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RESPONSE OF CLUSTERS OF *VITIS VINIFERA* GRAPES TO 2,4-D AND RELATED COMPOUNDS[1]

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INTRODUCTION

THE HERBICIDAL plant regulator 2,4-dichlorophenoxyacetic acid (2,4-D) and related compounds have been demonstrated to be very toxic to the grapevine even at low concentrations (Clore and Bruns, 1953; Weaver, Winkler, and McCune, 1958).[5] It is generally recognized that clusters are extremely sensitive to the compounds, especially at young stages (Weaver and Williams, 1951). However, there have been few studies where the relative sensitivity of clusters at each of several stages of development to 2,4-D have been made. This paper presents results of experiments performed in 1958 and 1959, to elucidate and add further information on response of clusters to 2,4-D and related compounds. One objective was to develop a test method whereby one could compare toxicities of various regulators on grape clusters, or could compare varietal sensitivity of clusters to the regulators.

MATERIALS AND METHODS

Mature vines of Thompson Seedless (syn. Sultanina), Tokay, and Ribier in an irrigated vineyard at the University of California, Davis, were used. The seedless variety, Thompson Seedless, was pruned to four canes (Winkler, 1931), while Tokay and Ribier, seeded table grapes, were cordon-trained and spur-pruned.

Aqueous solutions of the plant growth regulators, obtained from commercial sources, were prepared by adding sufficient ammonia to dissolve the compounds. All concentrations are expressed in parts per million on an acid-equivalent basis. Hereafter, 2,4-dichlorophenoxyacetic acid is referred to as

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[5] See "Literature Cited" for citations referred to in the text by author and date.

2,4-D; 2,4-5-trichlorophenoxyacetic acid as 2,4,5-T; and 4-chlorophenoxyacetic acid as 4-CPA. The wetting agent in all experiments was "Dreft," a proprietary compound of sodium laural sulfate. Sufficient Dreft was added so that test clusters were thoroughly wetted.

In the selection of clusters for experimentation a large number of vines were utilized so that uniformity could be attained. For example, in the first experiment on Tokay, 80 vines were used. Clusters of flowers or fruit chosen at random were immersed momentarily in a solution of one of the growth regulators.

The number of seeds per berry, except for small shot berries that failed to soften, was determined. At harvest berries were removed from the cluster, counted and weighed. The percentage of total soluble solids in the juice was determined with a hand refractometer.

EXPERIMENTATION AND RESULTS

Stage of Development of Tokay Clusters in Relation to Sensitivity to 2,4-D

On April 17, 1958, ten clusters about 1½ inches long were dipped in 2,4-D concentrations of 0 to 25 ppm. At later dates clusters were similarly dipped in 0 to 100 ppm solutions of 2,4-D (table 1). The stages of development of the vines at the dates of treatment were as follows:

April 17 —Shoots about 6 inches long, clusters about 1.5 inches long
April 24 —Shoots about 15 inches long, clusters about 3.5 inches long
May 5 —Shoots about 30 inches long, clusters about 5.5 inches long
May 15 —Shoots about 40 inches long, clusters about 7.0 inches long
May 21 —Full bloom, 50 per cent of calyptras fallen
June 16 —Berry-shatter stage, berries about 1.5 mm in diameter
August 2—Coloring just initiating, juice about 12° Balling.

The compound at 2 ppm caused no early visible injury at any time of treatment, but clusters dipped on the first four dates in 2,4-D at 8 ppm became yellowish within 7 to 10 days after dipping. Curvature of the rachis and its laterals was noticeable within two weeks after treatment, and shot berries had developed. Injury was usually progressively greater as concentrations increased.

Clusters dipped on May 5 in 2,4-D in range from 8 to 50 ppm developed many persistent calyptras which failed to fall at the normal time.

Clusters were harvested on October 8. 2,4-D at 2 ppm showed no visible injury to the cluster within several weeks after treatment. However, the harvest data showed that considerable damage was done by all the treatments, except the last, and especially by the first four applications (table 1, fig. 1). The treatment on August 2 resulted in no injury even from the compound at 100 ppm. In general, the earlier the treatment, the greater was the injury as expressed by decreased cluster and berry weight (table 1, fig. 2). There were some exceptions to this generality. For example, the treatment on April 24 with 2,4-D at 2 and 8 ppm usually resulted in more injury than that with the same treatments on April 17.

