THE INTERRELATION BETWEEN CO_2 METABOLISM AND PHOTOPERIODISM IN KALANCHOË. II. EFFECT OF PROLONGED DARKNESS AND HIGH TEMPERATURES[1]

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When *Kalanchoë Blossfeldiana* is exposed to repeated short-days there is a marked increase in both the amount and the rate of the fixation of carbon dioxide in the dark (3). Plants which have received a series of short day and long night cycles show an uptake of CO_2 in each dark period which begins slowly, increases to a maximum and falls again; finally it reaches zero and is followed by a brief period of CO_2 production just before the end of a 16-hour dark period. If similar plants were kept in darkness for a period longer than 16 hours, there was no further CO_2 uptake but rather a steady loss of CO_2 by respiration. During the light period, on the other hand, the opposite phenomenon occurred; CO_2 was given off soon after illumination had begun, and this also passed through a maximum and came to an end before the end of the 8-hour light period. It was also found that the amount of CO_2 evolved paralleled the amount of CO_2 absorbed during the preceding dark period. This uptake of CO_2 in dark and release in light only occurred to a very small extent in plants exposed to 8-hour nights or to long nights interrupted with a brief light period. Tentatively the phenomenon was ascribed to the formation of a light-labile or heat-labile CO_2 compound, probably an organic acid.

The parallelism between the effect of photoperiodic treatment on CO_2 metabolism and its influence on flowering in this short-day plant is obviously very suggestive. However, before this parallelism can be pursued, the exact role of the light period in causing release of CO_2 needs further investigation. In the preceding experiments (3) it was not determined whether the CO_2 release is due to light itself or to high temperature. This uncertainty stems from the fact that when the lights come on, although the temperature of the room remains constant, the temperature within the Lucite chamber containing the plant is raised to about 29° C. The temperature within the leaves doubtless rises even higher. It has been shown by Wolf (8) that organic acids are used up in Bryophyllum leaves kept in the dark at high temperatures, and other workers have demonstrated that acid formation by succulents is depressed at higher temperatures (1, 7). It was possible, therefore, that the production of CO_2 in light, particularly if it arises from an organic acid, might have been due merely to the temperature. It must also be borne in mind that the photoperiodic response of many plants, in regard to flowering, can be extensively modified by temperature.

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With this problem in mind, plants were exposed to successive short-day cycles and then held in prolonged darkness. The CO_2 metabolism was followed continuously. After the dark fixation of CO_2 had been completed, the plants were exposed to high temperatures while still in the dark.

MATERIALS AND METHODS

Kalanchoë Blossfeldiana var. Tom Thumb were raised from cuttings taken from plants which had been maintained on long days. They were grown in air-conditioned lightrooms at 19° C. and 75 % relative humidity with a light intensity of 1500 fc at plant level, as previously described (3). Prior to treatment, the daylength was maintained at 16 hours. The term "short-day cycle" refers to 8 hours light and 16 hours darkness.

The plants were inclosed in the Lucite gas-tight chambers previously described (3), and the CO_2 determinations were made with the infra-red gas analyzer. Because of variations in the composition of the air supply, the data are presented as a percentage of the CO_2 content of the incoming air (which averaged 0.04 %). Flow rates were maintained at 27.5 ± 0.5 liters per hour by means of a modification of the apparatus described by Porter, Pal and Martin (6).

RESULTS

EFFECT OF 30° C TEMPERATURES: As stated above, the dark fixation of CO_2, by plants given repeated short-day cycles is usually completed before the end of the 16-hour dark period. Exposure to a further 32 hours of darkness at 20° C caused only a steady production of CO_2. When the lights were turned on at the end of this 48-hour dark period, a burst of CO_2 production occurred very similar to that taking place after 16-hour dark periods. In figure 1 the behavior in light is compared for the same plant following 16-hour and 48-hour dark periods. It will be seen that in both cases photosynthesis (CO_2 uptake) begins immediately after the plants are illuminated, but that within about half an hour a vigorous production of CO_2 supervenes. This lasts 5 to 7 hours. There is some difference in the speed of onset and duration of the CO_2 production in the two cases. However, the close correspondence between the total amounts of CO_2 evolved in the two cases proves that the product of the dark fixation, which is decomposed in light, is still present after 48 hours, and hence fairly stable in the dark at 20° C.

