STUDIES IN ULTRA-VIOLET AND RESPIRATORY PHENOMENA. II

The Effects of Ultra-Violet on Respiration and Respiratory Enzymes of Higher Plants[1]

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The present paper reports observations on the respiratory rate and activity of oxidizing enzymes of tomato and bean plants as affected by ultra-violet radiation, and offers suggestions concerning the relationship of oxygenase and peroxidase to the respiratory mechanism. The pertinent literature has been discussed in the first paper of the series (Wynd and Reynolds, '35).

EXPERIMENTAL METHODS

From several hundred plants of Bonny Best tomatoes and Red Kidney field beans, those of uniform size and vigorous physiological condition were selected. They were irradiated under the conditions of the individual experiments by a Burdick quartz mercury vapor arc suspended above the plants. The distances reported were measured from the arc to the surface of the soil. Two types of filters were used: one a quartz-plate

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tray 5 mm. thick, containing 2 cm. of distilled water; the other, a tray made of Vita glass, containing a similar depth of water. The quartz water cell, according to Jones ('28), absorbs all wave lengths longer than 1.4 μ, and the Vita glass water cell eliminates not only this infra-red but also the ultra-violet shorter than 2894 Å.

Respiration was determined by weighing the amount of carbon dioxide eliminated per gram of fresh weight over a given period (Wynd, '32, '35). The intrinsic accuracy of this apparatus is very great. However, the respirational rates of the plants themselves were found to vary about ± 5 per cent in duplicate controls, and so this must be regarded as the maximum accuracy of the data obtained. The plants were cut off at the surface of the soil in the evening and placed in beakers of water in the respiration chambers, and the next morning the carbon dioxide absorption bulbs were weighed. Five plants were used for each determination, and observations on the control and the experimental plants were carried on simultaneously.

Since all of the enzymatic determinations were made on juice pressed from the plants *after* they had been rayed, the values obtained represent *in vivo* reactions. The necessary distinction between *in vivo* and *in vitro* experiments has been particularly pointed out by Fuller ('32) and by Wynd and Reynolds ('35). The upper leaves and the upper 2 inches of the stems were ground to a paste in a mortar with a little quartz sand, then squeezed through 2 layers of cheese-cloth, and aliquots of this solution used for the enzymatic determinations. Since the usual methods of measuring acidity are disturbed by oxidizing enzymes or by hydrogen peroxide, the final pH of all experimental solutions was checked by the glass electrode.

Extended experimentation by Wynd has shown that the determination of catalase activity can be more closely duplicated by titration than by gasometric methods. However, the gasometric method is entirely satisfactory as a *micro* procedure if the usual precautions are taken. Experiments involving relatively large amounts of material can be duplicated by titration with an error of 1 or 2 per cent, which is not possible by *macro* gasometric procedures. The experimental solutions contained

50 cc. of phosphate buffer at pH = 7.00, 20 cc. of 3 per cent commercial hydrogen peroxide, and 1 cc. of the expressed juice. The reaction time varied from 8 to 20 minutes in the different experiments, and it was stopped by adding 20 cc. of a 25 per cent solution of sulphuric acid. The time of adding the plant juice and of the subsequent addition of acid was carefully controlled by a stop watch. The amount of hydrogen peroxide remaining was then titrated with N/5 potassium permanganate. The reaction times of all the solutions were spaced 1 minute apart to allow time for the pipettings.

Peroxidase was determined by the method of Guthrie ('31). The substrate for the enzyme was prepared by adding 200 cc. of a citrate buffer at pH = 4.5 to 200 cc. of water. To this were added 1 gm. of p-phenylenediamine hydrochloride and 20 cc. of a 4 per cent solution of alpha-naphthol in 50 per cent alcohol. The final mixture was filtered and used immediately. At pH = 4.5, atmospheric oxidation of the substrate is slow, and catalase is so far depressed as not to seriously interfere. The experimental solutions consisted of 25 cc. of substrate, 1 cc. of commercial 3 per cent hydrogen peroxide, and .5 cc. of the plant juice. After 10 to 20 minutes, depending on the intensity of the color which developed, the reaction was stopped by the addition of 5 cc. of a .1 per cent potassium cyanide solution. Since the cyanide inhibits the enzyme but does not interfere with the atmospheric oxidation, the indophenol produced was determined as quickly as possible. This was done by shaking each experimental mixture with 50 cc. of toluene until the water layer was clear. The upper layer of toluene, after being poured off and centrifuged to remove bubbles and tissue fragments, was examined in a colorimeter. The error of this method is about 3 per cent.