TABLE 1

AVERAGE CLUSTER AND BERRY WEIGHT (GRAMS) OF TOKAY AT HARVEST ON OCTOBER 8, 1958 AFTER DIPPING IN 2,4-D AT VARIOUS CONCENTRATIONS AND AT VARIOUS STAGES OF DEVELOPMENT

(Figures are the average of 10 clusters)

Treatment, concentration 2,4-D (ppm)	Dates of treatment						
	4/17/58	4/24/58	5/5/58	5/15/58	5/21/58	6/16/58	8/2/58
	Weight per cluster (grams)						
0	689	689	689	689	689	689	689
2	726	537	388	591	810	766	826
8	182	99	163	363	379	624	694
25	109	114	248	29	406	610	661
50	...	65	165	0	207	540	773
100	...	32	123	228	418	355	720
	Weight per berry (grams)						
0	6.1	6.1	6.1	6.1	6.1	6.1	6.1
2	5.5	5.4	4.8	5.7	6.0	6.6	5.7
8	3.8	1.6	1.6	5.3	4.4	6.1	6.1
25	2.1	0.7	1.6	0.0	4.1	4.5	5.9
50	...	0.8	1.6	0.0	1.0	4.7	5.9
100	...	0.6	1.2	2.3	4.2	4.5	6.4

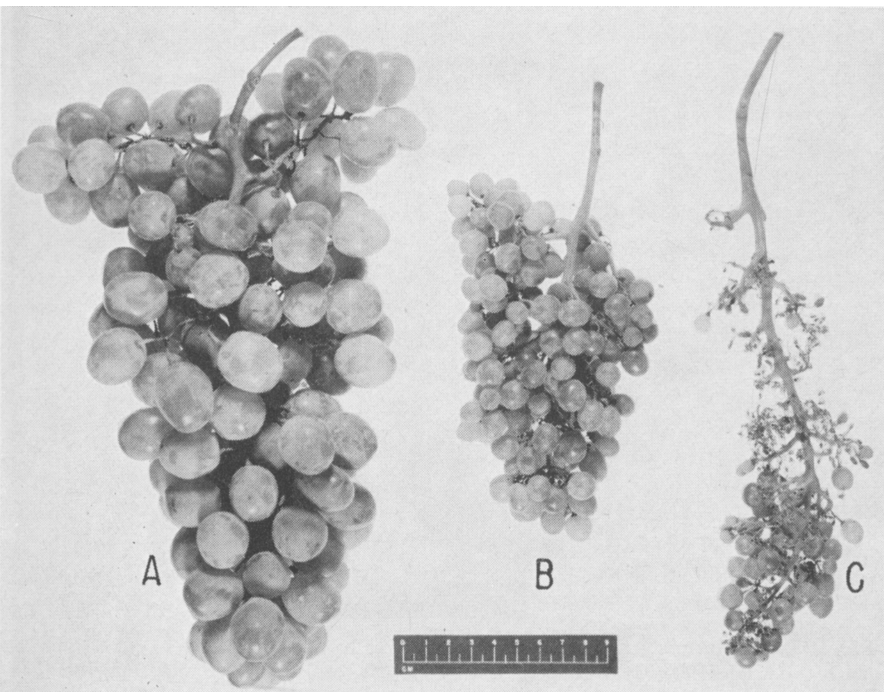

Fig. 1. Tokay clusters 168 days after dipping in 2,4-D at 8 (B) or 100 ppm (C) on April 24, 1958. A, control. Note that the compound at 8 ppm has greatly reduced berry size, and at 100 ppm cluster is mostly dead. (Photographed October 9, 1958.)