In order to ascertain whether the production of CO_2 in light could be explained by increased temperature alone, plants which had been pretreated with 36 short-day cycles were left in prolonged darkness, and

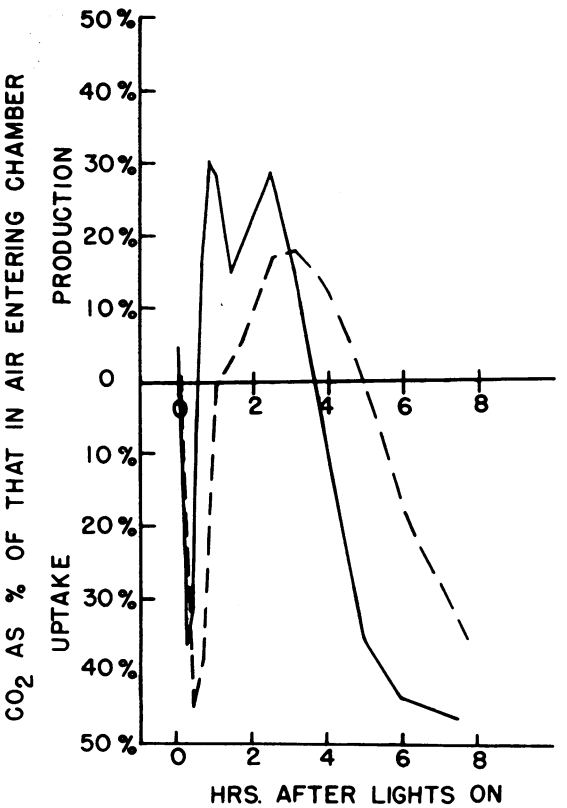

FIG. 1. The production of CO_2 during the light phase by a Kalanchoë plant pretreated with 32–34 short-day cycles. The solid line represents the metabolism of the plant in light after a prolonged dark period of 48 hours, while the broken line represents the average metabolism during the two previous light cycles after the normal 16-hour long nights.

after the dark fixation of CO_2 was completed, and the rate of respiration had become fairly constant, the chambers were transferred, in the dark, to a 30° C incubator.

One of two experiments of this sort is presented in figure 2. It will be seen that the plant pretreated with 36 long nights (solid line) has a considerable net uptake of CO_2 during the first 16 hours of the dark phase, but that, when continued in the dark, no further uptake occurs. The control plant (broken line) was given 36 long-night cycles, each interrupted by 10 minutes of 1500 fc given at the middle of the dark phase. This plant shows only very little net fixation of CO_2 and thereafter produces CO_2 steadily when continued in the dark. After the rate of respiration was stabilized, the short-day-treated plant (solid line) was transferred, in the dark, to 30° C.; an immediate large increase in the rate of production of CO_2 is evident. The rate of production of CO_2 reached a maximum and then declined, stabilizing at about the rate of production prevailing before the temperature was raised. After approximately 6 hours at the new stabilized rate, the chamber was removed from the incubator and the lights were turned on. A second, but reduced, burst of CO_2 production was now found to occur and the full photosynthetic rate was not achieved until at least 6 hours later. As previously reported, the control plant pretreated with interrupted long nights showed only a minute burst of CO_2 on illumination.

Similar, though not quite identical, results were obtained in a second experiment (fig 3). In this the plant was also pretreated with 36 long-night cycles and shows the typical, extensive CO_2 fixation during the first portion of the dark phase. By the end of the usual 16-hour dark period this fixation has given way to CO_2 production. When the rate of production had become fairly stable, the chamber was again transferred to a 30° C incubator in the dark, and again an immediate increase in production of CO_2 was evident. After reaching a maximum, the rate of production of CO_2 in the dark at 30° C stabilized at a somewhat higher value than it had been at 20° C, which is to be expected. When this rate fluctuated little for 8 hours, the chamber was returned to 20° C. and the lights were turned on. A second release of CO_2 was evident, and photosynthesis did not reach its full rate by 4.5 hours. Again, however, the CO_2 evolved in the light at 20° C was apparently less than at 30° C in the dark. The higher rate of dark uptake by this plant is probably due to its greater volume of leaf material.

EFFECT OF DARK PHASE TEMPERATURE ON FLOWERING: It was postulated that if the dark fixation reaction had any causal relationship to the flowering response, then exposing plants to high temperatures during the dark phase of induction