Oxygenase was determined similarly to peroxidase except that the hydrogen peroxide was not added to the substrate. Due to the feeble activity of oxygenase as compared with that of peroxidase, 1 cc. of plant juice was used for each determination, and the reaction time was usually extended to 20 minutes. The indophenol was extracted in 25 cc. of toluol. The error of this method is about 3 per cent.

Since the temperature, reaction periods, etc. varied from day to day, the absolute values of data obtained at different times may not be directly compared, and the tables contain values calculated as percentages of their corresponding controls. These percentage values obtained at different times may be compared directly. In the figures, the numbers on the ordinate represent the per cent of stimulation or depression, the graphical value of the control therefore being zero. The horizontal line at zero divides the stimulation from the depression values. The numbers on the abscissa represent minutes of daily exposure.

EXPERIMENTAL RESULTS

Experiment I.—Bonny Best tomato seeds were planted in greenhouse flats January 23, 1934, and were transplanted to 4-inch pots February 8. The raying began March 4, and was continued daily for 5 days. The distance was 24 inches and the dosages 4, 8, 16, and 32 minutes daily. The unscreened mercury arc was used as a source of illumination.

TABLE I

ENZYMATIC ACTIVITY OF TOMATO PLANTS RAYED AT 24 INCHES WITH THE UNSCREENED MERCURY ARC, CALCULATED IN PERCENTAGES OF THE CONTROL

	Control	Daily Dosage			
		4 minutes	8 minutes	16 minutes	32 minutes
After 5 days of irradiation					
Oxygenase	100	96	107	96	78
Peroxidase	100	97	159	166	213
Catalase	100	123	134	119	125
5 days after irradiation ceased					
Oxygenase	100	95	92	88	74
Peroxidase	100	189	263	345	385
Catalase	100	115	146	194	165

All plants showed definite injury at the end of the 5-day period of irradiation. The plants submitted to the heavier

dosages were very severely burned. One set of determinations was made at the end of the irradiation period and another 5 days later. The plants were not rayed during this intervening period. From table 1 and figs. 1 and 2, it is seen that with increasing amounts of daily irradiation the peroxidase activity greatly increased. Catalase also increased but less than the