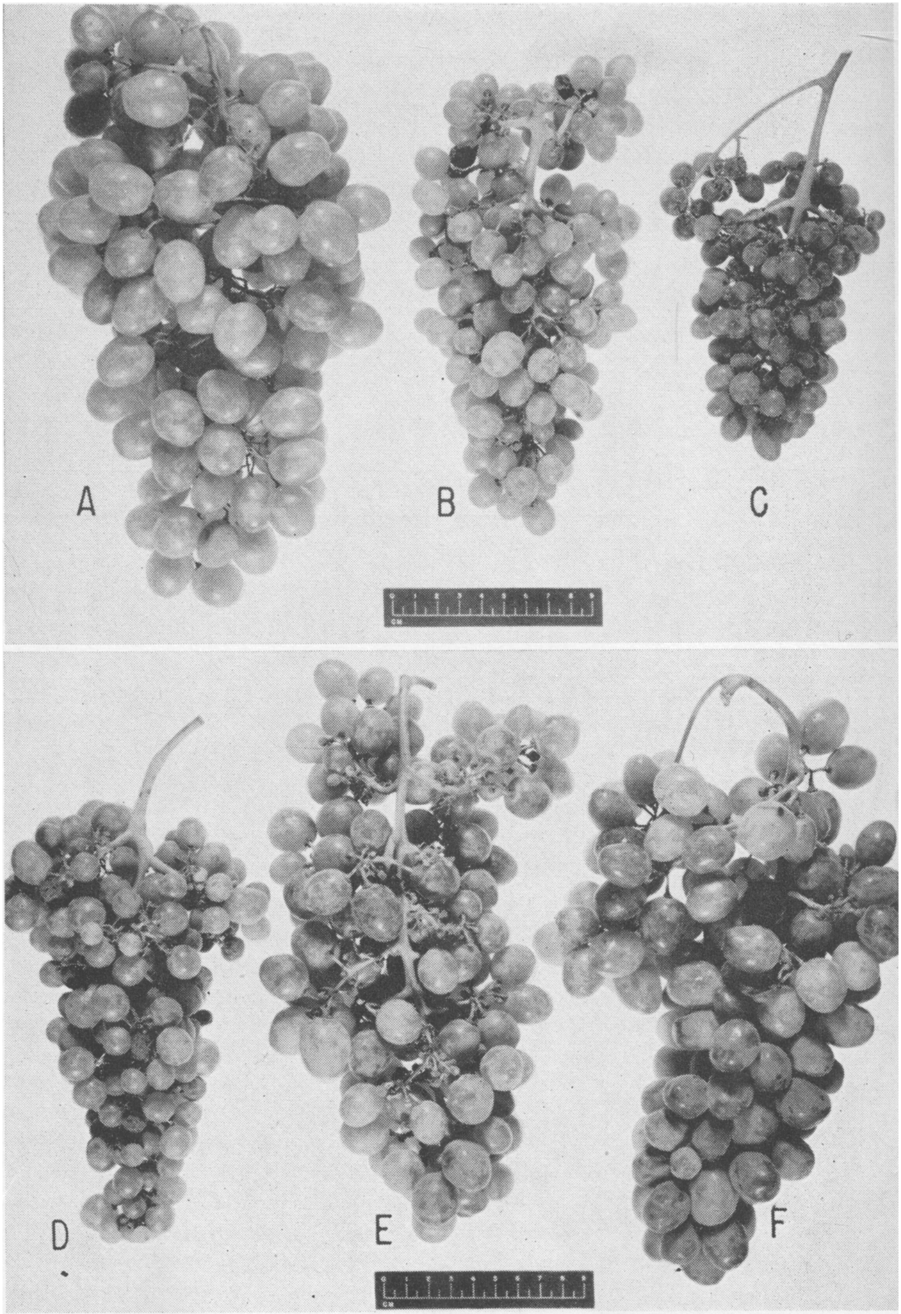

Fig. 2. Tokay clusters at harvest (October 8) after being dipped in 2,4-D at 25 ppm on April 17 (B), April 24 (C), May 5 (D), May 21 (E), or June 16 (F). A, untreated control. Note smaller berries and shot berries produced by 2,4-D, and that the earlier the treatment the greater is the injury, except that the second treatment (C) resulted in more injury than the first (B). Cluster treated on June 16 (F) appears about like the control (A). (Photographed October 9, 1958.)

Method for Testing Relative Toxicity of Plant Regulators on Clusters

It would be desirable to have a simple test to determine the relative toxicity of various plant regulators on clusters of a given variety and also to test the sensitivity of clusters of various varieties to a given regulator. The previous experiment has shown that the sensitivity of a cluster to 2,4-D varies tremendously with its stage of development. For a successful test method one must then select a fairly early stage when clusters are sensitive to 2,4-D, and also pick a definite physiological stage which can be duplicated among different varieties and over different years.

It was believed that full bloom (when 70 per cent of calyptras have fallen) and berry shatter stage (when all berries have shattered after bloom) would

TABLE 2

AVERAGE WEIGHT (GRAMS) PER CLUSTER AND PER BERRY OF TOKAY CLUSTERS AT HARVEST (SEPTEMBER 27, 1959) AFTER DIPPING IN 2,4-D AT FULL BLOOM OR SHATTER STAGE

(Figures are the average of 10 clusters)

Treatment, concentration of 2,4-D (ppm)	Full bloom stage		Shatter stage	
	Weight per cluster (grams)	Weight per berry (grams)	Weight per cluster (grams)	Weight per berry (grams)
0	768	5.17	708	4.84
0.1	853	5.76	768	5.26
1	791	5.62	642	5.17
5	589	4.15	659	5.16
25	344	2.56	402	3.61
50	119	1.60	613	4.70
$d_{.05}$	172	0.47	208	0.51

be the best two possibilities to meet these requirements. Studies were made on both Tokay and Thompson Seedless at full bloom and shatter stage to determine which stage results in most uniform injury in a test of toxicity, and also to determine whether reduction in cluster weight or berry weight would be the best criterion to use for degree of injury. Several concentrations of 2,4-D were used so that the optimum concentration for a suitable degree of injury could be ascertained.

