A Possible Role of Catalase in the Rest of Peach, *Prunus persica,* Sieb. and Zucc., Flower Buds[1]

W. Kaminski[2] and R. Rom
University of Arkansas, Fayetteville

Abstract. Catalase activity in peach flower buds was found to be comparatively high before winter dormancy. Chilling at 5°C resulted in a decrease in catalase activity. The lowest level was reached near the end of dormancy. Flower buds of cultivars requiring a longer chilling requirement had the greatest depression in catalase activity. The onset of 25°C temperature following a few days at 5°C delayed change in catalase activity during the next 5°C chilling period and resulted in a condition comparable to prolonged dormancy. Flower buds near the end of rest or in a post dormant rest when placed at 25°C showed a rapid increase in catalase activity. A flower bud's ability to rejuvenate its catalase level is correlated with it's flowering ability. Free internal O_2 due to the catalase system may trigger peach flower bud development.

Results of investigations concerning the biochemical transformation in dormant seeds of various species and potato tuber buds have shown an increase in catalase activity during or following emergence from the resting period (8). At temp unfavorable for emergence and responsible for secondary dormancy in seeds, the catalase activity was decreased (5, 10).

The explicit role of catalase in the rest period of dormant plant organs remains unknown. There is evidence that catalase functions in detoxification of harmful metabolic products and in intracellular oxidative processes requiring free O_2 (1). Some investigations (3, 9, 12) showed that the amount of O_2 in seeds or buds is the main factor in determining the direction of biochemical transformations leading to rest or it's termination. The authors' concern was, however, with the anaerobic condition in the plant organ because atmospheric O_2 is excluded by the seed coat or budscale. Our objective was to study the catalase activity in peach flower buds subjected to varying temp during the rest period.

Methods and Materials

Flower buds of cvs. Sunhigh, Dixigem, and Ranger were selected because of differences in chilling requirements 750, 850, and 950 hours, respectively, according to a published list of average chilling hours (11). Twigs of the previous season's growth were collected in mid-December from trees at the University of Arkansas Experiment Station peach cultivar orchard. The weather prior to sample collection was unseasonably mild and the peach buds had accumulated relatively few hours of temp below 10°C.

The basal ends of sampled twigs were placed in water filled containers and held at either 5°C and 25°C or alternated from 5°C to 25°C after 2 or 4 weeks and vice versa as indicated in Fig. 1. Only buds from the central portion of the twigs were used for catalase measurement and bud development observations.

Catalase activity was determined using a floating disc procedure based on the liberation of O_2 due to catalase activity and measured indirectly by recording the time required for an enzyme saturated paper disc to rise to the surface of a 3% hydrogen peroxide solution (6).

The discs (No. 57-G-H specially prepared by Schleicher and Schuell Co. Keene, N. H.) were submerged for 1 sec in the enzyme solution of ground buds and buffer. The enzyme solution was prepared by macerating buds in a mortar with pestle. Two ml of borax HCl buffer of pH 8.2 was added for each mg of fresh bud wt. The enzyme preparation was cooled in an ice water bath. Multiple measurement of catalase activity was done immediately following bud maceration. When buds were dormant, each measurement was based on the fresh wt of 10 buds. As visible differences in bud development became apparent, the catalase measurement was made on individually weighed buds sorted according to their development stage. A single bud stick served as a replicate and data presented are based on 3 replicates. Replicate variability was found to be less than treatment variability.

To relate observed enzyme activity to enzyme concn, a standard curve was developed by measuring enzyme activity in buds while using a serial dilution procedure in which increasing amounts of buffer were added for each determination (Fig. 4).

Results

The measured catalase activity in 'Ranger', 'Dixigem' and 'Sunhigh' flower buds was relatively high at the time of sample collection in mid-December. When buds were subjected to 2 weeks at 25°C, their catalase activity remained essentially unchanged (Fig. 1, line 1). Prolonging the 25°C regime beyond 2 weeks resulted in a decline in catalase acitivity of 'Ranger' and 'Sunhigh' flower buds but not in 'Dixigem'. At this time period, about 2% of 'Dixigem' buds flowered and had a high catalase activity. Those buds remaining dormant retained the same activity level noted in previous sampling.

Catalase activity in buds held constantly at 5°C decreased continually until the fourth or sixth week (675-1000 hr) of the experiment (Fig. 1, line 2). Continued exposure to 5°C chilling resulted in a rise in activity. Even after 10 weeks (1680 hrs) chilling, however, the activity level had not returned to that recorded at the experiment's beginning. Catalase activity in buds kept at 5°C was always highest for 'Sunhigh' the cultivar requiring shorter chilling.

