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(*) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

ABSTRACT

An investigation was undertaken to determine whether growth of a plant under different day lengths may bring about changes in such primary reactions as those intimately associated with the early steps of photosynthesis and thus may initiate the metabolic changes which lead to the differences in nitrogen and carbohydrate accumulation which are known to occur. Leaves from short- and long-day plants of Kalanchoë blossfeldiana were exposed to radioactive CO_2 under light for 5 minutes. The compounds which became radioactive in that time were determined by two-dimensional paper chromatography and exposure of film by chromatograms. Such experiments were performed at the onset of flowering and during fruit development. The total activity fixed per unit wet weight is greater for the short-day than for the long-day leaves in the earlier experiment but less in the later one. Short-day leaves fixed about one-half or less the activity in phosphoglyceric acid, serine and alanine and twice as much or more glycine and mannose as did the long-day ones in the earlier experiment. In the later one, short-

day leaves fixed one-half or less as much activity in aspartic acid, serine and alanine and twice as much phosphoglyceric acid and fructose as did long-day leaves. These differences in the amounts of radioactive compounds formed indicate that the reactions of early photosynthesis are indeed influenced by prior photoperiodic treatment of the plant.

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Numerous experiments during the last 30 years since the photoperiodic phenomenon has been recognized have shown that the nitrogen and carbohydrate accumulation by plants which respond to photoperiodic treatment differ with the day length under which they were grown.[1,2,3,4] The question arises whether these differences may be initiated by changes in such primary reactions as those intimately associated with the first steps of photosynthesis. An investigation of this problem was undertaken using

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(1) Murneek, A. E., Nutrition and Metabolism as Related to Photoperiodism. Vernalization and Photoperiodism Symposium, Waltham, Mass., Chronica Botanica Co., 1948.

(2) Parker, M. W. and Borthwick, H. A., Bot. Gaz. 100, 651 (1939).

(3) Sircar, S. M. and De, B. N., Proc. Natl. Inst. Sci., India, 14, 263 (1948).

(4) Hall, W. C., Plant Physiol., 24, 753 (1949).

tracer technique to determine the possible effect of different daylength treatments on the photosynthetic CO_2 fixation of Kalanchoë blossfeldiana.[5]

Experimental Methods

Cuttings of Kalanchoë blossfeldiana made from a plant which had long-day characteristics (i.e., large thin leaves and no flowers) were grown in temperature-controlled (75-80° F.), ventilated, light-tight chambers. Fluoroscent lights giving about 1000 foot candles at plant height were controlled by time clocks set to give 9 and 15 hours light in the short- and long-day chambers. After 16 weeks the plants in the short-day chamber had well-developed inflorescences and small thick leaves while plants in the long-day chamber had large thin leaves and no inflorescences. In order to permit comparison of our leaves with those of other investigators, the dry weight percentages and succulence values of these characteristic leaf types were determined by oven-drying leaves comparable to those used in the fixation experiments. Dry weight percentages of our leaves compared rather closely with those of Harder:[5] short-day leaves 3.2% and long-day leaves 5.4% as compared with Harder's values of 2.5% and 5.8%. Succulence values for short-day leaves were 2.85 (Harder, 2.65 and Bode, 2.00) and for long-day leaves 0.75 (Harder, 0.61 and Bode, 0.68).[5,6]

When the plants were 16 weeks old a carbon-dioxide fixation experiment (Experiment A) was performed using leaves from the third node below

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(5) Harder, Richard and von Witsch, Hans. Uber den Einfluss der Tageslänge auf den Habitus, besonders die Blattsukkuleng, und den Wasseraushalt von Kalanchoë blossfeldiana. Jb. Bot. 89, 354 (1940), in Soc. Exp. Biol. Symposia II, Growth in Relation to Differentiation and Morphogenesis, Cambridge, University Press, 1948.

(6) Bode, Otto, Uber die Zusanwerhange zwischen CO_2-assimilation und Photoperiodismus bei Kalanchoë blossfeldiana, Planta, 33, 278-289 (1943).

the inflorescence of the short-day plants and from the corresponding node of the long-day plants. After 22 weeks, when the flowers of the short-day plants were dried up and fruits had formed and the long-day plants still remained vegetative, another fixation experiment (Experiment B) was performed. Leaves from the fourth node above the soil surface from plants of both long- and short-day conditions were used.