Experiments on Tokay. On May 15, 1959, ten clusters selected at random at full bloom were dipped in 2,4-D at 0.1 ppm. Other series were dipped at 1, 5, 25, or 50 ppm, and another served as the untreated control. All clusters except the treated one were removed from the shoot before treatment. Clusters were harvested on September 27, 1959 (fig. 3). The compound at 0.1 ppm caused no apparent injury, but at concentrations of 1 and 5 ppm many shot berries had developed. Clusters dipped in 2,4-D at 25 ppm were straggly, with many small berries, and the apical halves of many clusters treated at 50 ppm were dead and dry.

The results (table 2) show both cluster and berry weight decreased as a result of 2,4-D. In both instances, 2,4-D at 25 ppm decreased weights by about half, as compared with the untreated clusters and berries.

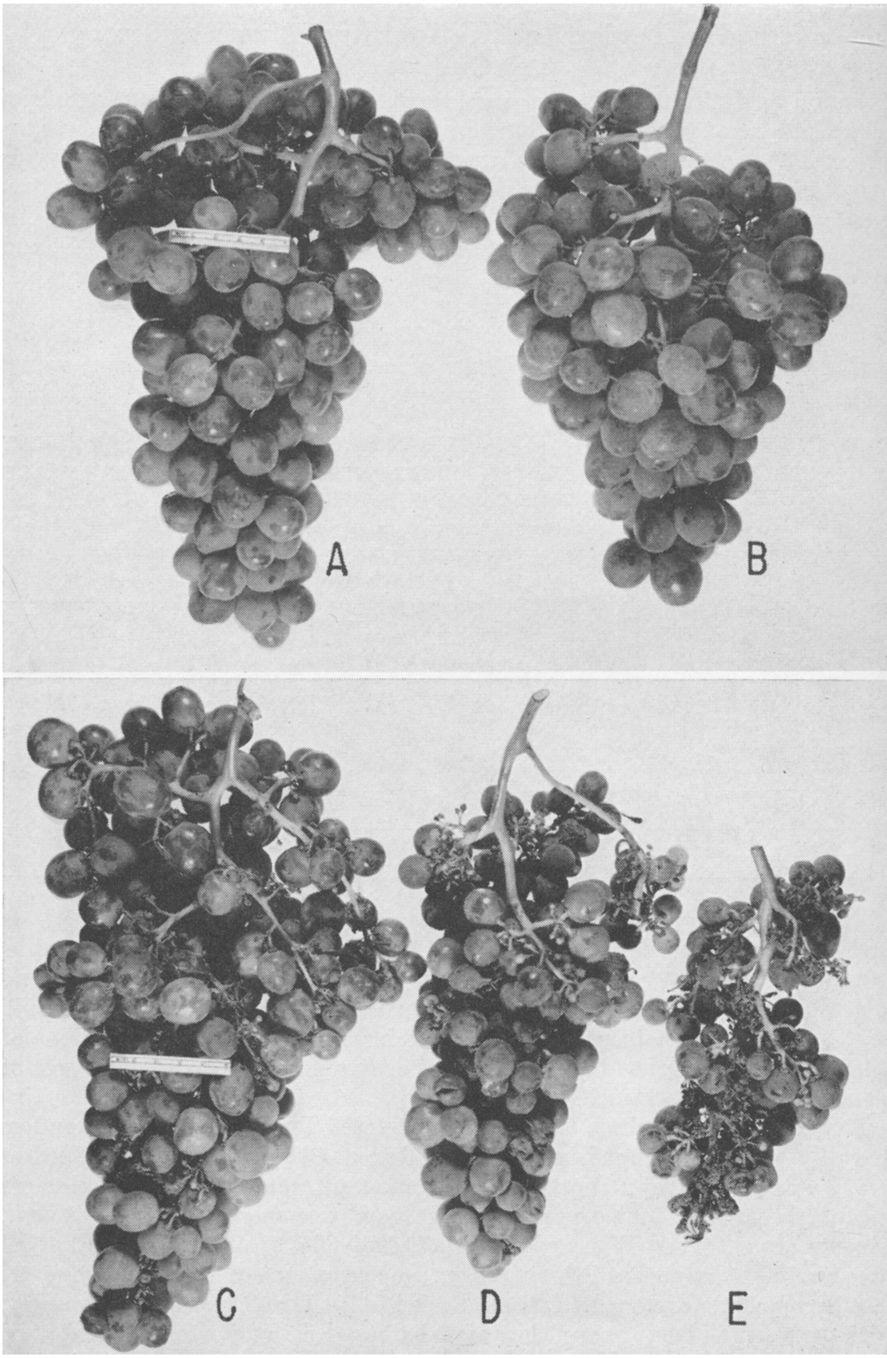

Fig. 3. Tokay cluster at harvest (September 27, 1959) 135 days after being dipped at full bloom in 2,4-D at 1 (B), 5 (C), 25 (D), or 50 ppm (E). A, untreated control. Note that the higher the concentration, the greater is the injury, and that the compound at 50 ppm almost completely killed the clusters. Note presence of shot berries in treated clusters. (Photographed September 27, 1959.)

