SOME EFFECTS OF TEMPERATURE AND CARBON DIOXIDE CONCENTRATION ON PHOTOSYNTHESIS OF MIMULUS[1]

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It has been proposed that photosynthetic responses to an altitudinal gradient of CO_2 might influence altitudinal distribution of some plants (1). The present work was undertaken as a preliminary test of this hypothesis and yielded no support to it. Additionally, the study yielded information about temperature effects on carbon dioxide compensation concentration, apparent photosynthesis at simulated high altitude, apparent respiration, and respiration during photosynthesis.

For simplicity of expression, the following definitions will be adhered to throughout this paper: photosynthesis—total amount of CO_2 reduced, regardless of origin; apparent photosynthesis—measurable decrease of CO_2 in a closed system containing an illuminated green leaf; respiration—amount of CO_2 produced endogenously and either reused or evolved; apparent respiration—measurable increase of CO_2 in a closed system containing an illuminated leaf.

MATERIALS AND METHODS

Mimulus was selected for this study because altitudinal distribution of races and species has been studied intensively (5) and because abundant clonal material was readily available. Two clones were used. One (clone 6546–5) was an individual of *M. cardinalis* Dougl. originally from an elevation of about 150 feet near the Pacific coast at Los Trancos Creek, San Mateo County, California. The other (clone 6546–3) was an F_1 hybrid between the Los Trancos form of *M. cardinalis* and a subalpine plant of *M. lewisii* Pursh. from 10,700 feet elevation in the Sierra Nevada Mountains at Slate Creek Valley, Mono County, California. According to Nobs (6) the Los Trancos strain grew well at low altitude but did not survive when transplanted to 4,500 feet altitude; whereas the hybrid flourished at low altitude, at 4,500 feet and at about 10,000 feet.

All propagules were from cuttings taken in May 1957, started in sand, and grown in soil in 4-inch pots in a greenhouse at Stanford, California. Experiments were done during July 1957. Six pairs of opposite leaves on 5 plants of the parent clone and 6 pairs on 3 plants of the hybrid clone were used.

The apparatus used was a modification of one already described (2). It consisted of a leaf chamber, a small air pump, and an infrared gas analyzer (Beckman L/B 15 coupled to a Varian recording potentiometer) in closed series. The analyzer recorded continuously the concentration of CO_2 in the system. A change of concentration was considered a direct measure of uptake or evolution of CO_2 by the plant material enclosed in the leaf chamber.

The routine procedure was as follows. An intact branch tip, still attached to an intact potted plant and bearing 1 pair of opposite leaves, was sealed in the chamber and left at a standard illumination of 2000 ft-c (300 watt, internal-reflector, tungsten filament flood lamp) and a constant temperature for 15 to 30 minutes before measurements were begun. Then CO_2 was added to raise the concentration to about 500 ppm. The subsequent uniformly decelerated decrease of CO_2 in the system was recorded. When concentration had fallen below 100 ppm, more CO_2 was added and a duplicate tracing was made (fig 1). This time, concentration was allowed to fall to compensation, that is, to that low value at which there was no net gain or loss of CO_2 by the leaf. Then a scrubber bottle of aqueous NaOH was put in the system, and CO_2 was reduced to less than 50 % of compensation concentration. The scrubber was removed, and the subsequent increase of concentration (apparent respiration) was recorded. This procedure was duplicated. The chamber was then darkened and duplicate tracings of CO_2 evolution were made at 300 ppm. The whole procedure was repeated at 2 other temperatures before the leaves were removed for area measurement. Leaf area was computed from the weight of a piece of aluminum foil cut to the pattern of the leaf.

Each of the 6 possible sequences of 3 temperatures (20°, 30° and 40° C) was used once with each clone. This design was intended to eliminate any subtle but consistent extraneous effect (e.g., sequence, length of time under the lights, exposure to low CO_2 concentration, etc.) from comparisons between temperatures or between clones by distributing it uniformly over temperatures and clones. Preliminary tests revealed no massive effect of the experimental procedure that needed to be taken into further account.

RESULTS

Rates of CO_2 uptake and evolution were computed from slopes of lines drawn tangent to recorder tracings as shown in figure 1 (example: at 300 ppm, 1 mv = 2.34 μg CO_2 in the 239 ml system; $2.34 \times 21.5 / 60/0.23\ dm^2 = 3.65\ \mu g/sec/dm^2$ for the tracing shown). Duplicated rates of apparent photosynthesis at 300, 200 and 100 ppm, of apparent respiration at varied low concentrations, of dark respiration at 300 ppm, and a single observation of the CO_2 compensation concentration were thus obtained for each pair of leaves at each temperature (except that there was usually no apparent photosynthesis at 100 ppm and 40°). Mean rates and concentrations computed from these data are shown in figure 2.

