CATALASE ACTIVITY AND RESPIRATION IN THE LEAVES OF GROWING BARLEY

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INTRODUCTION

There has been some difference of opinion as to whether there is an association between catalase activity and respiration. In 1910 Appleman (1) noted a correlation between the two in the potato tuber and later (2) observed the same thing in potato tubers manipulated in various ways to modify respiration and in stem and seed ends of the same tuber. He found a similar correlation in stored sweet corn (3), and concluded that catalase activity is a fair index of the comparative intensity of respiration in the tissues of sweet corn and potato tubers. Morinaga (11), from studies of rice seedlings grown under differing conditions of aeration, concluded that there is a close positive connection between the two phenomena, and Burge (4) and Burge and Burge (5) are convinced, from studies of a number of organisms, that catalase activity is definitely associated with metabolism and with respiration in particular.

Others, however, have not obtained such convincing results. Crocker and Harrington (6) found a positive correlation between respiration and catalase activity in seeds of Johnson grass (Sorghum halepensis (L.) Pers.) and probably in those of Avena fatua L. but not in Amaranthus seeds. Rhine (13), working with germinating seeds, concluded that catalase activity is a measure of metabolism only when there is no rapid change in respiration. Lantz (10) did not find a close correlation between catalase activity and respiration in germinating corn of different chemical composition (high and low oil, high and low protein).

The writer has demonstrated widely different degrees of catalase activity in different parts of the same barley (Hordeum distichon) plant. The relationship between catalase activity and respiration in leaves of different age is shown in this paper.

MATERIALS AND METHODS

Plants of pure-line Hannchen barley, C. I. 531, growing in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), were tagged for identification. A daily growth record of each leaf, based on measurements from the soil surface to the leaf tips, was kept for several days before the experiment. For each experiment three plants were selected, the homologous leaves of

1 Received for publication Mar. 25, 1932; issued February, 1933.
2 Reference is made by number (italic) to Literature Cited, p. 40.
3 Unpublished data.
4 C. I. denotes accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.
which had made nearly equal growth. In these plants the developing spikes were small and still inclosed in the twelfth or boot leaf. From the first plant the six youngest leaves were removed, quickly weighed, and dried to constant weight at 80° C. at a pressure of approximately 6 cm of mercury. The second plant was laid away in a moist towel until the respiration run was started, when its leaves were individually tested for catalase activity. The leaves of the third plant were used for measuring the rate of respiration.

The respiration chamber was an Erlenmeyer flask of 125 c c capacity fitted with a 2-hole rubber stopper through which two glass tubes were passed, the upper ends projecting about 2 cm above the stopper. One of the tubes extended to within 2 cm of the bottom of the flask and the other only through the stopper. To the external ends of these glass tubes were fitted short pieces of rubber tubing with pinchcocks. The leaf to be investigated was loosely rolled on the finger, tied with a small cord, and suspended in the flask by slipping the end of the cord under a rubber band around the long glass tube. The chambers were set up in series with alternating blanks. The train was swept free of carbon dioxide, and 10 c c of nearly saturated barium hydroxide was introduced into each chamber through the hole stoppered by the short tube. The flasks were then shaken gently in a rocker shaker in the dark. In experiment 1, shaking was continued for 21 hours, and in experiment 2, for 23 hours. Temperatures ranged from about 24° to about 27° C. Blanks and respiration chambers were titrated alternately through the hole in the stopper, due precaution being used to prevent admission of atmospheric carbon dioxide. Titrations were made with 0.1362 normal hydrochloric acid, with phenolphthalein as an indicator. After the respiration run, the leaves were dried to constant weight and respiration rates were determined on both wet and dry weight bases. The slight error introduced by exposure to light and consequent assimilation during titration could not be determined.

Catalase determinations were made on leaves of the second plant by using the apparatus previously reported (12). Each leaf was triturated to a thin paste with washed sand, powdered calcium carbonate, and a small quantity of water. Enough water was added to make a 1 : 50 dilution based upon the green weight of leaf tissue. Two cubic centimeters of the suspension (equivalent to 0.04 g of green tissue) was then shaken with 3 c c of hydrogen peroxide in experiment 1 and with 2 c c in experiment 2. The volume of oxygen liberated for a dry-matter content of 0.008 g was calculated by using the average percentage of dry matter in homologous leaves of the other two plants.

EXPERIMENTAL DATA

The respiration rates which are based on dry matter in the leaves after the run probably are somewhat high. (Table 1.) Homologous leaves were reasonably similar in growth rate, amount of dead tip tissue, and size of sheath and blade, and the data should be comparable.

The youngest leaf gave off carbon dioxide most rapidly. The quantity given off by the next older leaf was much smaller and dropped much more gradually to the fourth youngest. Carbon dioxide
evolution possibly was due in part to oxidation of dead tissues in the two oldest leaves. This factor may account for variability in that part of the curves.

