and the latter especially gives an almost perfect single recessive ratio when grown in the field.

This paper deals primarily with the white mutant. Extensive tests show this white strain to be devoid of carotenoids, but nevertheless it is a vigorous seedling, grafts freely, and as a scion sets seed to its own pollen. The seedling reacts to gravity and light, and under suitable conditions produces one-half to two-thirds as much protochlorophyll, chlorophyll a and chlorophyll b, as do seedlings of its green sib under like conditions. The ratios of these three pigments is stable over a wide range of conditions.

A wide range of studies on light intensities and exposure time show maximum pigment production within 72 hours at 0.5 fc of light.

Protochlorophyll is formed in both light and darkness, but varies somewhat with the age of the seedlings.

Long exposures from 2 to 16 hours to light intensities of 250 to 2000 fc cause severe fading. Preliminary tests show that if dark-grown seedlings are exposed to sufficiently strong light, their capacity to produce chlorophyll is destroyed.

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THE DARK FIXATION OF CARBON DIOXIDE AS A FACTOR IN PHOTOPERIODISM [1,2]

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It has been shown that long dark periods are essential for floral induction of short day plants (6) and are prohibitory of induction of long day plants (4). Therefore, some reaction or reactions which occur in the absence of light are specifically involved in photoperiodic induction. The observation that biotin and pantothenic acid contents of leaves were altered by photoperiodic induction (8) suggested the possibility that the dark fixation of CO_2 might be related to photoperiodic phenomena in plants.

The present paper represents a study of the possible relationships existing between the dark fixation of CO_2 and photo-induction in some long and short day plants.

MATERIALS AND METHODS

The plants used in these studies include one long day species, Wintex barley, and two short day species, Biloxi soybean and cocklebur (Xanthium commune Britton). These plants were grown in the greenhouse under non-inductive daylengths until each experiment was conducted. Long and short days consisted of daylengths of 18 and 9 hour durations respectively.

For experiments concerning CO_2-free atmospheres, potted plants were placed in 20-liter large mouth bottles which were sealed at the start of each dark period. Carbon dioxide-free conditions were made possible by the use of a circulating air pump which continuously circulated the atmosphere surrounding the plants through solutions of sodium hydroxide. Barium hydroxide turbidity tests showed that all detachable CO_2 had been removed after a

period of five minutes. Such treatment not only essentially removed the existing CO_2 in the air but also lowered the partial pressure to such an extent that respiratory CO_2 was presumed to be rapidly removed. Circulation through a water solution was utilized for the control treatment. The air circulation was started 30 minutes before commencement of each dark period. While such conditions may have led to a certain amount of photo-oxidation in the foliage in the light, its effects did not alter the photoperiodic response of the barley plants (table I), a fact which suggests that the photoperiodic function was not seriously altered by such photo-oxidations. At the end of each dark period the plants were removed from the bottles and placed under natural

TABLE I

EFFECT OF REMOVAL OF CO_2 DURING THE DARK PERIOD UPON PHOTOINDUCTION OF BILOXI SOYBEAN, XANTHIUM AND WINTEX BARLEY

SPECIES	LENGTH OF DARK PERIOD	NUMBER OF PLANTS	FLOWER PRIMORDIA PER PLANT		FLORAL INHIBITION
			AIR	CO_2-FREE AIR	
	hrs				%
Biloxi soybean	9	10	0	0	..
	15	10	3.67	0.67	82
Xanthium	6	10	0	0	..
	10	10	4.0	0.2	95
	18	10	14.6	3.2	78
Wintex barley	9	20	15.4	13.0	15
	15	20	0	0	..

TABLE II

RELATIONSHIP OF QUANTITIES OF C*O₂ FIXED DURING THE FIRST AND SECOND HALF OF THE DARK PERIOD BY DETACHED LEAVES OF BARLEY, SOYBEAN AND XANTHIUM

TREATMENTS	RADIOACTIVITY IN COUNTS PER MINUTE *		
	BARLEY	SOYBEAN	XANTHIUM
8½ hrs dark C*O₂	168	511	246
9½ hrs dark + 8½ hrs dark C*O₂	265	109	64
18 hrs dark C*O₂	453	541	270

* Each figure represents an average of at least 6 leaves.

light intensities. After the plants had received a given number of photoinductive cycles they were again placed under non-inductive daylengths and at the end of 3 weeks the degree of induction was determined by micro-dissection.