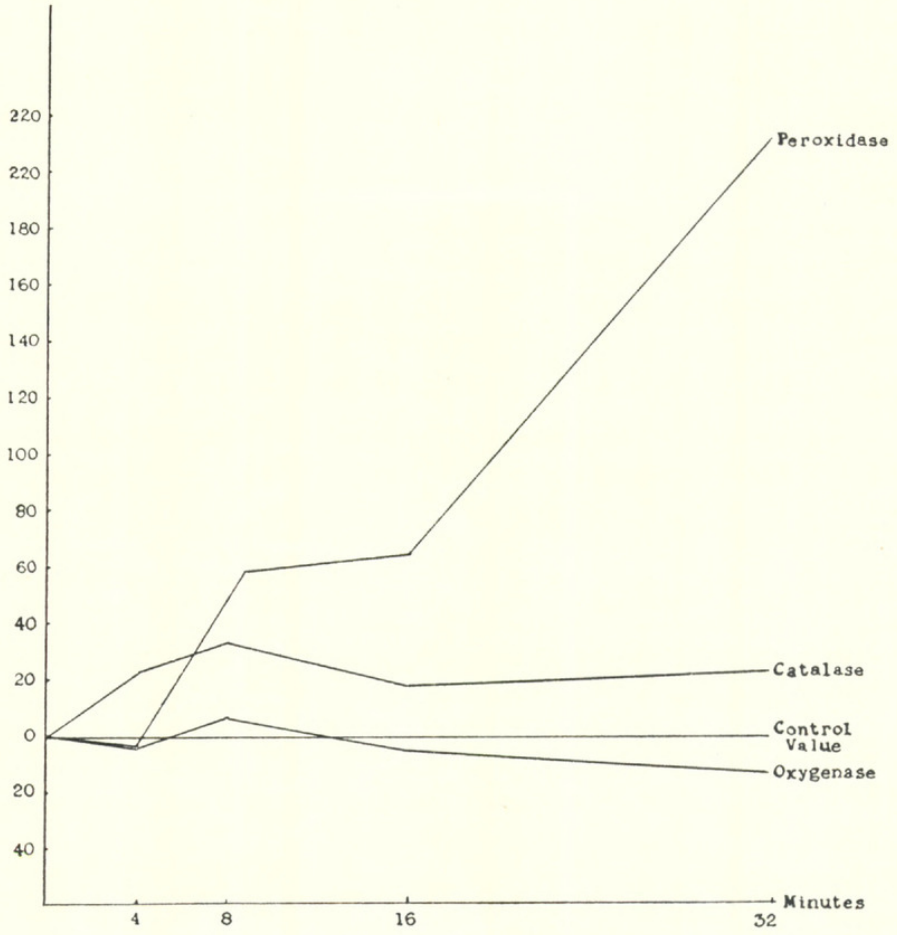

Fig. 1. Determination of enzymatic activities of tomato plants after being irradiated daily for 5 days at 24 inches, through quartz water-cell filter.

peroxidase, while oxygenase was progressively inhibited. These results were of greater magnitude 5 days after irradiation ceased.

Experiment II.—Bonny Best tomatoes were planted February 15, 1934, and transplanted from the flats to 4-inch pots on

April 5. The raying began April 16, at a distance of 100 inches, and continued in 10-minute doses daily for 5 weeks. According to Fuller ('31), these are the optimum conditions for growth stimulation. Some of the plants were rayed through the quartz