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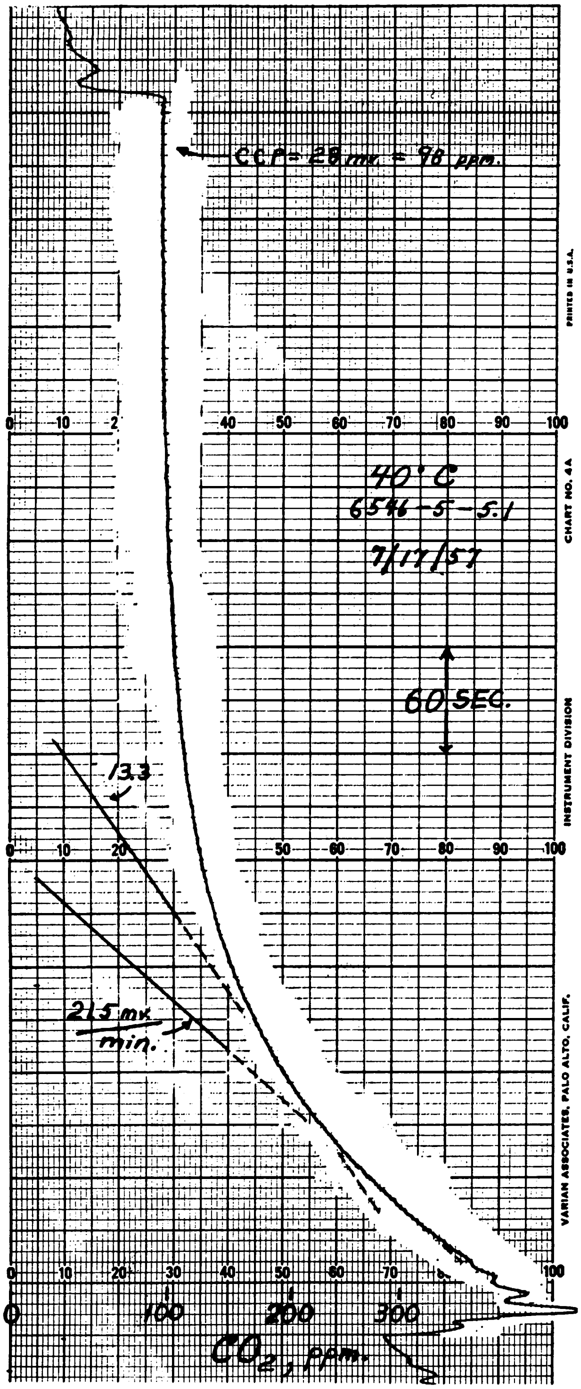

FIG. 1. Photograph of a recorder tracing showing the decrease of CO_2 concentration in a closed system containing a pair of intact Mimulus leaves illuminated at 2000 ft-c. Some grid marks were washed over with white ink to increase contrast. Recorder pen moved upward. Straight lines are drawn tangent to the tracing at 200 and 300 ppm. CCP is CO_2 compensation concentration.

TABLE I

AVERAGE RESPIRATION RATES* FOR 6 SETS OF MIMULUS LEAVES IN DARKNESS; AND RATIO OF APPARENT PHOTOSYNTHESIS OF SAME LEAVES AT 2000 FT-C AND 300 PPM TO RESPIRATION (P/R)

	R			P/R		
	20°	*30°*	*40°*	*20°*	*30°*	*40°*
Parent clone	0.15	0.29	0.46	21.8	9.8	4.7
Hybrid clone	0.22	0.39	0.63	12.3	7.2	2.9

*In μg $CO_2/sec/dm^2$.

The confidence interval (fig 2) was computed from the error term of an analysis of variance (table II). It can be interpreted as indicating that if the experiment were rerun with similar conditions and materials, mean values should be expected to deviate from those shown by more than this amount only about once in 20 trials.