Berries of the control and those treated with 2,4-D at 25 ppm were cut open to determine the number of seeds per berry. The data (table 3) indicate 2,4-D produced a smaller percentage of 2- and 3-seeded berries and more 0- and 1-seeded berries. However, more berries with 4 seeds developed as a result of the treatment with 2,4-D. The average weight per berry was reduced in all seed classes, and clusters treated with 2-4-D had a higher degree Balling reading.

On June 1, 1959, after shatter of berries, the experiment performed at full bloom was repeated. The fruit was harvested on September 27. Much less injury resulted than when 2,4-D was applied at full bloom (table 2, fig. 4).

TABLE 3

EFFECT OF DIPPING TOKAY CLUSTERS AT FULL BLOOM IN 2,4-D AT 25 PPM ON SEEDEDNESS, WEIGHT PER BERRY, AND DEGREES BALLING

(Figures are the average of 10 clusters)

Treatment	Number of seeds per berry				
	0	1	2	3	4
	Percentage of total number of berries in each seed class				
Untreated	0	40.1	40.2	15.8	3.9
2,4-D	4.3	43.6	34.3	11.5	6.3
	Weight per berry in each seed class				
Untreated	2.00	3.98	5.68	6.78	6.67
2,4-D	1.16	2.15	2.89	3.39	3.38
	Degrees Balling in each seed class				
Untreated	22.4	23.7	21.8	21.6	18.8
2,4-D	24.7	26.1	25.6	29.8	25.4

For some reason, 2,4-D at 50 ppm caused less injury than at 25 ppm. The shatter stage appears less accurate as a measure than the full bloom stage. However, at higher concentrations the shatter stage might be a useful stage to use.

Experiments on Thompson Seedless. The experiments on Tokay were repeated on Thompson Seedless on May 15, 1959 (full bloom) and on May 25 (shatter stage). Thompson Seedless were thinned to 5 clusters per cane (20 clusters per vine) and berry-thinned by removing the apical half of the cluster (Winkler, 1931). All clusters were harvested on August 19. Clusters dipped at full bloom at 0.1 and 1 ppm appeared normal at harvest, but many dipped at 25 ppm were dead and others had small clusters and small berries (fig. 5, table 4). Most clusters treated with the compound at 50 ppm were dead and dry. Treatment at shatter stage often caused less injury than treatment at full bloom. For example, berry size was depressed by 2,4-D at both 25 and 50 ppm applied at full bloom but not significantly so by 50 ppm applied at the shatter stage. However, 2,4-D at 25 ppm applied at shatter stage

Fig. 4. Cluster of Tokay 119 days after dipping at berry shatter stage in 2,4-D at 25 ppm (right). Left, control. Note treated berries (right) appear only slightly smaller than the controls. (Photographed September 27, 1959.)

TABLE 4

DATA AT HARVEST (AUGUST 19, 1959) FOR THOMPSON SEEDLESS CLUSTERS DIPPED AT FULL BLOOM OR AFTER SHATTER IN 2,4-D AT VARIOUS CONCENTRATIONS

(Figures are the average of 10 clusters)

Treatment, concentration of 2,4-D (ppm)	Full bloom		Shatter stage	
	Weight per berry (gram)	Percentage of total number of berries dead and dry	Weight per berry (gram)	Percentage of total number of berries dead and dry
0	1.99	0	2.19	0
0.1	1.72	0	2.08	0
1	2.12	0	2.14	1
5	1.73	3	2.09	1
25	0.73	43	0.86	55
50	0.11	93	1.87	5
$d_{.05}$	0.53	..	0.40	..