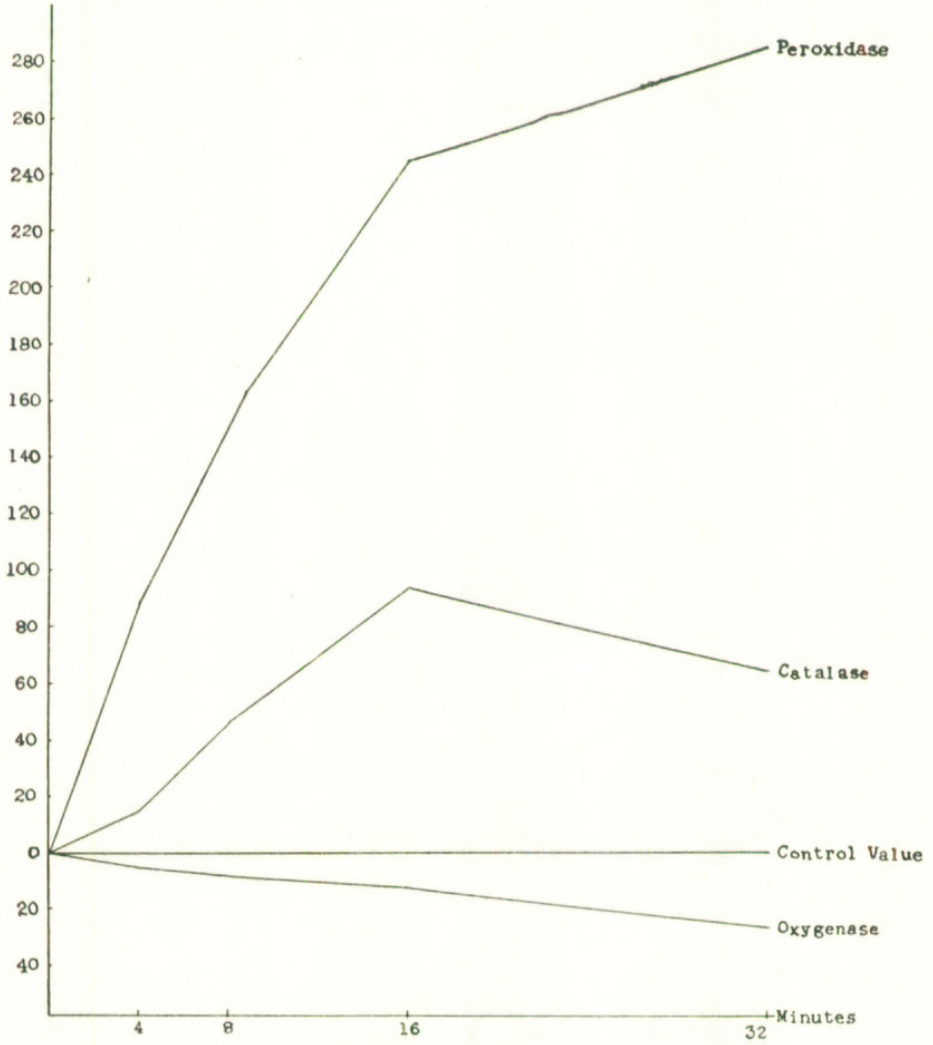

Fig. 2. Determination of enzymatic activities of tomato plants 5 days after irradiation ceased.

water cell, and others through the Vita glass water cell. Two determinations of enzymes and respiration were made on consecutive days at the end of the period of irradiation.

The data in table II, graphically represented in fig. 3, indicate that the plants rayed through the Vita glass water cell

TABLE II

RESULTS OF IRRADIATING 5 TOMATO PLANTS AT 100 INCHES FOR 10 MINUTES DAILY FOR 5 WEEKS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	One day after irradiation ceased						
	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity		
	Actual	Relative	Per gm. fresh wt.	Relative	Catalase	Oxygenase	Peroxidase
Control	95.6	100	.00098	100	100	100	100
Rayed through Vita glass water cell	107.5	112	.00128	131	164	104	100
Rayed through quartz water cell	93.5	98	.00102	104	190	117	103
Two days after irradiation ceased							
Control	84.5	100	.00200	100	100	100	100
Rayed through Vita glass water cell	102.0	121	.00218	109	142	103	100
Rayed through quartz water cell	90.0	107.0	.00208	104	118	106	102

were stimulated in growth from 12 to 21 per cent. Since only 5 plants were used for the determination, this result is not statistically significant, but there is qualitative agreement with the results published by Fuller ('31) on the stimulation of growth under these conditions. Respiration is also increased. Catalase showed a very great increase, while oxygenase was but slightly increased, and peroxidase was not affected. The variations given for oxygenase probably are not far beyond the experimental error. There was qualitative agreement between the data obtained on successive days, and consequently on different samples of tissue.

Irradiation through the quartz water cell, which allows much of the short, injurious ultra-violet rays to pass, had no con-

sistent effect on growth, although, as noted above, the number of plants was too small to present convincing evidence. Respiration was stimulated only slightly, less than when the Vita glass water cell was used as the filter. Again catalase was greatly stimulated, while oxygenase showed a smaller increase. Peroxidase was slightly increased, although the increase is too close to expect experimental error to be accepted as significant. It is thus seen that the physiological effects of rayings through