Activity in buds moved to 25°C after an initial 2 weeks at 5°C showed a reversal of the inhibitive effect of chilling on catalase activity (Fig. 1, line 3). This reversal was temporary. Extending the 25°C treatment beyond 2 weeks resulted in a drop in activity. None of the buds developed during this 25°C regime.

Buds held first for 2 weeks at 25°C and then placed at 5°C had a stronger catalase activity depression than those held continuously at 5°C (Fig. 1, line 2 vs line 4) and the activity did not show as marked an increase as the cold period was extended. This response was typical for all 3 cultivars.

Changes in catalase activity of buds chilled for 2 weeks at 5°C followed by 2 weeks at 25°C and then returned to 5°C varied with the cultivar (Fig. 1, line 5). 'Ranger' and 'Dixigem' buds in this second chilling regime continued to lose enzyme activity until the study was terminated; yet their catalase activity did not reach the low level of other treatments.

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[2]Present address, Institute of Pomology, Skierniewice, Poland.

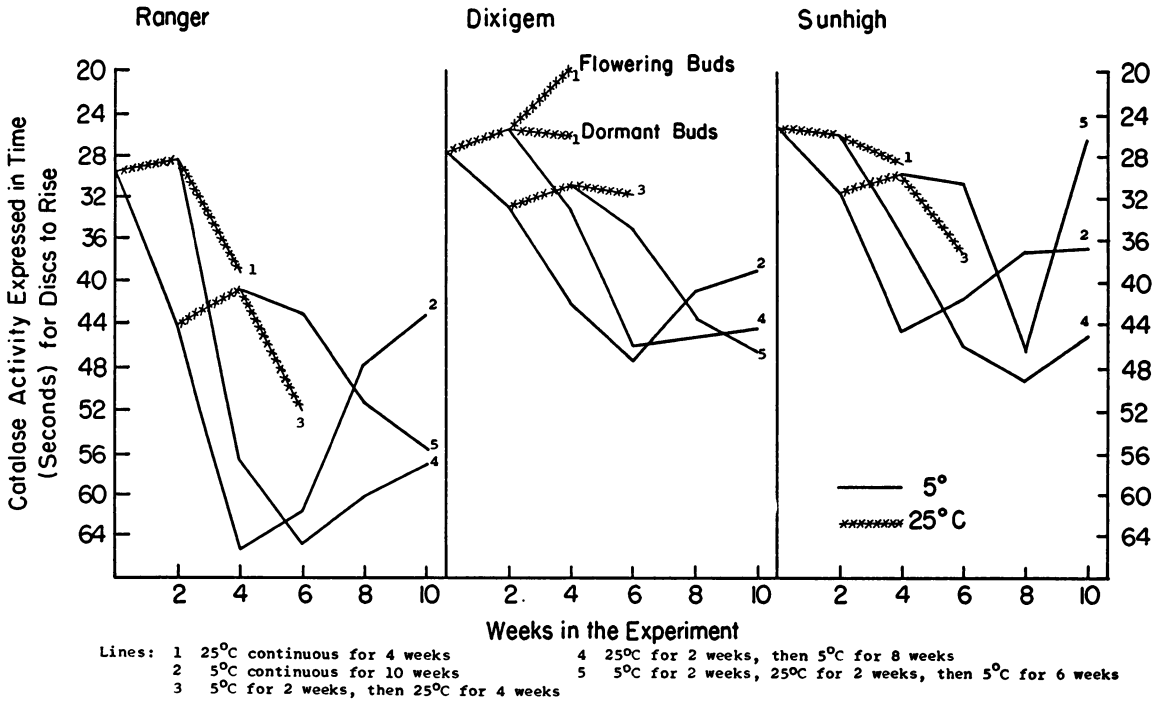

Fig. 1. Effect of 5°C and 25°C on the catalase activity in peach flower buds during dormancy.

'Sunhigh' flower buds given this alternating temp regime reached their lowest activity level in the 8th week and then increased to an activity level higher than buds of that cultivar held continuously at 5°C.