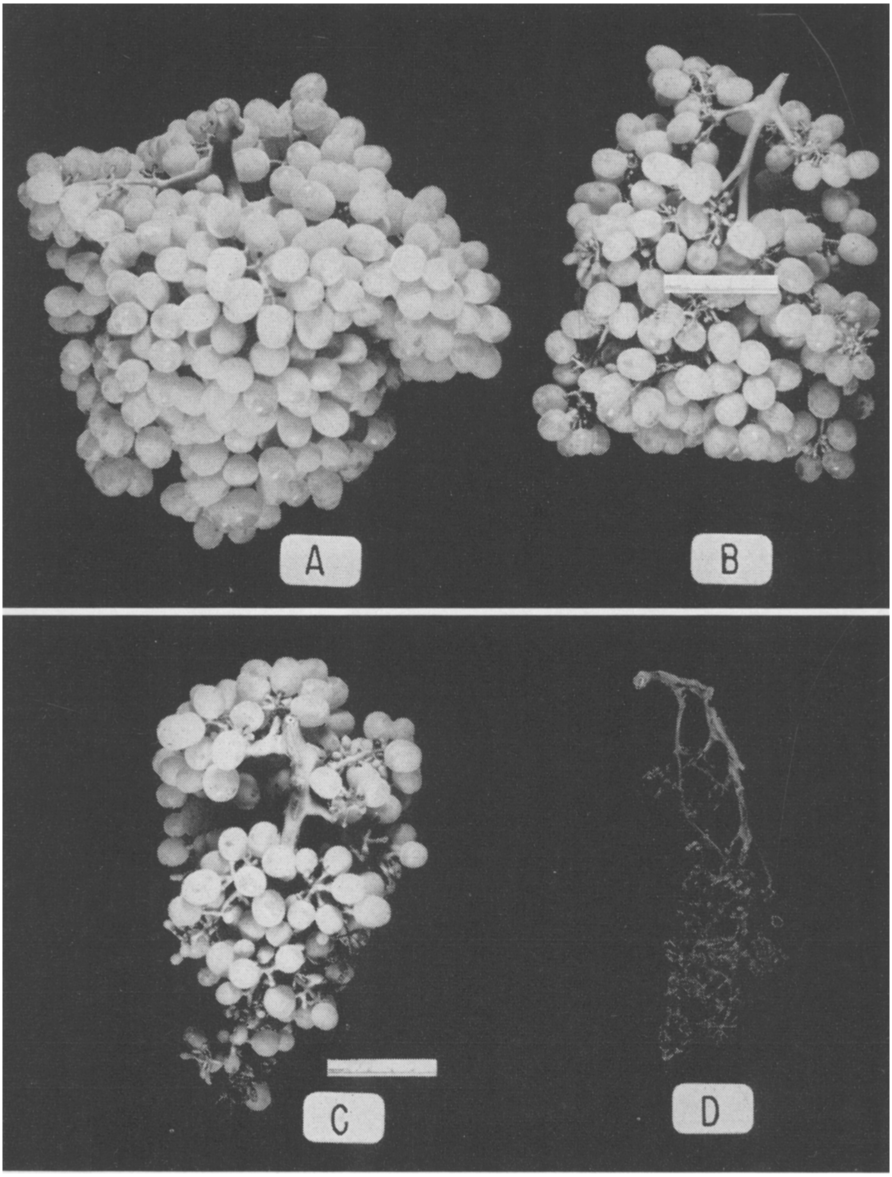

Fig. 5. Thompson Seedless clusters 96 days after being dipped at full bloom (May 15, 1959) in 2,4-D at 5 (B), 25 (C), or 50 ppm (D). A, control. Note occurrence of shot berries as a result of 2,4-D at 5 ppm, and that injury becomes progressively greater with compound at 25 or 50 ppm. (Photographed August 15, 1959.)

caused much callusing and splitting of stems and dead berries. The compound at 25 ppm applied at shatter stage resulted in more injury than at 50 ppm. The percentage of dead and dried berries usually increased with increasing concentrations of plant regulator.

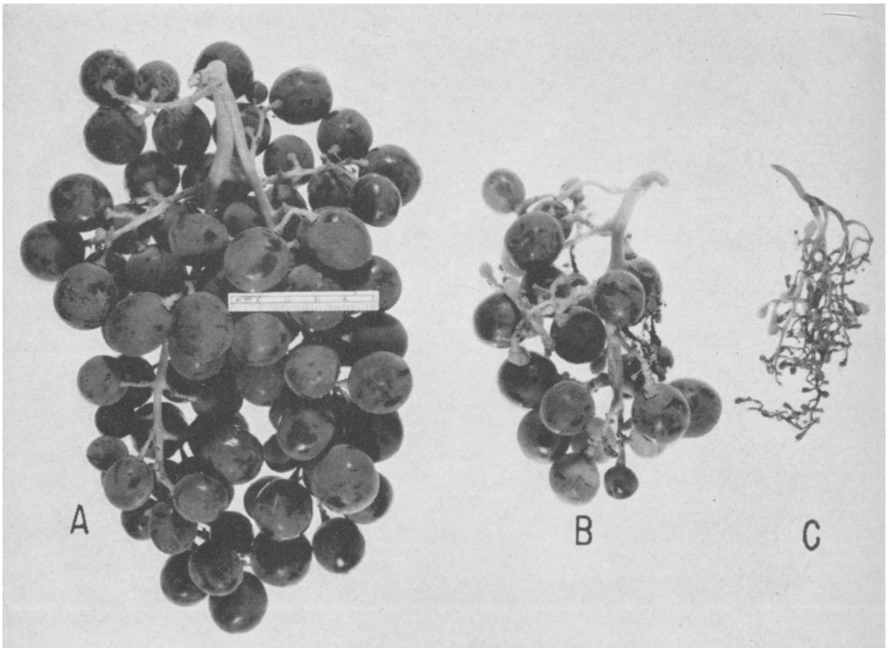

Fig. 6. Ribier clusters at harvest (August 25) 92 days after dipping on May 25 (shatter stage) in 2,4-D (B) or 2,4,5-T (C) at 25 ppm. A, control. Note that 2,4-D (B) had caused much injury, but that 2,4,5-T (C) had killed clusters. (Photographed August 25, 1959.)