These CO_2-fixation experiments were carried out in the following manner: The petioles of selected leaves of both short- and long-day plants were put in small tubes of water within a thin gas-tight chamber with parallel glass sides. This chamber was illuminated by two lamps and kept at room temperature by means of water-cooled heat filters between the chamber and the lamps. The chamber received 13,000 foot candles during Experiment A and 5,000 foot candles during Experiment B. The leaves were illuminated 15 minutes with air being drawn through the chamber; following this period the chamber was partially evacuated and radioactive CO_2 entered the chamber. After 5 minutes the short- and long-day leaves were removed to separate mortars containing liquid nitrogen and ground. A boiling 80% ethanol extract was made of the frozen ground leaves. Aliquots of this extract were dried on aluminum plates and the radioactivity counted with a wide-mouth Geiger-Müller tube to determine the total amount of radioactive CO_2 fixed. The amount of CO_2 fixed in the 80%-ethanol-soluble compounds was similarly determined by plating and counting an aliquot of the supernatant solution

The distribution of the radioactivity in various compounds of the 80%-soluble portion was determined by 2-dimensional chromatography of the

concentrated extract.[7] Phenol-water was used as the first solvent and a butanol-propionic acid-water mixture as the second solvent. No-screen X-ray film was exposed to the chromatograms to determine the location of radioactive compounds. The identity of the compounds causing the exposed spots was established by their relative positions and, in cases of doubt, by co-chromatography of the eluted spots with known compounds. The activity of each compound was counted and tabulated in terms of counts per minute per gram of leaves per 5 minutes of photosynthesis.

Results

Table I shows the results of these two experiments in terms of the total amount of radioactivity fixed, the amount fixed in the 80%-ethanol-soluble portion and the distribution of activity in various compounds. The total activity fixed is greater for short-day than for long-day leaves in Experiment A (1.7×10^6 c/m/g as compared with 1.2×10^6 c/m/g) but less in Experiment B (1.5×10^6 c/m/g as compared with 9.5×10^6 c/m/g). The amount of activity fixed in the ethanol-soluble portion is less for short-day than for long-day plants in both experiments.

The amount of activity in the various compounds on the chromatograms varies considerably, depending on the previous day-length treatment of the leaves. The ratio of the activity fixed in each compound by short- and long-day leaves has been entered in the table in order to present a clearer picture of what these activity values mean in terms of the effect

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(7) Bassham, J. A., Benson, A. A., Kay, L. D., Harris, A. Z., Wilson, A. T., and Calvin, M., The Path of Carbon in Photosynthesis. XXI. The Cyclic Regeneration of Carbon Dioxide Acceptor, J. Am. Chem. Soc., 76, 1769 (1954).

of photoperiodic treatment. In addition, this S/L* ratio for each compound has been reduced to the same number of counts on the chromatogram by dividing it by the S/L ratio of the total number of counts on the chromatogram. Because of the experimental errors inherent in this type of experiment, some deviation of this ratio from 1 is to be expected. However, variations of about 100% are considered to be well beyond the range of experimental error and to be indicative of changes in metabolism brought about by growth in different day lengths.

In Experiment A short-day leaves fixed about one-half or less the activity in phosphoglyceric acid, serine, and alanine as did long-day leaves. Glycine and mannose were twice or more as active in short-day as in long-day leaves. In Experiment B fixation by short-day leaves was one-half or less that by long-day leaves in the case of aspartic acid, serine, and alanine, but twice or more that of long-day leaves in the case of phosphoglyceric acid and fructose.

Discussion

Although these experiments are of a very preliminary nature, the results clearly indicate that in the case of Kalanchoë blossfeldiana the metabolism of early products of photosynthesis is altered by growth in two different day lengths, one of which induces flower formation and the other of which does not.