Bud development in 'Sunhigh' and 'Dixigem' peaches was observed after 4 weeks of chilling at 5°C (672 hrs) when followed by 2 weeks at 25°C (Fig. 2). The percent of buds developing and their development stage during the 25°C period increased as the chilling time increased. 'Ranger' buds responded to a 25°C regime only after 8 weeks of chilling at 5°C (1344 hrs). Changes occurred in catalase activity in peach flower buds which reached different development stages during a 2 week period at 25°C preceded by 6 weeks at 5°C (Fig. 3). The increased catalase activity in 'Dixigem' and 'Sunhigh' peaked when the flower buds were in early stages of swelling and then systematically decreased as bud wt increased and growth proceeded to flowering.

The relationship between enzyme concn in a bud to the time required to float the disc in this test for catalase activity was determined (Fig. 4). Using these data and the results shown in Fig. 1, line 2, a 4 fold decline in 'Ranger' flower bud catalase activity occurred during the first 4 weeks of chilling at 5°C. Similarly the changes in magnitude of catalase activity for 'Dixigem' and 'Sunhigh' during the same treatment period was 2 to 3 fold. These later 2 cultivars showed a 5 to 8 fold activity increase from their lowest activity level (Fig. 1, lines 2 and 5) to their highest activity level at first bud swell (Fig. 3).

Discussion

A relationship exists between dormancy in seeds of different species and their catalase activity (4, 5, 7, 10). Dormant seeds have a minimum catalase activity level after harvest which increases during after-ripening up to germination. By contrast, we found that peach flower buds, at the beginning of this study, had not yet attained their deepest dormancy as their catalase activity level was relatively high. Some 'Dixigem' flower buds, for example, were capable of flowering and did without a cold treatment. This ability to flower at 25°C was lost, however, after 2 weeks at 5°C chilling. Probably the onset of cold temp is

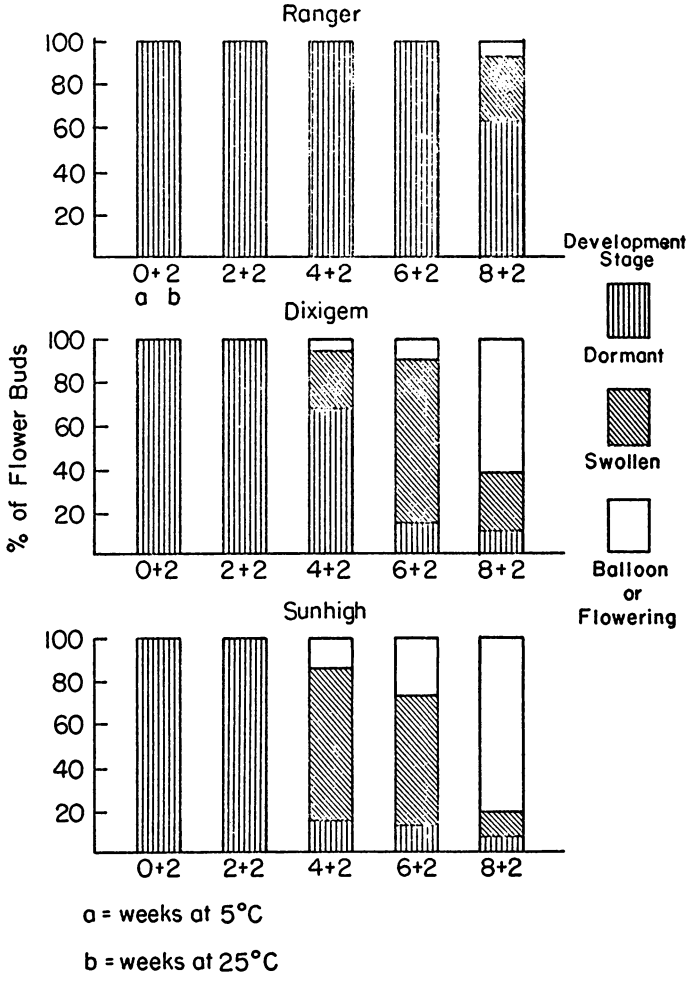

Fig. 2. Effect of temperature regime on flower bud development.