TABLE 5

DATA AT HARVEST (AUGUST 19, 1959) FOR RIBIER AND THOMPSON SEEDLESS CLUSTERS DIPPED AT SHATTER STAGE IN 2,4-D, 2,4,5-T, OR 4-CPA AT 25 PPM

(Figures are average of 10 replicate clusters)

Item	Untreated controls	Treatment			
		2,4-D	2,4,5-T	4-CPA	$d_{.05}$
	Ribier				
Weight per berry (gm)	2.6	1.25	0.04	4.08	1.03
Percentage of total number of berries that were shot berries	1.2	90.0	99.0	2.5	....
	Thompson Seedless				
Weight per berry (gm)	2.16	0.80	0.01	2.25	0.34
Percentage of total number of berries that were shot berries	0	15.0	100.0	0	....

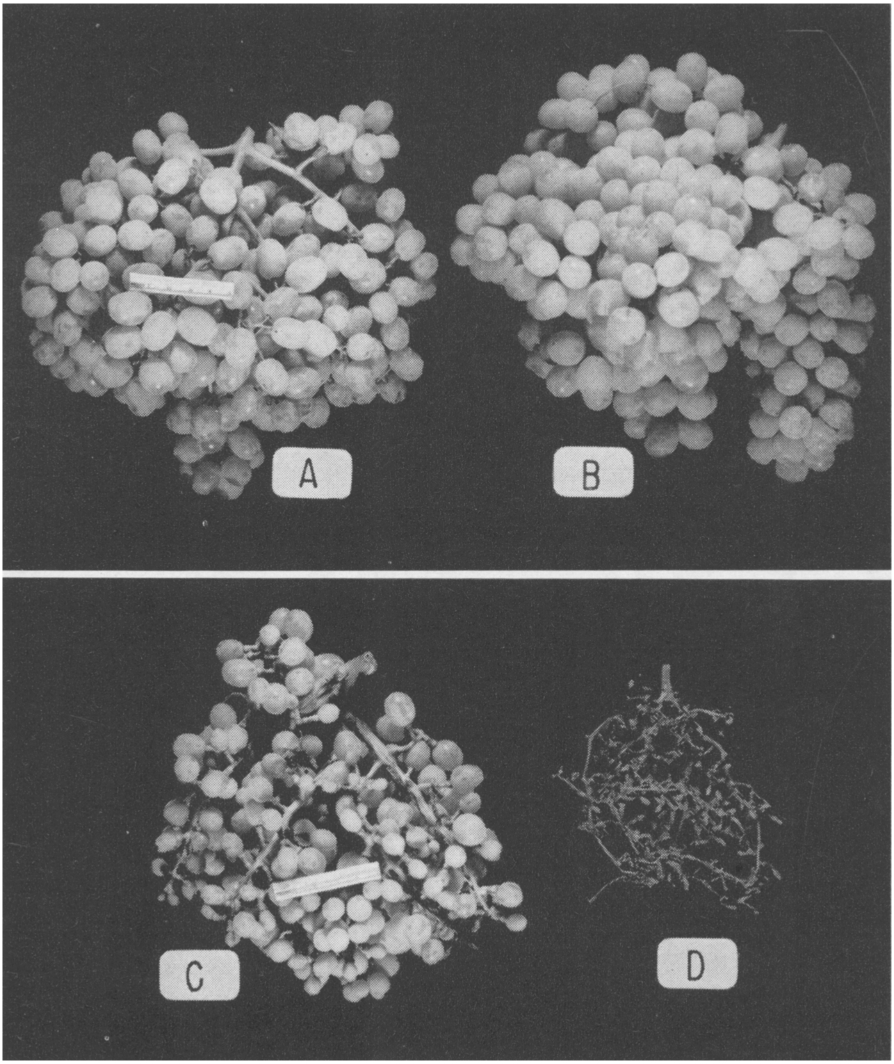

Fig. 7. Thompson Seedless cluster 86 days after dipping on May 25 (shatter stage) in 4-CPA (B), 2,4-D (C), or 2,4,5-T (D) at 25 ppm. A, untreated control. Note that 4-CPA has produced no injury, 2,4-D severe injury, and that clusters treated with 2,4,5-T are dead. (Photographed August 19, 1959.)