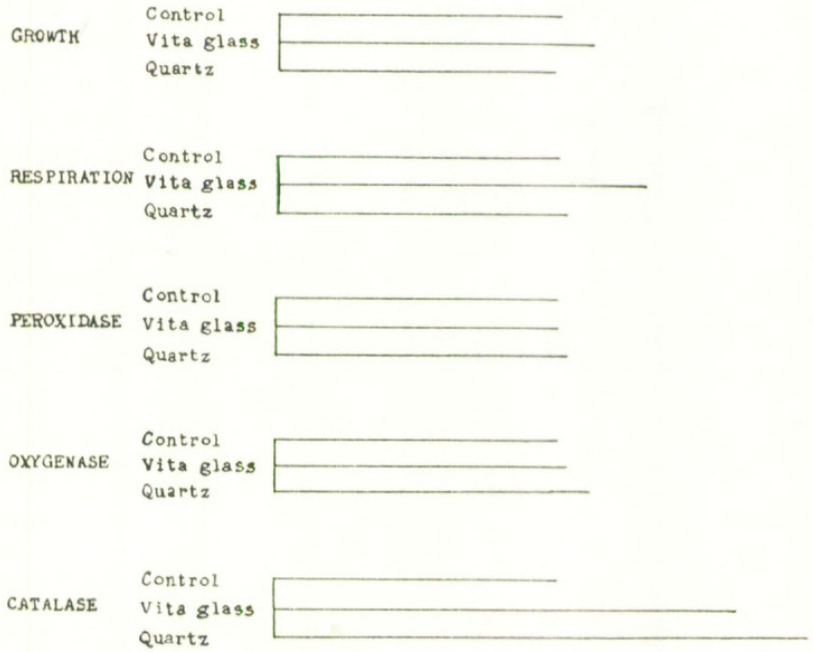

Fig. 3. Determinations made on tomato plants day after irradiation ceased. Plants had been irradiated at 100 inches for 10 minutes daily for 5 weeks. Linear values calculated as percentages of the control.

the quartz water cell were very similar to those obtained by the use of the Vita glass water cell, with an indication of a possible injury of the plants.

Experiment III.—The tomato plants used for this experiment were planted February 15, 1934, and transplanted from the flats to 4-inch pots on April 5. The raying began April 20, at a distance of 36 inches from the arc. The quartz water cell was used as a filter. One group was rayed 15 minutes, and another group 30 minutes, daily for 7 days. Determinations

were made daily for 4 days following the irradiation period. The data are included in table III, and are represented graphically in figs. 4–7 inclusive.

All of the rayed plants in this experiment were greatly injured. The first set of determinations, made the day after the

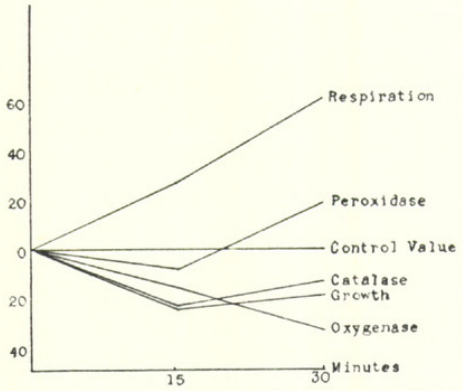

Fig. 4. Determinations made on tomato plants after being rayed at 36 inches for 7 days with quartz water-cell filter.

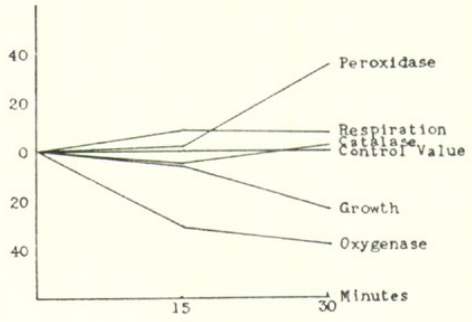

Fig. 5. Determinations made on tomato plants on the second day after irradiation ceased.

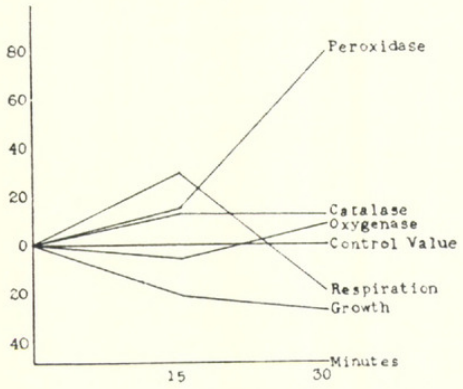

Fig. 6. Determinations made on tomato plants on the third day after irradiation ceased.