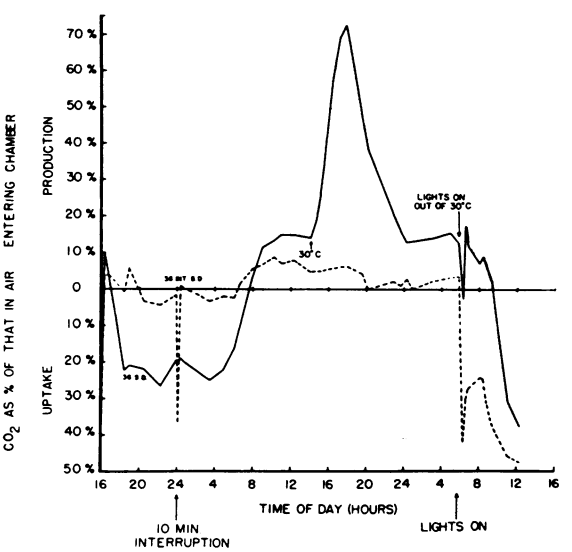

FIG. 2. The CO_2 metabolism of Kalanchoë during a prolonged dark period of almost 38 hours. The broken line represents a plant pretreated with 36 interrupted long nights and the solid line a plant that was pretreated with 36 uninterrupted long nights. The plant designated by the solid line was placed in a 30° C incubator for the period shown.

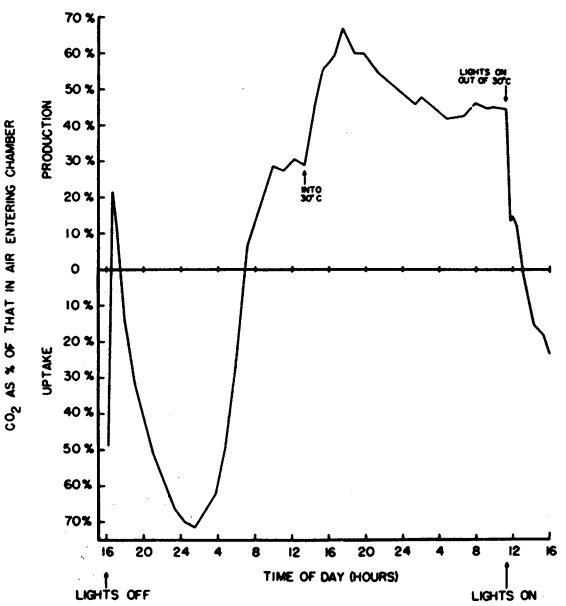

FIG. 3. The CO_2 metabolism of Kalanchoë during a prolonged dark period of 43 hours, after pretreatment with 37 long-night cycles. The plant was placed in a 30° C incubator for the period shown.

should decrease the flowering. Effects of the dark-phase temperature on the flowering response have already been demonstrated for a number of plants (2, 4) and, for a fairly low temperature range, for Kalanchoë (5). It seemed desirable, nevertheless, to study the effects of high temperature under our conditions.

Plants were therefore exposed to fifteen short-day cycles (a marginal treatment for flowering induction under these conditions). During the light phase the air temperature was 19 to 20° C, but during the dark phase the plants were placed in incubators at temperatures between 15 and 35° C. The results of two preliminary series are shown in table I. It can be seen that, in general, higher temperatures strongly decreased the initiation of flowers. The only plants which produced open fertile flowers, however, were the plants maintained at 30° C in the second series, although these plants developed fewer buds and the flower primordia were evident at a later date than plants kept at 15° C.

METABOLISM IN PROLONGED DARKNESS AFTER PRETREATMENT WITH NON-INDUCTIVE CYCLES: In figure 2 it is seen (broken line) that a plant pretreated with non-inductive, interrupted long nights has little net dark fixation of CO_2, and that CO_2 fixation does not take place even though the plant is now kept in prolonged darkness. The effect of pretreatment with interrupted long nights followed by long days is shown in figure 4. In this experiment, plant A (broken line) was pretreated with 36 short-day cycles with interrupted long nights, while plant B (solid line) received 21 such interrupted long-night cycles followed by 15 long-day cycles. Both received, in addition, 8 hours of light preceding the start of prolonged darkness. Neither of these treatments led to flowering.

As in figure 2, we find that with both of these plants there is some fixation of CO_2 during the early part of the dark period (as evidenced by decreased respiration), but that subsequently there is a continuous production of CO_2, presumably at the normal respiration rate. When the lights were turned on after 43 hours of darkness, both plants showed only a small burst of CO_2 in light.

DISCUSSION AND CONCLUSIONS

The large quantity of CO_2 released when short-day-treated plants are raised to 30° C in the dark (figs 2 and 3) indicates that at least part of the fixation product is thermo-labile. The secondary burst obtained on exposure to light, following this 30° C treatment, can only be interpreted as the light destruction of additional material. Since the thermal destruction at 30° C had obviously reached completion, it follows that part of the CO_2 fixation product is thermo-stable and is decomposed only in light.