Table II is based on only the 144 measurements of apparent photosynthesis represented in figure 1 by the 12 points for 20° and 30° C at 100, 200 and 300 ppm. Because there were few measurements at 100 ppm and 40° C, all measurements at 40° were excluded to simplify computations. Measurements of apparent respiration were excluded because they were made at a variety of concentrations and were thus subject to additional variance. Because compensation concentration was subject to an entirely different kind of variance (resulting in horizontal rather than vertical dispersion as seen in figure 2), a separate analysis of variance was made for these measurements (table III).

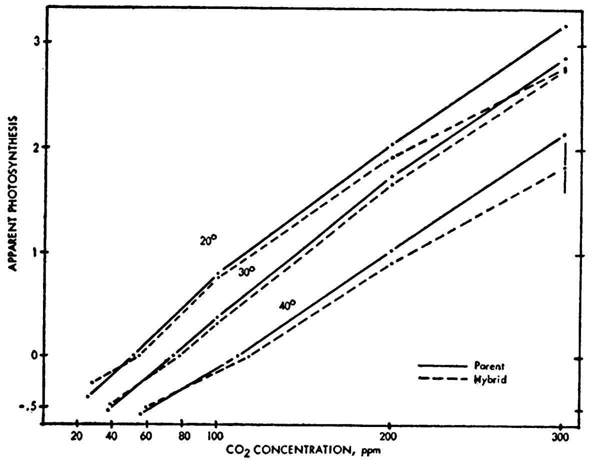

FIG. 2. Effects of air temperature and of CO_2 concentration on apparent photosynthesis of intact leaves of Mimulus. Illumination was 2000 ft-c. Ordinal unit: μg CO_2 per second per dm^2 of leaf area. Points at zero apparent photosynthesis represent means of 6 observations each, all others are means of 12.

Table II

Analysis of Variance of Data Represented in Figure 1 by Upper 12 Values at 20° and 30° C

Source of variation	Degrees of freedom	Variance
Clones (Cl)	1	0.355
Individuals (I)	10	1.989
Subtotal	11	
Temperatures (T)	1	1.532
T-Cl	1	0.058
T-I	10	0.224
Subtotal	23	
Concentrations (Co)	2	33.028
Co-Cl	2	0.076
Co-T	2	0.112
Co-T-Cl	2	0.041
Error (Co-I + Co-T-I)	40	0.172
Total	71	

Discussion

Similarity of Responses of the Two Clones: Inspection of figure 2 suggests only slight differences between the clones. This similarity is confirmed more rigorously by table II. Marked and consistent differences in the responses of the 2 clones to temperature or concentration would have resulted in much larger interaction variances (T-Cl and Co-Cl). A differential response to concentration that varied with temperature would have resulted in a larger 3-way interaction (Co-T-Cl).

This work does not establish that the 2 clones are necessarily similar in their photosynthetic responses to altitude changes. It simply establishes that no consistent difference was revealed. There are several possibilities that need investigation before the original hypothesis can be ruled out for even these 2 clones: 1) Perhaps differences in photosynthetic mechanism develop only when plants are actually grown at high altitude. 2) Perhaps reduced partial pressure of CO_2 does not simulate high altitude realistically. 3) Variance between individuals of the same clone was surprisingly high and perhaps masked otherwise significant differences.

Temperature Dependence of CO_2 Compensation Concentration: The increase of compensation concentration with temperature shown in figure 2 is consistent with that reported by Egle and Schenk (4) and by Thomas and Hill (8). It is at variance with results of earlier studies reviewed by Rabinowitch (7.) Thomas and Hill ascribe this dependence to the fact that the temperature coefficient for respiration is probably much larger than that for photosynthesis, and no alteration of their interpretation is proposed here.

Table III

Analysis of Variance of Data Represented in Figure 2 by the 6 Values for Compensation Concentration (apparent photosynthesis = 0)

Source of variation	Degrees of freedom	Variance
Clones	1	165
Individuals	10	84
Subtotal	11	
Temperatures	2	11,643
T-Cl	2	12
T-Cl-I	20	107
Total	35	

Suggested Explanation for Compensation Concentration: The well-known fact, here demonstrated again, that compensation concentration exceeds zero probably indicates that a photosynthesizing leaf does not reuse directly all endogenous CO_2, even when photosynthesis is CO_2-limited. At this low concentration, light-saturated photosynthesis is clearly CO_2-limited. If all endogenous CO_2 were reused directly, a leaf should continue to take up external CO_2 in proportion to its availability (because ability to consume CO_2 photosynthetically exceeds ability to produce CO_2 in respiration), and the curve of apparent photosynthesis vs CO_2 concentration would pass through the origin. The fact that the curve actually passes to the right of the origin indicates that some endogenous CO_2 diffuses into the external system before it can be reused; and at compensation, uptake of external CO_2 is diminished until it just equals this leakage.