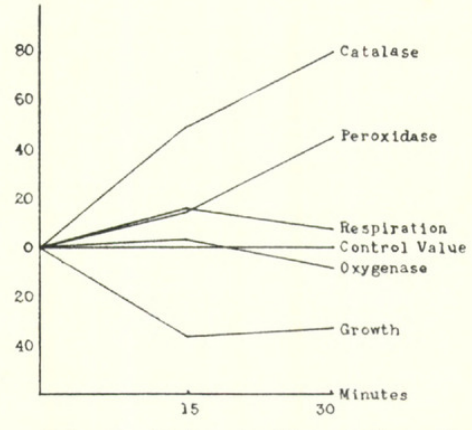

Fig. 7. Determinations made on tomato plants on the fourth day after irradiation ceased.

irradiations ceased, showed a heightened rate of respiration that was proportional to the length of exposure. On subsequent days the amount of carbon dioxide eliminated per gram of fresh weight remained at the heightened level in those plants rayed for 15 minutes daily, but there was a great drop in respiration on subsequent days in those plants which

TABLE III

RESULTS OF IRRADIATING 5 TOMATO PLANTS THROUGH QUARTZ WATER CELL AT 36 INCHES DAILY FOR 7 DAYS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity		
	Actual	Relative	Per gm. fresh wt.	Relative	Catalase	Oxygenase	Peroxidase
First determination							
Control	60.2	100	.00130	100	100	100	100
Rayed 15 minutes	46.5	77	.00165	127	77	85	92
Rayed 30 minutes	49.0	81	.00209	161	87	67	119
Second determination							
Control	57.4	100	.00219	100	100	100	100
Rayed 15 minutes	53.9	94	.00235	108	75	67	102
Rayed 30 minutes	43.8	76	.00223	107	104	62	135
Third determination							
Control	80.4	100	.00174	100	100	100	100
Rayed 15 minutes	64.2	79	.00208	129	117	94	115
Rayed 30 minutes	58.7	73	.00153	89	112	108	179
Fourth determination							
Control	86.7	100	.00228	100	100	100	100
Rayed 15 minutes	54.4	63	.00264	116	149	103	115
Rayed 30 minutes	59.1	67	.00248	109	180	91	145

had been rayed for 30 minutes daily. This drop is only apparent, because there were progressively larger amounts of withered and dead tissue in these greatly injured plants on each succeeding day. Since these non-respiring tissues were unavoidably included in the recorded fresh weight, a false value is obtained. This was not an important source of error in those

TABLE IV

RESULTS OF IRRADIATING 5 BEAN PLANTS AT 70 INCHES FOR 10 MINUTES DAILY FOR 5 WEEKS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity	
	Actual	Relative	Per gm. fresh wt.	Relative	Peroxidase	Catalase
First determination						
Control	92.5	100	.0053	100	100	100
Rayed through Vita glass water cell	74.5	81	.0053	100	150	91
Rayed through quartz water cell	59.8	64	.0049	92	237	100
Second determination						
Control	70.0	100	.0062	100	100	100
Rayed through Vita glass water cell	58.0	83	.0057	92	119	95
Rayed through quartz water cell	45.0	63	.0068	109	145	108

plants rayed 15 minutes daily. Since the enzymes were determined in the extracted sap, these values represent more truly the comparative activities of the actually living tissue. The peroxidase, with the exception of a single determination, followed the upward trend of respiration. Oxygenase was greatly decreased during the first 2 days after the irradiations ceased,

the decrease being proportional to the length of exposure, but normal values were regained on the third and fourth day. Catalase showed a progressive increase for the four days following the termination of the raying. The activity of catalase shows no apparent relationship to respiration nor to the activities of peroxidase and oxygenase.

