 2,4-D, the sodium, ammonium, and triethanolamine salts, and the methyl, ethyl, and butyl esters. Also included were studies on 2-methyl-4-chlorophenoxyacetic acid. These compounds were selected because they were the active ingredients in most of the hormone-like herbicides available during 1945–46. The results of such a study should assist in the interpretation of comparative field tests when these compounds are used as weed killers.

The chemicals used in this study came from several sources. The 2,4-D (m.p., 139°C.) was prepared in the laboratory of the Chemistry Section, Colorado Agricultural Experiment

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Station. This was dispersed in Carbowax 1500. The ammonium salt of 2,4-D was obtained from the Du Pont Semesan Company. The monohydrated form of the sodium salt was obtained from the J. T. Baker Company. The 2,4-D dispersed in triethanolamine was made in this laboratory by dispersing purified 2,4-D in 10 times its weight of triethanolamine. The methyl ester used was the Dow Chemical Company product called Dow-G-652 and contained 22.75 per cent of the ester. The ethyl ester was the 1945 product (Formula No. 2) of the Sherwin Williams Company. This contained 75 per cent of the ester. The butyl ester was the Sherwin Williams Company 1946 product ("Weed-no-more 40"), which contained 40 per cent by weight of the ester. The compound 2-methyl-4-chlorophenoxyacetic acid was the 1946 Du Pont Chemical Company product called "Methoxone," which contained 85 per cent of the active ingredient.

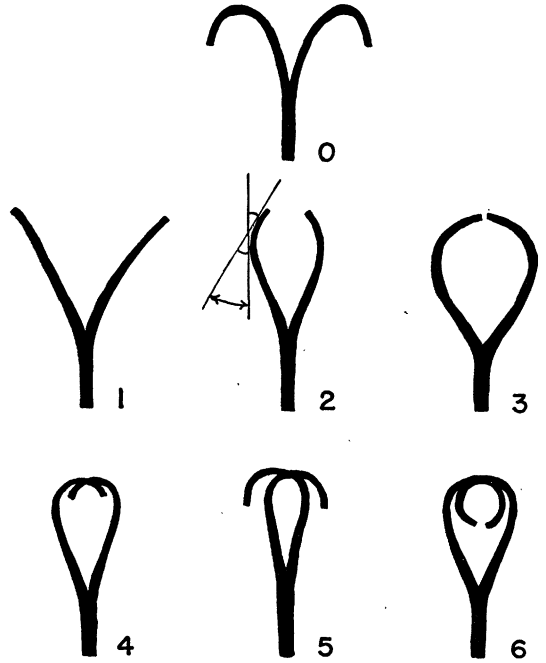

FIG. 1. Pea-stem growth-curvature classes: 0 = zero inward curvature, typical of distilled water; 1 = slight inward curvature; 2 = tips parallel to and inward curvature of 45°; 3, 4, 5, 6 = average inward curvatures between 45° and 90°, between 90° and 135°, between 135° and 180°, and greater than 180°, respectively.

Samples of all chemicals were treated by placing them in sterilized Petri dishes and irradiating for a 12-hour period. The layer of material in each case was approximately 2 mm. thick. At hourly intervals each dish was stirred, shaken, and leveled. Two sources of light were employed: a GE Mazda mercury-arc lamp, type A-H4, with a 100-watt capacity, used without a filter, and the same type lamp of 250-watt capacity, used with a black light filter. The two standard filters used were Nos. 5860 (51) and 5970 (41). Separate series of each chemical were treated with each filter. Transmission measurements of the filters were made in the Physics Department, Colorado Agricultural Experiment Station. It was found that the range of No. 5860 (51) was between 3,407 and 3,888 A., with a peak at 3,600 A.; that of No. 5970 (41), between 3,306 and 4,000 A., with a peak at 3,700 A. and a small amount of transmission in the red between 6,800 and 7,500 A. Transmission in the ultraviolet for No. 5860 (51) was 35 per cent and for No. 5970 (41), 45 per cent.

Among the numerous biological tests for measuring the growth-regulating properties of organic chemicals are those involving measurements of root-growth inhibition, epinastic

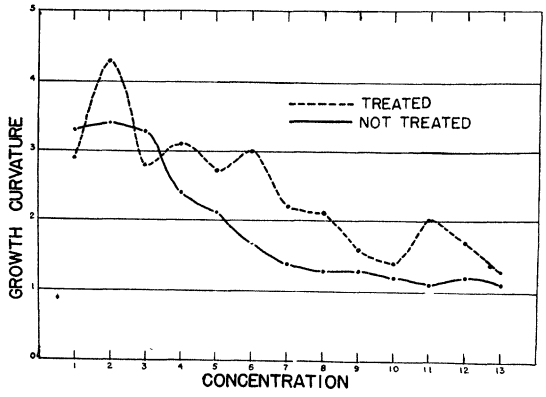

FIG. 2. Pea-stem growth-reaction curves of 2,4-D and of the same compound treated with ultraviolet light (3,407–3,888A.).

response, and growth curvatures of the oat coleoptile and of the split stem-tips of peas. Went's pea test (*4*) was selected for this study because it provided an adequate number of observations for statistical study and because of the ease of obtaining uniform plants.

