TEMPERATURE CHARACTERISTICS FOR THE PRODUCTION OF CO₂ BY GERMINATING SEEDS OF LUPINUS ALBUS AND ZEA MAYS

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I

Temperature characteristics for the consumption of oxygen by germinating seeds of Lupinus albus and Zea mays have been discussed in a previous paper (Tang, 1930-31). The present account deals with the production of CO₂ by these seeds as a function of temperature. It was hoped that such a study might contribute to knowledge of the mechanism of the two phases of respiration, and also to a qualitative investigation of the "respiratory quotient" as a function of temperature.

II

It is of course desirable to use single seeds, as was done for the measurement of oxygen consumption. Several attempts to use single seeds or a very small number of seeds (six to twelve) failed to give significant results, owing to the small amount of CO₂ produced (cf. Navez, 1928-29, and Crozier and Navez, 1930-31), and finally a mass method was adopted. Thirty seeds in the case of Lupinus, and fifty in the case of Zea, were used for each test. The usual Ba(OH)₂ absorption technic was used for collecting the CO₂ excreted. A stream of outside air freed of dust particles by passing through about 20 cm. of loosely packed cotton wool was sucked through metal piping. The CO₂ in the air was eliminated by passing it through about 120 cm. of soda-lime, and then through three bottles of concentrated NaOH solution. The CO₂-free air was saturated with water vapor by passing through a moist chamber containing distilled water and glass beads, and was then led into the respiration chamber. The air, together with the CO₂ excreted by the seeds, was then allowed to pass out of the chamber and bubbled through a special Pettenkofer tube (cf. Crozier and Navez, 1930-31) containing 50 cc. of about 0.383 N Ba(OH)₂ where the CO₂ was completely absorbed. The suction was by a water pump and the rate of air passage was about 250 cc. per minute, maintained constant by means of a Hoffman clamp between the respiration chamber and the inlet to the Pettenkofer tube.
The respiration chamber was made of a piece of straight Pyrex glass tubing 32.5 cm. long and 2.5 cm. in diameter, with three outlets for the insertion of thermocouples (cf. Crozier and Navez, 1930-31). The chamber was closed at the ends by one-holed rubber stoppers through which the inlet and the outlet tubes passed. The seeds were placed on a piece of wire netting (about 5 mm. by 5 mm. mesh) about 26 cm. long and 2.3 cm. wide, of which the wires were thoroughly covered with paraffin. The seeds were so placed on the netting that they were isolated from one another and their hypocotyls or radicles were free from contact and gravitational stimuli. The netting, with the seeds on it, was then carefully inserted in the chamber which was held horizontally in the thermostat.

The Ba(OH)₂ solution was prepared according to Kostychev (1927). 7 gm. of crystalline Ba(OH)₂ was dissolved in each liter of water to which was added 1 gm. of BaCl₂. The concentration of such a solution was about 0.383 N, varying slightly each time it was prepared. The actual concentration was ascertained by titrating against the HCl solution which in turn was titrated against Na₂CO₃ solution. The HCl solution was prepared by diluting concentrated acid (C.P.) to about 5/100 N and titrated against Na₂CO₃ solution of known strength, using phenolphthalein as indicator. When so prepared, each cc. of HCl solution was found to be equivalent to 0.845 mg. CO₂.

The amount of CO₂ excreted by the seeds was ascertained in the following manner. The Ba(OH)₂ solution from the Pettenkofer tube was run into an Erlenmeyer flask and tightly stoppered, and let stand for an hour for the carbonate to settle. A sample of 10 cc. was then pipetted out into a second Erlenmeyer flask (150 cc. capacity) and two drops of 1 per cent phenolphthalein solution added. HCl from a 25 cc. burette graduated to 5/100 cc. was run into the flask with constant shaking of the latter until neutrality was reached. The end point was observed under a daylight lamp. The titrations were done in triplicate, and they seldom showed a variation of one part in a thousand. At the beginning, and sometimes also at the end of an experiment, a blank titration was performed in which no seed was placed in the chamber. The volume of HCl used was recorded, and from this quantity was subtracted the volume of the solution used in each actual experiment. The difference, multiplied by the "CO₂ equivalent" (5 × 0.845) gave the mg. of CO₂ produced in a given period by the group of seeds.

The thermostat used is of the type described by Crozier and Stier (1926-27) and is capable of maintaining a temperature constant to ± 0.005⁰ between 0 and 50⁰C. For each kind of seed a test was usually started at 18⁰; the temperature was then lowered to 14⁰, and then to 12.5⁰. After that the temperature was successively raised to 16⁰, 20⁰, 22⁰, and 24⁰. This was then repeated in another experiment at the same temperatures or at different temperatures, so that the resulting data, when brought together, gave rates of CO₂ production between 12.5⁰ and 25⁰ about 1⁰ apart. The values of the temperature characteristics for the individual experiments were found to be identical.