The total amount of CO_2 incorporated into the leaf constituents has been shown to vary depending upon the previous day length treatment and possibly depending upon the reproductive state of the plants at the

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(*) S/L ratio = $\frac{\text{Short-day plant}}{\text{Long-day plant}}$

time of the experiment. The results of the two experiments cannot be closely compared because experimental conditions such as light intensity, CO_2 availability, etc., were not the same in the two cases. However, the fact that the amount of CO_2 fixed by short-day leaves as compared with long-day leaves is sharply reduced from the time of flowering to the time when fruit has been formed may be significant. This fact corresponds with the results of Bode's manometric experiments which showed lower CO_2 fixation of K. blossfeldiana leaves from plants which were past flowering than from those which were in bud.[6] The fact that in Experiment A more CO_2 was fixed by short-day than by long-day leaves also corresponds approximately with his findings of CO_2 fixation rates using leaves from plants of about the same age and state of floral development.

It is impossible on the basis of these preliminary experiments to speculate as to the mode of action by which different photoperiods involk quantitative changes in the reactions responsible for the several compounds shown to vary between short-day and long-day leaves. However, that there are such actual differences in the amounts of numberous compounds which have been shown to be formed very early in the photosynthetic process is a significant fact in itself. Perhaps the clearest manifestation of these differences is to be seen in the relative amounts of the three amino acids, glycine, serine, and alanine, which are synthesized in short- and long-day plants. While the total amount of soluble compounds newly made is considerably greater in the long-day plant than it is in the short-day plant, the amount of newly made three carbon amino acids, serine and alanine increases much more than proportionately, while the amount of glycine is increased much less than proportionately: in fact

in Experiment A the glycine newly made is actually less, absolutely, in the long-day plant. Whether this shortage of labeled glycine in the long-day plant is due to a decrease in the relative rate of its synthesis or an increase in the relative rate of its utilization remains to be seen.

While the results of these experiments demonstrate metabolis changes which are the result of different photoperiodic treatments, they do not shed any light on the question of whether similar changes in photosynthetic products occur during the period of floral induction. The findings of an investigation of this problem coupled with the results of Leopold and co-workers on enzymatic reactions carried on in the dark periods during photoinduction could well lead to an explanation of the actual chemical reactions involved in photoinduction.

Table I

Radioactive CO_2 Fixed by Short- and Long-day Kalanchoë Leaves During 5 Minute Photosynthesis Experiments

	A 16 wks. old plants		B 22 wks. old plants	
	Short	Long	Short	Long
Total c/m fixed/g. wet wt./5 min.	1.7×10^6	1.2×10^6	1.5×10^6	9.5×10^6
c/m fixed in 80%-ethanol-soluble compounds/g. wet wt./5 min.	0.3×10^6	0.6×10^6	0.35×10^6	3.0×10^6
% soluble in 80% ethanol	17.8	50.0	23.3	31.6

c/m of compounds fixed/g. wet wt./5 min.:*

	S	L	S/L	$(S/L)/(S/L)_t$	S	L	S/L	$(S\ L)/(S\ L)_t$
Sugar diphospates	0	t	-	-	0	5,300	-	-
Uridine diphosphate glucose, UDP-galactose	2,800	5,800	.48	1.2	6,200	98,700	.063	.90
Hexose monophosphates	15,500	22,500	.69	1.8	21,800	347,000	.063	.90
Phosphoglyceric acid	t	1,400	-	-	1,000	4,800	.21	3.0
Phospho-enol-pyruvate	t	t	-	-	t	t	-	-
Aspartic acid	0	t	-	-	0	75,700	-	-
Serine	1,300	6,000	.22	.56	2,000	61,700	.032	.46
Glycine	4,200	3,800	1.1	2.8	5,700	53,300	.11	1.6

Table I, Cont.

	S	L	S/L	$(S/L)/(S/L)_t$	S	L	S/L	$(S/L)/(S/L)_t$
Alanine	600	3,800	.16	.41	1,800	47,700	.038	.54
Mannose	1,700	t	-	-	1,000	13,800	.073	1.0
Fructose	t	t	-	-	1,800	9,000	.20	2.9
Sucrose	21,100	80,600	.26	.67	59,200	689,000	.086	1.2
Total in acid region	1,600	2,300	.70	1.8	3,000	65,200	.046	.66
Total on chromatograms	48,800	124,000	$.39(S/L)_t$		103,500	1,471,200	$.070(S/L)_t$	

(*) 0 counts indicates no visible exposure of the film.

t indicates exposure of film but counts too low above background counts to be considered accurate enough for comparative purposes.