Dark fixation studies using CO_2 tagged with C^{14} were conducted by exposing detached leaves of Xanthium, soybean and the entire aerial parts of barley plants to initial atmospheres of 0.25 % C^*O_2 in glass containers. While some dilution of the radioactivity by respiratory CO_2 may have occurred, it could not have conspicuously altered the fixation for it can be seen in table II that fixation for two half nights approximately equalled fixation for one entire night period. At the end of each dark period the remaining C^*O_2 in the atmosphere was rapidly removed and the plants dried in a drying oven at 65° C for twenty-four hours. Radioactivity determinations were made on the dried material after it had been ground to pass a 60 mesh screen. All samples were counted at infinite thickness using a windowless flow counter attached to a preset scaler. Sufficient numbers of ionizing events were recorded so that the error in counting was less than 2 %. All counts were corrected only for background.

Red light for night interruption treatments was supplied by encasing a 60 watt incandescent bulb in a red glass filter which transmitted wave lengths longer than 5200 Å.

RESULTS

THE REQUIREMENT FOR CARBON DIOXIDE: In an effort to determine whether CO_2 is essential for the dark reaction in photoperiodism, experiments were carried out using barley and soybean, depriving the plants of CO_2 for 15 hours each day, beginning one half hour before the start of the dark period. Dark periods of 9 and 15 hours were given in the presence and absence of CO_2. The treatment was repeated for 5 days, after which the plants were maintained in non-inductive photoperiods until dissected. Xanthium plants were studied similarly under dark periods of 18, 10, and 6 hours, depriving the plants of CO_2 for 18 hours each day. The results of representative experiments are shown in table I.

The data indicate that under the conditions of these experiments photoinduction in the short-day species, soybean and Xanthium, was grossly inhibited when CO_2 was removed during the long dark periods. Floral induction in soybean was inhibited 82 %, and in Xanthium it was inhibited 78 %. Further, in Xanthium this inhibition was enhanced when the dark period was shortened to a threshold period of 10 hours of darkness, which resulted in an inhibition of 95 %. It can also be seen in table I that floral initiation of the long day barley was only slightly inhibited (15 %) by the CO_2-free atmosphere under the daylengths tested.

PATTERNS OF CARBON DIOXIDE FIXATION: In an effort to determine the effect of various degrees of induction on the extent of the dark fixation of CO_2, barley, soybean and Xanthium plants were exposed to from 0 to 4 inductive cycles. Excised leaves were then taken from these plants and exposed to C^*O_2 atmospheres during a single non-inductive night period—8 hours for soybean and Xanthium and 18 hours for barley. The inductive cycles were so arranged that all plants could be studied during the same dark period with all treatments of each species being exposed to the same atmosphere in a single container. The results of this study are shown in figure 1.

The data suggest that dark fixation of CO_2 was promoted by the first two inductive cycles in all of the plants when compared to leaves of non-induced plants. These increases for barley, soybean and Xanthium were 50, 26 and 21%, respectively. In the case of barley and Xanthium the greatest total fixation occurred during the first inductive cycle, whereas soybean reached a peak after 2 inductive cycles.
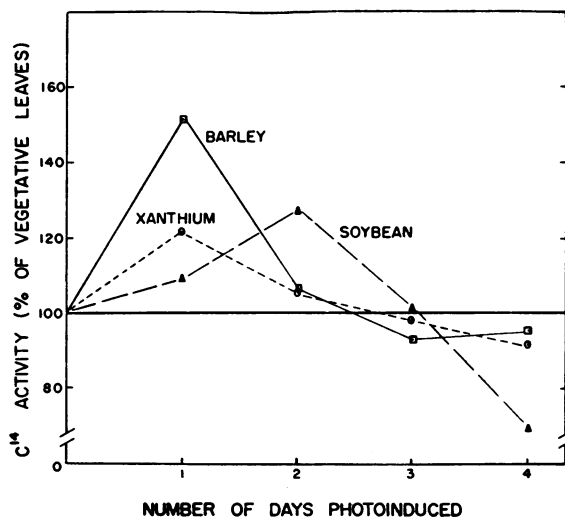

FIG. 1. Uptake of C^*O_2 during a dark period by detached leaves of Wintex barley, Biloxi soybean and Xanthium. Each point represents an average of three separate composite samples taken from at least 4 detached leaves.