For each compound, four pea tests (one for each light treatment and control) with 13 concentrations were used, and 10 pea stems were employed in each concentration. Each compound was irradiated according to the three treatments described above.

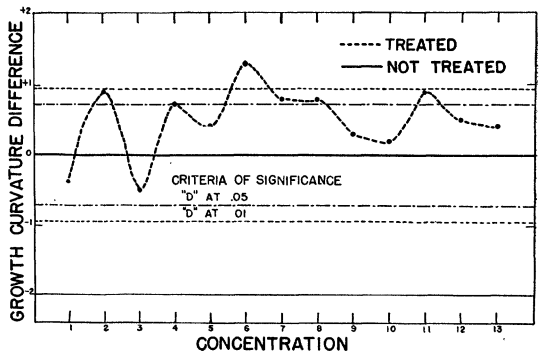

FIG. 3. Criteria of significance of the growth-curvature differences of data shown in Fig. 2.

All solutions were made on a molar basis, such that the weakest concentration (No. 1) corresponded to .000029 M and the strongest (No. 13) to .122000 M. The highest concentration was made by direct weighing and making up to volume; all others, by diluting in such a manner that each succeeding dilution was one-half the concentration of the preceding one. This particular series of concentrations was selected because of the relationship to the sodium salt of 2,4-D expressed in parts per million, *i.e.* concentration No. 1 corresponds to 8 ppm and No. 13 to 31,942 ppm.

The growth curvature of each pea stem was classified according to the system indicated in Fig. 1.

In studying the growth curvature results for each compound, variance analyses were made for each treatment with its control (chemical without light treatment), in order to eliminate error due to treatments and within treatments. Then the criterion of significance of difference, or d-value, between means of each pair (treated chemical and untreated) was found. To facilitate the reading of these differences, graphs were made of those between the two means at each concentration, and the criteria, or d-lines, were drawn at the .05 and .01 levels. This procedure is illustrated by the pea reaction curves for 2,4-D and for the same compound treated with ultraviolet light (filter 51) Figs. 2 and 3. A similar procedure was used to study the effect of ultraviolet light on all other compounds investigated. Results and their significance are summarized in Table 1.

TABLE 1

Chemical	No.	Light treatment	Concentration												
			1	2	3	4	5	6	7	8	9	10	11	12	13
2,4-D	31	Mercury arc	I**	I**	S**	I**	I**	I	S	0	I	I	I		
		Filter 41	I	I**	I	I*	I	I**	*	*	S	I	S**	S	S
		" 51	I	S	I	S	S**	S	S**	S**	S*	S	S	S	S
Sodium salt of 2,4-D	124	Mercury arc	S**	S	S	S	S	S	S**	S**	S	S	I	S	S
		Filter 41	I	I	I	S	S	S	I*	I	I**	I	S	I	I**
		" 51	S	S			S	S	S	S	S		S	I	I
Butyl ester of 2,4-D	150	Mercury arc	F value not significant												
		Filter 41	"	"	"	"									
		" 51	* S	** S	S	S	* S	I	I	S		I			
Ethyl ester of 2,4-D	151	Mercury arc	F value not significant												
		Filter 41	"	"	"	"									
		" 51	"	"	"	"									
Methyl ester of 2,4-D	184	Mercury arc	"	"	"	"									
		Filter 41	"	"	"	"									
		" 51	"	"	"	"									
2,4-D dispersed in triethanolamine	152	Mercury arc	I		I	I**	I**	I	I**	I**	I**	I	I	* I	** I
		Filter 41	F value not significant												
		" 51	S	S**	I	I	S	S	I	S	S	S	S	S	S
2-methyl-4-chlorophenoxyacetic acid	204	Mercury arc		I	I	I		I	I	S	S	S	S	S	S
		Filter 41	F value not significant												
		" 51	* S**	S**	**	I**	0**	I**	** S**	** S**	S**	** S**	** S	* S	
Ammonium salt of 2,4-D	149	Mercury arc	I	I	I	I	I*	I**	I**	I	I	I	I		*
		Filter 41	I**	I**	I**	S**	S	I**	I**	S	I	I	I	I	I
		" 51	I	I	I	I	I	I	I	S	I	I	I	I	I

The letter I indicates inferiority of the irradiated chemical; the letter S, superiority. A single asterisk indicates significance at the .05 level; a double asterisk, at the .01 level.

The trend of these results indicates that ultraviolet light of the range and intensity described, when transmitted by filter 51, can be used to activate 2,4-D, the sodium salt, the butyl ester, and 2-methyl-4-chlorophenoxyacetic acid. Comparative tests of the herbicidal effects of these chemicals activated with ultraviolet light and those of untreated chemicals are suggested.

It is recognized that, with the exception of 2,4-D, pure compounds were not used in this investigation. Possibly purified salts and esters might give different reactions than the impure commercial mixtures used. Had this work been done first on the purified compounds, the question of its action on commercially available herbicides would still have been unanswered, and this was the question of immediate importance.

The results further suggest a possible explanation of the variable results secured from uniform trials of 2,4-D and similar compounds at different times and places. Since the amount of ultraviolet light reaching the earth varies with change in atmospheric conditions, altitude, and season of the year, the herbicidal effects might be expected to vary accordingly. Field tests designed to settle this question are suggested.

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