1 hour was allowed for thermal adaptation, during which time the air stream was allowed to proceed. At the end of the period of adaptation, the stream was
temporarily cut off by proper turning of the stop-cocks on the Pettenkofer tube, and 50 cc. of Ba(OH)₂ solution was introduced from an 8 liter reservoir placed above and connected with the tube. The passage of the air was then resumed and the CO₂ absorbed by the baryta water. At the end of an hour the air stream was again stopped for about 2 minutes to allow the collection of the baryta water for titration, after which the streaming was again resumed. At the same time the temperature of the thermostat was changed, and the seeds were allowed to adapt for another hour at the end of which the entire procedure was repeated. The experiments were carried out in darkness except during the time of collection and changing of the temperature of the thermostat when the light was turned on for a few minutes, the thermostat being covered with red cloth and boards to prevent the light from reaching the respiration chamber.

The seeds used in this study belong to the same lots as those used in the measurements of the rate of oxygen consumption, and were germinated in the same manner, i.e., on moist maple sawdust, in darkness, at 23 ± 1°C. Seeds were soaked in distilled water for 12 hours, then incubated on sawdust for 12 hours (Lupinus) or 36 hours (Zea).

III

Fig. 1 presents the data for Lupinus albus. The ordinate is logarithm of rate of CO₂ production, the abscissa the reciprocal of absolute temperature. The values from several experiments were brought together by factors. Within reasonable limits (± 8 per cent at the maximum) the points fall well on two lines intersecting at a critical temperature of 20°. The temperature characteristic (μ) for the line above the critical temperature is 16,100 ± calories and that below, 24,000 ± calories.

The results of the experiments with Zea mays are given in Fig. 2, plotted in the same manner as Fig. 1. There is no evidence of any discontinuity of the line on which the points fall. The deviation from the straight line of the two points at the lowest temperatures is mainly due to the difficulty in estimating the small amounts of CO₂ at those temperatures. But for these two exceptions, all points fall well on a line from the slope of which μ = 20,750.

Plotted in a slightly different way, we have Figs. 3 and 4 corresponding to Figs. 1 and 2 respectively. The relative rates of CO₂ production in mg. per hour per 30 or per 50 seeds are plotted on the ordinate and the temperature in degrees C. on the abscissa. For Zea, the curve so obtained is of course continuous; while for Lupinus there is no way of fitting the data but by two curves—each exponential.
CO₂ PRODUCTION OF GERMINATING SEEDS

Fig. 1. Log rate of production of CO₂ by germinating seeds of *Lupinus albus*, plotted against reciprocal of absolute temperature. When the data from several experiments are brought together by factors, they can be fitted by two lines intersecting at 20°, with a maximum scatter of about ± 8 per cent.

Fig. 2. Data for the rate of production of CO₂ by germinating seeds of *Zea mays* plotted as in Fig. 1.
Fig. 3. Data from Fig. 1, plotted in a different way. The ordinate represents relative rate of production of CO₂ in mg. per hour per 30 seeds, the abscissa temperature in degrees C. The cusp formed by the two intersecting lines (from Fig. 1) brings out the occurrence of the critical temperature. The points tend to scatter with increasing temperature—especially so at the region of the critical temperature (cf. Crozier and Stier, 1926–27).

Fig. 4. Data from Fig. 2 plotted as in Fig. 3. The points can be fitted by only one curve, with no evidence of a critical temperature at 20°. The points tend to scatter more as temperature increases.
in nature, intersecting at 20° and forming a cusp at that point. This fact is interesting not only because it illustrates the necessity for the discontinuous graph in Fig. 1 (cf. Crozier, 1924–25; Brown, 1926–27) but also because it brings out the fact that in this type of experiment, it is essential to determine the rates at short intervals of temperature instead of as a few scattered points, as has been done by many workers (e.g. Warburg, cited by Harvey, 1930, p. 327; cf. Crozier and Stier, 1926–27). Thus if we obtained only the points at 13°, 16°, and 24°, we could have drawn a smooth curve through these three points, completely failing to detect the "break" at 20° in the case of the Lupinus seeds.