Since the data shown in figure 1 suggest that the dark fixation of CO_2 may be altered by photoinduction, it appeared of interest to examine the respective rates of dark fixation of CO_2 throughout single long dark periods. Excised leaves of barley, soybean and Xanthium were placed in a single container such that leaves of all three species were exposed to the same atmosphere of 0.25% C^*O_2. In nine of these containers C^*O_2 was generated at essentially the same time, and the fixation was then stopped at intervals of two hours by removing the leaves from the C^*O_2 atmospheres and killing them as previously mentioned. The results of such a study are shown in figure 2.

The data suggest that barley continued to fix CO_2 throughout the entire dark period while the soybean and Xanthium may reach a fixation equilibrium at about the middle of the long night period.
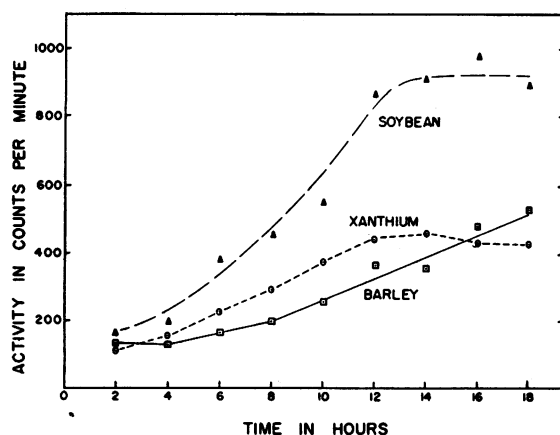

FIG. 2. Effect of previous photoinduction on the fixation of C^*O_2 in the dark by detached leaves of Wintex barley, Biloxi soybean and Xanthium during a single dark period. Each point represents an average of 3 separate composite samples taken from at least 4 detached leaves.

In order to establish more exactly the differences in CO_2 fixation by the species indicated in figure 2, it appeared of interest to compare the quantity of C^*O_2 fixed during the first and last halves of an 18 hour dark period. The results of such a study are shown in table II.

It can be seen that the barley leaves fixed more C^*O_2 during the second half of the dark period than during the first half, while the soybean and Xanthium fixed the bulk of the C^*O_2 during the first half. Such data essentially confirm those presented in figure 2, indicating that barley leaves fix CO_2 at an undiminished or increasing rate throughout the entire long dark period, while the short day species tend to reach a point of maximum fixation rate at about the middle of the dark period.

EFFECTS OF NIGHT INTERRUPTIONS ON CARBON DIOXIDE FIXATION: Since it is well known that small quantities of red light in the middle of a long dark period effectively destroy the photoperiodic effective-

TABLE III

THE EFFECTS OF RED LIGHT UPON THE C^*O_2 FIXED BY DETACHED LEAVES DURING THE FIRST HALF OF A LONG DARK PERIOD

TREATMENTS	RADIOACTIVITY IN COUNTS PER MINUTE *		
	BARLEY	SOYBEAN	XANTHIUM
8½ hrs dark C^*O_2	277	450	319
8½ hrs dark C^*O_2 + 1 hr red light	264	258	176
8½ hrs dark C^*O_2 + 1 hr dark	163	311	259

* Each figure represents an average of at least 6 leaves.

ness of such dark periods, it appeared of interest to determine the effect of such night interruptions upon the fate of the C^*O_2 fixed during the first half of a long dark period and upon subsequent fixation in the second half of the dark period.

Excised leaves of barley, soybean and Xanthium were exposed to C^*O_2 for a dark period of 8.5 hours; and one third of these was then killed and their activity measured; another third was exposed to red light in the presence of unmarked CO_2 for a period of one hour, and the remaining third was held in darkness for a similar time period under a similar atmosphere. The results obtained are shown in table III.

It appears from these data that red light has a stabilizing influence upon the CO_2 fixed by the barley leaves in the preceding darkness. The opposite appears to be true for the soybean and Xanthium; red light caused a much more rapid disappearance of the C^*O_2 previously fixed when compared to those leaves which remained in darkness for the same length of time.