Table 1.—Respiration and catalase activity in homologous leaves from similar plants of Hannchen barley

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Green weight Mg</th>
<th>Dry weight Mg</th>
<th>Catalase activity as O₂ evolved in 5 minutes Cc</th>
<th>For 0.04 g green tissue Cc</th>
<th>For 0.008 g dry tissue Cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.446</td>
<td>4.108</td>
<td>4.89</td>
<td>9.31</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.302</td>
<td>3.066</td>
<td>7.24</td>
<td>14.51</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.294</td>
<td>2.801</td>
<td>8.26</td>
<td>14.75</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.262</td>
<td>2.627</td>
<td>7.42</td>
<td>13.26</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.227</td>
<td>2.683</td>
<td>6.77</td>
<td>13.35</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.204</td>
<td>2.585</td>
<td>4.51</td>
<td>7.92</td>
<td></td>
</tr>
</tbody>
</table>

In both experiments catalase activity was low in the youngest, most rapidly elongating leaf, but was much greater in the second youngest, which was elongating at about one-third the rate of the youngest, and was still greater in the next older. During senescence—indicated by greater length of dead leaf tip—catalase activity dropped off rapidly. Figure 1 gives a direct comparison between the respiration and catalase activity of homologous leaves on the two pairs of plants.

DISCUSSION

Hover and Gustafson (8, p. 39) state that “as the leaves of corn, sorghum, wheat, and oats increase in age there is a decrease in rate of respiration; but that as the leaves become still older (past about middle age) the rate gradually increases.” Their data, however, show this conclusion to be fully justified only in the case of sorghum. In their experiments the oldest corn leaves at time of pollination showed a higher rate of respiration than the youngest; in oats both plants used showed a rise with age, and in one wheat plant the youngest leaf showed a lower rate than did the next older, whereas in the other wheat plant used the situation was reversed.

In the present experiments it is assumed that the quantity of carbon dioxide given off is a measure of the respiration of the living tissues in the plant part. The results show a relatively high respiration rate for the youngest leaf of barley. There is a sharp decline in the curve to the next older leaf, after which the decrease in successively older leaves is much less, and at the fourth youngest leaf an approximate level is reached. This leaf had finished growth in length a week or
more before respiration was determined and had died back about 2 cm at the tip. Leaves still older had still more dead tissue, and the shape and irregularities of the respiration curve probably can be explained in part by the accumulation as age progresses of nonrespiring structural and senescent tissues. Rate of respiration, highest in the young leaf, decreased rapidly with advancing age to a point where it probably remained constant on the basis of living tissue.
Knott (9) found the youngest and oldest leaves of spinach and celery usually low in catalase activity as compared with those of intermediate age. Ezell and Crist (7) found a significantly negative correlation between catalase activity and plant size in lettuce but not in radish or in spinach.

In the experiments herein described catalase activity was relatively low in the youngest leaf of barley but increased in successively older leaves up to maturity. It then fell off rapidly with increasing necrosis of the oldest leaves.

On comparing catalase activity and respiration (fig. 1) a negative correlation is found between the two phenomena for the three youngest leaves. In the older leaves the curves are irregular and the relationships less definite, since catalase activity appears to decrease as living material decreases in the tissues, and apparent respiration possibly includes the production of carbon dioxide by oxidation of dead tissues.

Since in these experiments the only correlation discernible is negative, and since positive correlations have been found in stored potato tubers and sweet corn (1, 2, 3), in germinating seeds (6, 14), and in rice seedlings (6), it seems highly probable that in no case is the relationship causal.

The nature of these results, contrary to those of most investigators, raises the question of the significance of catalase activity. The writer has assumed that the total catalase activity of the sample can be measured by prescribed methods. This assumption is true only if the agency responsible is a by-product of metabolism or if it is some part of the metabolic machinery which remains relatively stable and is not used up in the process. If either of these hypotheses is correct it must be concluded from the contradictory nature of the experimental data available from this and other studies that there is no causal relation between respiration and catalase activity.

On the other hand, the definition of catalase or catalase activity includes nothing but its reaction with hydrogen peroxide, which is the sole basis of its detection and determination. If this reaction occurs in normal tissues it is impossible to make an accurate determination of the agent producing it by shaking a tissue suspension with hydrogen peroxide, since the results then will be produced by the unused portion and will indicate only the excess of catalase manufactured over that utilized. It is conceivable that respiration and total catalase produced are positively correlated. In that case, in order to explain the results of these experiments it would be necessary to assume that in barley leaves high respiration is associated with a proportionately greater consumption of the catalase-activating agency, leaving a determinable surplus actually smaller in amount than when respiration is low.

SUMMARY

The rate of respiration was high in young barley leaves and decreased in proportion to the rapidity with which the leaves matured.

Catalase activity was low in young barley leaves and increased to a maximum at about the time of leaf maturity, after which it decreased with decrease of living material in the tissues.

A negative correlation was found between respiration rate and catalase activity in homologous younger leaves of comparable barley.
plants. In older leaves relationships were indefinite, probably
masked by increasing amounts of structural material and necrotic
tissues.

Since, contrary to the results of other investigators, a negative
correlation was found between catalase activity and respiration, it is
highly probable that any correlation is fortuitous.

Current methods of determination probably can measure total cata-
lase activity only if that phenomenon is a by-product of metabolism or
has a function in the tissues other than the breaking down of hydrogen
peroxide.

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