Whether the thermo-labile fraction (which is the major fraction) is also decomposed by light (at 20° C) is not established. Thus the CO_2 which is released by short-day-treated plants at the beginning of each light phase may be due to light alone or may be made up of both thermo- and photo-labile components. That there is a truly photo-labile material is supported by the organic acid determinations of Bonner and Bonner (1) on three Bryophyllum species. They found that when excised leaves were incubated for 48 hours at 25° C in the dark there was

TABLE I

EFFECT OF DARK PHASE TEMPERATURE ON FLOWERING

TEMP. °C	BEHAVIOR IN 60 DAYS FOLLOWING START OF SHORT DAY TREATMENT *	
	FLOWERING RESPONSE, NO. OF PLANTS FLOWERING PER NO. OF PLANTS TREATED	NO. OF DAYS TO FIRST VISIBLE FLOWER PRIMORDIA
	Series 1	
15	3/3	49–59
20	1/3 **	58
30	0/3	..
	Series 2	
15	2/2 †	35
20	1/2	38
25	1/2	54
30	1/2 ††	48
35	0/2	..

* 15 days of 8 hrs light and 16 hrs dark, then returned to 16-hr days at 20° C.

** One of the other plants showed a questionable flower response.

† Numerous unopened flowers.

†† The other plant formed a single flower by the 75th day.

a small increase in the organic acid content, while incubation for the same period at 25° C in the light gave considerable decreases in the organic acid content.

Plants pretreated with cycles such as 8-hour nights or interrupted long nights, which do not induce flowering, fail to show much fixation of CO_2 even when exposed to a prolonged dark period (figs 2 and 4, also figs 5 and 6 of the preceding paper). The plants in figure 4 do show a small amount of fixation (as evidenced by an apparent decrease of respiration) during the first portion of the dark phase. Following the prolonged dark period there is only a small amount of CO_2 production in light. It follows that, as previously reported (3), a succession of long uninterrupted nights is required for the development of a large net dark fixation and subsequent light production of CO_2. A single uninterrupted long night, even though of extensive duration, will not substitute for such a succession. The data confirm the conceptions, already brought forward, that (1) the CO_2 released in the light must come from the dark fixation products, and that (2) dark fixation requires a preceding light period. The effects of light and dark periods are thus closely interwoven.

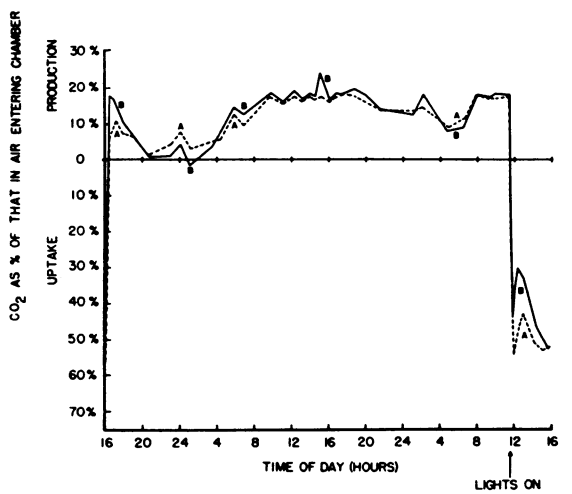

FIG. 4. The CO_2 metabolism of Kalanchoë during a prolonged dark period of 43 hours after pretreatment with 36 interrupted long nights (plant A), or 21 interrupted long nights followed by 15 long days (plant B).

The preliminary experiments presented in table I indicate that high temperature, a factor shown to destroy part of the dark fixation product (figs 2 and 3), also decreases the initiation of flower primordia. It is evident, therefore, that three different treatments which decrease the amount of CO_2 fixed in the dark, i.e., short nights, interruptions of the night, and long nights at high temperature, all decrease the flowering response. The role of the dark temperature may be complex, however, since temperatures favoring the initiation of flower primordia do not favor their subsequent development into open fertile flowers, but lead instead to phyllody of bracts.

SUMMARY

Plants of *Kalanchoë Blossfeldiana*, previously exposed to short days, were allowed to fix CO_2 in a prolonged dark period and then the temperature was raised to 30° C. A large quantity of CO_2 was evolved. On now exposing to light at 20° C a further (smaller) evolution of CO_2 occurred. It is deduced that the dark fixation product comprises both thermo-labile and photo-labile fractions.

Plants given the minimum number of short-day cycles to induce flowering, but kept at different temperatures during the dark period, showed a decreased flowering response at high temperatures. This suggests that the product of dark fixation of CO_2 may be causally related to flowering.

Exposure to a single very long dark period causes little or no CO_2 fixation, and correspondingly there is little CO_2 evolution on subsequent exposure to light.

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