Experiment IV.—For this experiment, Red Kidney field beans were planted in flats January 23, 1934, and transplanted

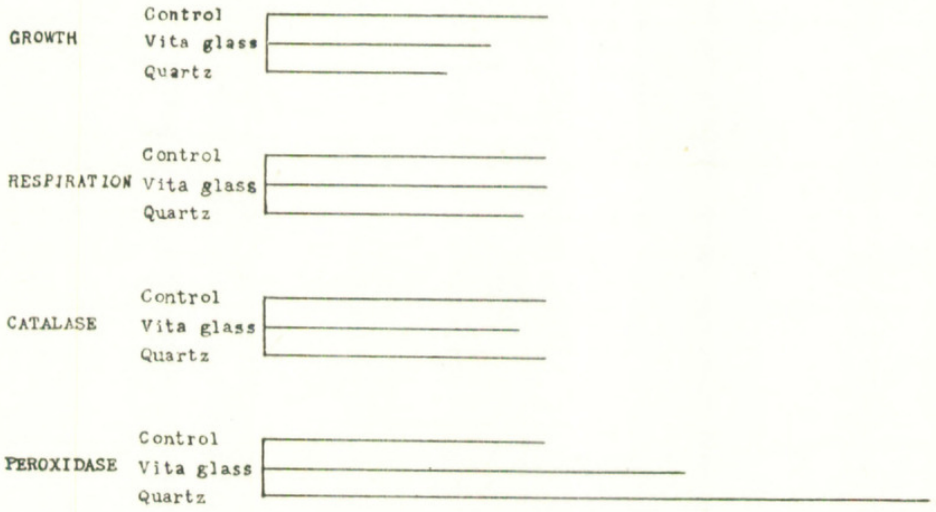

Fig. 8. Determinations made the day after irradiation ceased on bean plants rayed at 70 inches for 10 minutes daily for 5 weeks. Linear values calculated in percentages of the controls.

into 4-inch pots February 8. The irradiation began February 15, and continued for 5 weeks. The distance was 70 inches, and the daily exposure was 10 minutes. One group was rayed through the quartz water cell, and one through the Vita glass water cell. One set of determinations was made the day after the irradiations ceased, and another 4 days later. The results are presented numerically in table IV and graphically in figs. 8 and 9.

All irradiated plants showed injury. The injury and stunting were slight in those plants rayed through the Vita glass water cell, but quite pronounced in those rayed through the quartz water cell. Respiration and catalase were not signifi-

cantly affected, but peroxidase was very markedly increased in both rayed groups, particularly when the quartz water cell was used as the filter. Five days after irradiation ceased these differences were still evident although of lesser magnitude. The tomato plants in experiment II were rayed under approximately similar conditions, but they were stimulated in growth, respiration was slightly increased, peroxidase was insignificantly affected, and catalase was very greatly stimulated.

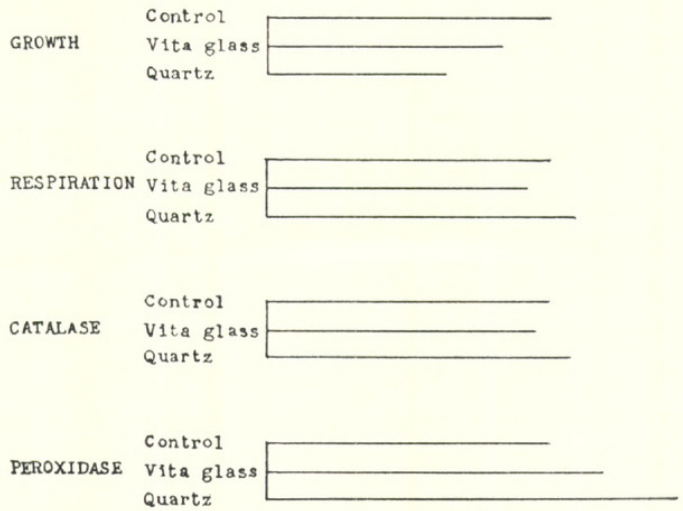

Fig. 9. Determinations made 5 days after irradiation ceased on bean plants rayed at 70 inches for 10 minutes daily for 5 weeks. Linear distances calculated in percentages of the controls.

