Effects of Heat and Cold Treatment Upon Enzyme Activity in Bulbs and Corms

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Among the commonly used methods of breaking the dormancy of plant organs is their treatment with various stimulating gases such as ethylene chlorhydrin, and their exposure for varying periods of time to temperatures considerably above or below normal temperatures prevailing during the rest period. In the present work, the storage stems of hyacinth Marconi, gladiolus Picardy, and Ornithogalum umbellatum were exposed (in the mid-portion of their dormant period) to temperatures of 50°C and 40°C for 24 hours; controls were those kept at 20°C throughout their dormant period. The experimental groups were placed in damp sphagnum and kept for the 24-hour period in constant-temperature cases in the greenhouse. At the end of this period, the bulbs and corms were prepared from enzyme tests in the following manner:

The outer coverings were removed and 20-gram portions were cut from the bulbs. These were run through the nut-chopper of a kitchen grinder. To the ground pulp 10 cc. of distilled water were added. In the case of catalase, 2 grams of powdered calcium carbonate were added to the tissue as it passed through the grinder. The ground pulp and juice were then centrifuged, except in the case of catalase which is partially inactivated by centrifuging. For the determination of oxidase and peroxidase, the alpha-naphthol paraphenylenediamine hydrochloride method of Guthrie (1931) was used. One cc. portions of the centrifuged juice were added to the buffered substratum and the reaction was allowed to proceed for 15 minutes, at the end of which the color was extracted by toluol and differences in color intensity determined colorimetrically. Catalase was determined by the method of Waksman and Davison (1926) in which at the end of two hours' reaction of the juice with hydrogen peroxide, the remaining peroxide is oxidized by N/5 potassium permanganate. Peptase was determined by the method of Sorensen (1908) which uses thymolphthalein as indicator. Invertase was determined by the Fehling method, the cuprous oxide measured by dissolving the oxide in Bertrand's solution and titrating the reduced iron with N/20 potassium permanganate. Diastase activity was measured by the Fehling method, the cuprous oxide being determined as in the case of invertase.

The results of the enzyme tests are presented in the following table. The controls are represented by the value 100, the others by relative numbers for individual enzymes.

	Gladiolus			Hyacinth			Ornithogalum		
	5°	20°	40°	5°	20°	40°	5°	20°	40°
Catalase	178	100	106	127	100	25	130	100	58
Oxidase	152	100	124	141	100	58	121	100	50
Peroxidase	129	100	110	128	100	69	133	100	78
Diastase	114	100	101	104	100	61	106	100	62
Invertase	110	100	102	101	100	78	110	100	109
Peptase	101	100	89	98	100	81	102	100	92

The table shows clearly the following:

1. Catalase, oxidase, and peroxidase show definitely accelerated activity in the 5° bulbs for all three species. These results coincide with those obtained by Denny, Miller, and Guthrie (1930) from experiments on bulbs and tubers treated with stimulating gases.

2. In the 40° bulbs, a slight acceleration in gladiolus and definite decrease in activity of these enzymes in hyacinth and ornithogalum are apparent. In the two latter species, injury to the outer parts of the bulbs resulted from the 40° treatment. The outer scales were soft and mucilaginous as though they had been partially broken down by the high temperature.

3. The activity of diastase, invertase, and peptase were relatively little different in the various sets, except that in hyacinth and ornithogalum there was some depression of activity in the 40° bulbs, due undoubtedly to heat injury of the tissues. The 5° bulbs showed slightly greater degree of activity in these enzymes as compared with the controls, but whether these differences are much beyond experimental error is problematical. One may conclude that the oxidizing enzymes are those especially affected by the heat treatment.

The 5° treatment was most effective in shortening the dormant period and in promoting growth. This effect coincides with the increased activity of the oxidizing enzymes.

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