Relative Toxicity of Various Compounds on Clusters of Ribier and Thompson Seedless

The purpose was to use the test method described in the preceding section to determine the relative toxicity of 2,4-D, 2,4,5-T, and 4-CPA by dipping clusters at the shatter stage. Ribier clusters were dipped on May 25, 1959, 10 clusters per treatment, with plant regulators at 25 ppm. Fruit was harvested on August 25, 1959. Clusters dipped in 2,4-D had small berries and thickened and crooked peduncles and pedicels (fig. 6). All clusters dipped in 2,4,5-T were dead and dry, while clusters dipped in 4-CPA were larger than the controls (table 5). It is not known why Ribier berries were enlarged by the 4-CPA; perhaps it was a result of thinning (fewer berries per cluster). The 2,4,5-T was the most toxic and 4-CPA the least.

Thompson Seedless grapes were similarly treated on May 25, at shatter stage, and harvested on August 19. Results were similar to the Ribier experiment. Clusters dipped in 2,4-D had small berries, and thickened and cracked cluster frameworks. 2,4,5-T had killed all clusters, while 4-CPA had caused no injury (table 5, fig. 7).

Sensitivity of Clusters of Various Varieties to 2,4-D

The purpose was to determine how clusters of various varieties differ in their sensitivity to 2,4-D. Ten clusters of Thompson Seedless, Sultana, Tokay, and Ribier grapes were dipped in 2,4-D at 25 ppm at the shatter stage. Ten

TABLE 6

EFFECT OF DIPPING SEVERAL VARIETIES OF CLUSTERS IN 2,4-D AT 25 PPM AT SHATTER STAGE ON CLUSTER AT HARVEST

(Figures are average of 10 replicate clusters)

Variety	Control	2,4-D	Percentage decrease of treated cluster
Thompson Seedless			
Weight per cluster (gm)	537*	482	10
Weight per berry (gm)	1.85	1.75	5
Sultana			
Weight per cluster (gm)	711	328	54
Weight per berry (gm)	1.37	1.20	12
Tokay			
Weight per cluster (gm)	708	402	43
Weight per berry (gm)	4.84	3.61	26
Ribier			
Weight per cluster (gm)	313	51	84
Weight per berry (gm)	3.09	1.25	60

* Differences required for significance between controls and 2,4-D treated for any one variety are 85 for cluster weight and 0.36 for berry weight.

clusters of each were the controls. Thompson Seedless, Sultana, and Ribier were treated on May 29, 1959, and Tokay on June 1. Thompson Seedless and Sultana were harvested on August 19, Tokay on August 25, and Ribier on September 29.

The data (table 6) show that Ribier suffered the greatest, and Thompson Seedless the least injury as judged by degree of suppression of cluster and berry weight.

DISCUSSION

In general, the earlier clusters were treated with 2,4-D, the greater was their sensitivity. This is in agreement with the general observation that it is the young meristematic tissues that are the most responsive to growth regulators.

Dipping clusters at full bloom, and then determining suppression of berry growth appeared to be the best method for determining sensitivity of clusters to growth regulators. It would be desirable to use this test in addition to another bioassay developed to determine sensitivity of growing shoots to growth regulators (Weaver and McCune, 1959) in assessing the sensitivity of the vine to plant regulators.

Dipping flowering clusters of Tokay in solutions of 2,4-D increased the number of seedless berries. This may have been a result of an injurious effect of 2,4-D to the germination of the pollen grains preventing fertilization. In other words the 2,4-D may have acted as a pollenicide. Within each seed class the weight per berry was lessened as a result of 2,4-D, and the degree Balling was increased. Perhaps the increased degree Balling reading was inversely correlated with berry size, or perhaps the 2,4-D served to mobilize the sugars into the treated fruit.

SUMMARY

An experiment in which Tokay clusters were dipped in solutions of 2,4-D at various concentrations showed that in general the earlier the time of treatment, the greater the injury. A method was developed for testing the relative toxicity of plant regulators on grape clusters. Tokay and Thompson Seedless clusters were dipped at full bloom or berry shatter stage in 2,4-D at varying concentrations. Full bloom appeared to be the best time for treatment, and decrease in berry weight at harvest the best criterion for injury.

When 2,4-D at 25 ppm was applied to flowering Tokay clusters, a large number of berries containing 0 or 1 seed per berry occurred. The average weight per berry was reduced in all seed classes, and clusters treated with 2,4-D had a higher degree Balling reading.

The bioassay was used to determine relative toxicity of regulators on grape clusters. 2,4,5-T was most toxic, 4-CPA the least toxic, and 2,4-D intermediate in toxicity to Ribier and Thompson Seedless clusters.

The increasing order of sensitivity of clusters of four varieties to 2,4-D was Thompson Seedless, Sultana, Tokay, and Ribier.

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