DISCUSSION

These experiments on irradiated tomato plants show that the rate of respiration as indicated by the amount of carbon dioxide eliminated per gram of fresh weight markedly increases proportionally to the extent of injury. However, it is not possible to obtain accurate numerical data on plants in the most advanced state of injury because the development of dead, non-respiring tissue confuses the weights. This increased rate of respiration is approximately paralleled by the activitiy of peroxidase in the expressed juice. Oxygenase, on the other hand, is inhibited proportionally to the extent of raying. Experiment III shows that normal oxygenase activity is regained

soon after irradiation stops, although the plants remain in a very disturbed physiological condition. Catalase reacts variously. In experiment I it was stimulated, yet in experiment III determinations made the day after raying ceased gave subnormal values. On successive days its activity increased until a considerable degree of stimulation was reached. These results agree with those of Fuller ('32).

The irradiated bean plants reacted somewhat differently. Their growth was not stimulated under conditions stimulative to tomatoes. In experiment IV, the only decided effect was a greatly stimulated peroxidase activity, while the tomato plants under the approximately similar conditions of experiment II showed a greatly stimulated catalase activity as the chief physiological reaction. Bean plants do not give the colorimetric test for oxygenase, and this is itself sufficient evidence that the physiology of beans is different from that of tomatoes. It is not surprising, then, that beans and tomatoes react differently in regard to other features of their physiology.

The data obtained in these experiments have a bearing on the nature of the oxidation processes in plants. The numerous contributions of Bach and Chodat on the intracellular oxidation mechanism have been responsible for the concept that oxygenase unites molecular oxygen to some cellular component to form a peroxide. This peroxide is then activated towards some oxidizable substance in the protoplasm by peroxidase. It is obvious that if this relationship be true, then oxygenase and peroxidase would necessarily be related quantitatively to each other. This is indeed suggested by numerous experiments upon diseased plant tissues in which both enzymes were markedly greater in activity than in normal plants. A summary of these experiments has been published by Wynd ('34).

The necessity of oxygenase for the respiratory process is, however, open to several serious objections. Onslow ('21, '31) has shown that only about 60 per cent of the higher green plants give the oxygenase reaction and that any fundamental component of the respiratory mechanism would be more universally distributed. Kertesz ('34) has shown that fresh tis-

sue of some varieties of peaches does not discolor upon exposure to the air. Qualitative and quantitative studies showed the lack of catechol compounds in the non-discoloring varieties. This indicates that the oxygenase-catechol system may be absent even in very closely related plants, and consequently could not be of fundamental importance.

Wynd ('34) has shown, in an extended study of the tomato plant, that there is no quantitative relationship between the activities of oxygenase and peroxidase. Plants stimulated to great respirational activity by the addition of potassium iodide to the nutrient solution showed a corresponding increase of peroxidase but oxygenase was greatly inhibited. These results, together with those of the present paper, tend to indicate that the oxygenase-catechol-peroxidase system does not represent a fundamental respiratory mechanism in the tomato plant.

SUMMARY

1. Tomato and bean plants were rayed under various conditions by a mercury arc, and the effect on respiration and oxidizing enzymes determined.

2. In tomato plants, *injurious* raying stimulated respiration and peroxidase. Oxygenase was inhibited, and catalase reacted variously but in general was stimulated. The most pronounced effect of *non-injurious* raying was greatly stimulated catalase.

3. In bean plants, comparatively weak dosages produced greatly stimulated peroxidase as the chief reaction. Bean plants did not exhibit oxygenase activity, which indicates a different chemical physiology consistent with their different reactions to ultra-violet light.

4. The comparative activities of peroxidase and oxygenase show that the oxygenase-peroxide-peroxidase system as proposed by Bach and Chodat does not represent a fundamental respiratory mechanism in the tomato plant.

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