IV

It is unfortunate that most of the recent data dealing with production of CO₂ by seedlings as a function of temperature have not been obtained in a manner warranting the mode of treatment used here. Experiments with wheat seedlings reported by Mack (1930) are complete and well planned in many ways, but the failure to eliminate geotropic and contact stimuli renders the data unfit for quantitative treatment. Those reported by Kurbatov and Leonov (1930) with Phaseolus aureus are decidedly not comparable with the present work, due to a number of technical difficulties such as crowding of the seedlings, insufficient thermal adaptation, and control; these have been discussed by Crozier and Navez (1930–31). In Harvey's recent book (1930, p. 327) there are given some data for wheat seedlings (without any experimental detail). A plotting of his data between 0 and 25° shows a critical temperature at 11° with a temperature characteristic of 12,550 ± calories above and 17,150 ± calories below that temperature. Above 25° the relation no longer holds. Since the technic was not given, and the temperature intervals are too great, little significance can be attached to these values. However, they do show a striking agreement with many of the existing values for respiratory process. Navez (1928–29) with Vicia faba reported a temperature characteristic of 16,250 between 7.5° and 20°. Crozier and Navez (1930–31) obtained a value of 16,500 for Phaseolus aureus between 12° and 20°. These values are in accord with the one reported here for Lupinus albus above the critical temperature only. Whether the
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difference between the values obtained here and the values obtained by Navez and Crozier and Navez is due to the difference of the seeds used or due to the difference in the stages of development we are not yet in a position to ascertain, but these values have all been observed before in experiments of this nature (cf. Crozier, 1924–25).

Table I summarizes the temperature characteristics for both the oxygen consumption and production of CO₂ by *Lupinus albus* and *Zea mays*.

In going over the figures in Table I it is at once apparent that the temperature characteristics obtained with the same seeds at the same developing stage are not necessarily the same with respect to oxygen consumption and production of CO₂. For *Lupinus*, the critical temperatures for oxygen consumption and for production of CO₂ come at very nearly the same place (20°), but the respective temperature characteristics are quite different. For oxygen consumption it is 11,700 calories above the critical temperature, and 16,100 calories below; the latter agrees with that for production of CO₂ above the critical temperature, but not below. For *Zea*, the temperature characteristic for production of CO₂ is of the same order of magnitude as that for oxygen consumption below the critical temperature (20°). Such a temperature was not found in the case of production of CO₂. Above the critical temperature the value of μ is 13,100 for oxygen consumption.

These differences are rather striking, considering the fact that the
seeds are germinated alike and are from the same lots. Two interpretations suggest themselves. The first is that if temperature characteristics indicate in any way the mechanism of the chemical reactions involved in respiration, then such differences in the values obtained would suggest that the mechanisms of the reactions involved in the two phases of respiration—oxygen consumption and production of CO₂—may not necessarily be the same, and may be quite independent of one another, a concept not entirely novel to students of plant respiration.

A corollary of the above is that since the temperature characteristics for rates of oxygen consumption and production of CO₂ are different, the ratio of the two—the respiratory quotient—should be a function of temperature. Superimpose two lines of different slopes so that they intersect at a point corresponding to a given temperature with reference axes similar to those in Fig. 1, and the R.Q. at that temperature is 1; at temperatures above and below that point the values of R.Q. should be either greater or less than 1, according to the slopes of the lines. Such a picture is merely qualitative,—nevertheless the fact is significant.

A perhaps serious objection which may be made against this interpretation is supplied by the second alternative suggestion as to the reason for the differences between μ's for consumption of O₂ and production of CO₂ by the same seeds. This is the fact that although the seeds are from the same lots and are treated in the same way with respect to conditions of germination, the technic with which the rate of oxygen consumption was obtained is different from that used in ascertaining the rate of production of CO₂. In the former case the seeds were in a closed moist chamber, while in the latter case the seeds were subjected to a rather rapid stream of moist air. If this difference does introduce an effect determining the apparent temperature coefficient, however, it is difficult to account for the identical values of temperature characteristics for oxygen consumption and production of CO₂ below the critical temperature in the case of Zea; likewise it is difficult to account for the occurrence of the critical temperatures for the two processes at so very nearly the same place in Lupinus. Before any definite conclusion can be drawn this point must be more carefully tested, preferably with single seeds in both cases.
SUMMARY

The rates of production of CO₂ by germinating seeds of Lupinus albus and Zea mays were studied between temperatures 12.5° and 25°C. with the HCl-Ba(OH)₂ titration method. The temperature characteristics found are different from those previously obtained for the oxygen consumption of the same seeds germinated in the same manner. For Lupinus, the temperature characteristics above and below the critical temperature of 20° are 16,100 ± and 24,000 ± calories respectively. For Zea, no evidence of a critical temperature was found in this region, and the temperature characteristic is 20,750 ± calories throughout the range of temperature tested. The possible interpretations of the difference in the values of temperature characteristics for oxygen consumption and for production of CO₂ are noted.

CITATIONS