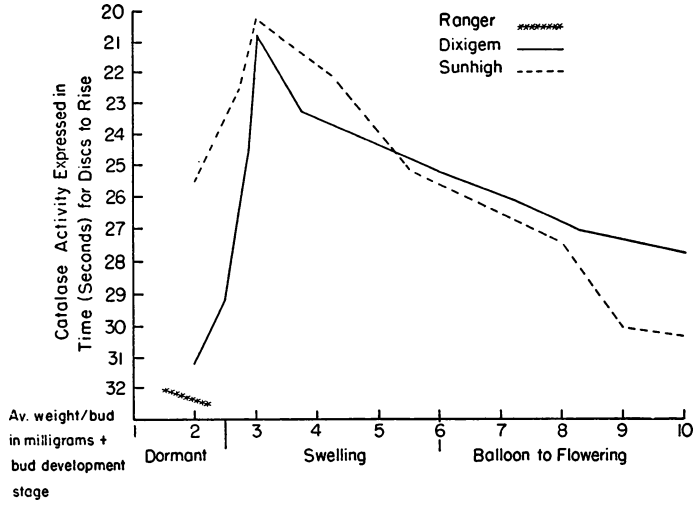

Fig. 3. Catalase activity in peach flower buds at different development stages measured after 6 weeks at 5° followed by 2 weeks at 25°.

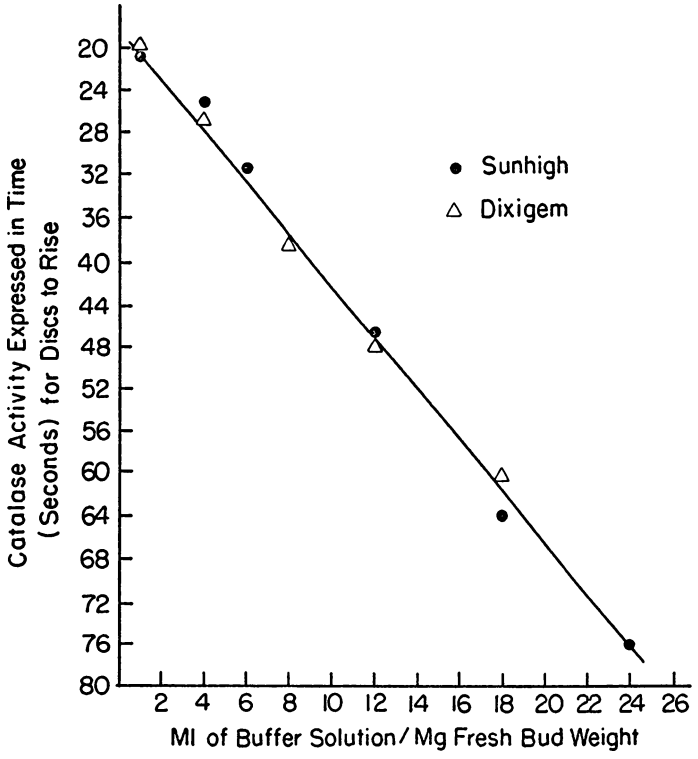

Fig. 4. Effect of different concentrations of catalase from peach flower buds on the time required for discs to rise. Determination was made using buds in the swelling stage following 6 weeks at 5°C and 2 weeks at 25°C.

necessary to put peach flower buds into their deepest dormancy which is directly connected with their most hardy condition. Chaplin (2) noted that peach flower buds were most hardy near the end of their rest. From our data the conclusion might be reached that the end of rest in peach flower buds is preceded by or coincides with low catalase activity level, after which in high temp, responsible for dehardening, catalase activity is increased in these buds. During early dormancy, however, the increase in catalase activity as a response to high temp was small in all 3 cultivars, yet it was only an initial response. Ultimately, continued high temp at this stage of dormancy resulted in decreased catalase activity. Warm temp depression of metabolic processes leading to the end of rest is reported as prolonged dormancy (14) or secondary dormancy (13).

Flower buds advanced toward their rest termination could and did progress to flowering following a prolonged warm period stimulus. They were not capable of flowering until catalase activity increased about 2 fold over the level determined at the start of the experiment, or 5 to 8 fold from the lowest level noted. Thus, a flower bud's ability to quickly rejuvenate it's catalase activity is correlated with the length of the chilling period.

The changes in catalase activity found to be associated with the phenomena of dormancy, prolonged dormancy, time for bud development and possible hardiness indicate that the catalase enzyme possibly plays a heretofore unreported part in these processes. What is its function? Inhibitors are reported as being significant in the induction and breaking of the rest period in plant organs (12). Is it possible that the mode of action for inhibitors, associated with dormancy in seeds and buds, is through a control of the catalase system which in turn regulates the internal O_2 supply to the dormant organ? The catalase enzyme in supplying free O_2 might be the "trigger mechanism" in bud development. Further research may provide clues for understanding the resting stage.

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