Increased Importance of High Temperature at Simulated High Altitude: Although proportional concentration of atmospheric CO_2 remains nearly constant at 300 ppm irrespective of altitude, actual concentration varies directly with total atmospheric pressure and thus decreases with altitude. Actual concentration at 15,000 feet altitude is about one half that at sea level. Presumably, apparent photosynthesis at 15,000 feet and 300 ppm would be the same as at sea level and 150 ppm. As shown in figure 2, an increase of temperature from 20° to 40° C decreased average apparent photosynthesis of parent plants about 33 % at 300 ppm and about 73 % at 150 ppm. This suggests that limitation of apparent photosynthesis (and therefore of dry weight increment) by high temperature may become more important at high altitude.

Effects of Temperature and CO_2 Concentration on Apparent Respiration: Mean values for apparent respiration are shown as negative values for apparent photosynthesis in figure 2. These fit nearly linear extensions of the lower ends of the curves for apparent photosynthesis and thus tend to validate linear extrapolations made in an earlier study (2). Apparent respiration increased with temperature and with decreased CO_2 concentration.

Effect of Temperature on Respiration During Photosynthesis: In an earlier study (2) respiration during photosynthesis was estimated from a curve of apparent photosynthesis vs CO_2 concentration by inserting a hypothetical curve for photosynthesis vs CO_2 concentration and measuring vertical distance between the 2 curves. This method is nearly identical with one that is perhaps more familiar—that of extrapolating an empirical curve back to zero CO_2. It is more complete logically in that it makes explicit the assumptions that are implicit in the latter method: the curve for photosynthesis vs CO_2 originates at the origin, is linear at the lower end, and is parallel to the corresponding curve for apparent photosynthesis. Although these assumptions are reasonable, they are untested and are not required in the following and 3rd method.

For simplicity, discussion is limited to 2 curve segments of figure 2: parent plants at 20° C, from 100 to 300 ppm; parent plants at 40° C, from compensation to 300 ppm. These segments are nearly parallel, that is, temperature effect on apparent photosynthesis was independent of CO_2 concentration.

Apparent photosynthesis is the resultant of photosynthesis minus respiration, and any effect on apparent photosynthetic rate is explainable in terms of rate changes of photosynthesis and respiration. Photosynthesis is CO_2-dependent, and thus a temperature effect on photosynthesis would vary with CO_2 concentration. If such an effect were involved in the response of apparent photosynthesis to temperature, the segments would diverge at high CO_2 concentration. They do not, and this leaves 1 other obvious possibility: respiration.

Respiration of a photosynthetic leaf in darkness is essentially independent of CO_2 concentration over the 100 to 300 ppm range (3). Presumably, respiration in the light is likewise independent. Therefore, the CO_2-independent effect of temperature on apparent photosynthesis most likely resulted entirely from a temperature effect on respiration; and vertical distance between the 2 curve segments yields a measure of the difference of respiration rates during photosynthesis at 20° and at 40° C (1.04 μg/sec/dm^2).

Appropriateness of this interpretation for the present data depends on whether the apparent parallelism is real or fortuitous. Although the analysis of variance reveals no divergence, this cannot be established with certainty because of the large experimental error. However, the fact that the corresponding segments for hybrid plants are also nearly parallel is confirmatory.

COMPARATIVE EFFECT OF TEMPERATURE ON RESPIRATION IN LIGHT AND DARKNESS: As indicated in the preceding section, average respiration rate of parent plants during photosynthesis probably increased 1.04 μg/sec/dm^2 as temperature increased 20°. Average respiration rate of the same leaves in darkness increased 0.31 μg/sec/dm^2 with the same temperature increase (table I). Respiratory acceleration was thus about 3.3 times greater in light than in darkness. Presumably, rates were proportional to accelerations, and thus respiration rate was about 3.3 times greater in light than in darkness.

SUMMARY

Effects of 3 temperatures (20°, 30°, 40° C) on apparent photosynthesis of 2 clones of Mimulus were studied over a wide range of CO_2 concentrations. Compensation concentration increased with temperature. High temperature inhibition of apparent photosynthesis was markedly greater at simulated high altitude than at sea level. Apparent respiration increased with temperature. Respiration during photosynthesis was evidently about 3.3 times greater than respiration in darkness.

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