Further studies were conducted to determine the

TABLE IV

EFFECT OF RED LIGHT ON THE FIXATION OF C^*O_2 BY DETACHED LEAVES IN THE SECOND HALF OF A LONG DARK PERIOD

TREATMENT	RADIOACTIVITY IN COUNTS PER MINUTE *		
	BARLEY	SOYBEAN	XANTHIUM
8½ hrs dark + 1 hr dark + 8½ hrs dark C^*O_2	88	253	107
8½ hrs dark + 1 hr red light + 8½ hrs dark C^*O_2	155	115	87

* Each figure represents an average of at least 6 leaves.

effect of night interruption upon the fixation of CO_2 in the second half of a long dark period. Excised leaves of barley, soybean and Xanthium were exposed to a period of 8.5 hours of dark in ordinary air. One half of these leaves was then exposed to red light while the other half remained in darkness. At the end of one hour all the plants were exposed to $C*O_2$ for a period of 8.5 hours following which their activities were recorded. The results as shown in table IV indicate that in the case of barley, previous treatment with red light promoted $C*O_2$ fixation in the following dark period, while the soybean and Xanthium showed a decrease in dark fixation of $C*O_2$ when the leaves had been exposed to red light.

DISCUSSION

On the basis of the studies reported here, it is suggested that the dark fixation of CO_2 is an essential part of the dark reaction in the photoperiodic induction of the short day species tested. This suggestion is based upon the following evidence: a) carbon dioxide fixation does occur during inductive dark periods as shown by the experiments with $C*O_2$; b) inductive dark periods are essentially ineffective in the absence of CO_2; c) night interruption treatments strongly bring about a reduction in the amount of CO_2 fixed.

Because it is essentially impossible to remove from the leaves all of the CO_2 formed by respiration, it can hardly be expected that the "CO_2-free" treatment entirely deprived the leaves of CO_2. The fact that a few of the short day plants did differentiate some flower primordia in that treatment may be accounted for on the assumption that some respiratory CO_2 was available within the leaves. It is likely that the quantity of CO_2 released in respiration is much greater than that fixed metabolically, as has been demonstrated in microorganisms (1).

Floral induction in the long day species—barley—was only slightly inhibited by a CO_2-free atmosphere during the dark periods as might have been expected since it is well known that many long day species initiate flowers in continuous light (10).

There are several striking analogies between the physiological factors which influence the inductive dark period of Xanthium and soybean and the factors which influence the dark fixation of CO_2. Each of the processes is identified with darkness, of course. The effectiveness of a photoinductive dark period is dependent upon the intensity and duration of the preceding light (6), and the dark fixation of CO_2 is similarly dependent (12). Young mature leaves are commonly the most responsive to photoinduction (3), and the same leaves are most active in CO_2 fixation (12). Moderately cool night temperatures are most effective for photoinduction (11) and are most promotive of CO_2 fixation (2).

It is of interest that night interruption treatments had a two-fold effect on the dark fixation of $C*O_2$ in Xanthium and soybeans. First, they promoted an actual loss of $C*O_2$ fixed in the preceding

part of the dark period and second, they lowered the rate of fixation in the following part of the dark period. Exposure to red light would thus necessitate a longer dark period for maximum fixation, a situation analogous to inhibitory effects of night interruptions upon the photoperiodic effectiveness of both the preceding and the subsequent portions of the long dark period recently demonstrated by Carr (5) and by Hamner (7).

The opposite effects of night interruption upon the CO_2 fixed by barley and by Xanthium and soybean closely parallel the effects of these treatments upon respiration of the same two groups of plants reported by Leopold and Guernsey (9).

The non-photosynthetic fixation of CO_2 has attracted wide attention from plant and animal biochemists in recent years, but principally for its biochemical interest. The present study suggests that this reaction may play a specific and essential role in the developmental process of some plant species.

SUMMARY

Studies were conducted to determine the relationships existing between the fixation of CO_2 in the dark and photoperiodic induction in a long-day species, Wintex barley, and in two short-day species, Biloxi soybean and Xanthium commune. The results obtained are as follows:

1. Inhibition of floral initiation occurred when soybean and Xanthium were exposed to long dark periods which were maintained essentially free of CO_2. Similar conditions only slightly inhibited initiation of barley.

2. The photoinduction of leaves promoted the fixation of $C*O_2$ in the dark in all three species tested.

3. CO_2 fixation rates during a long dark period appeared to be different in barley than in Xanthium and soybean.

4. Night interruption treatments showed an opposite effect on $C*O_2$ fixation in barley as compared to Xanthium and soybean. Night interruption treatments promoted a loss of $C*O_2$ fixed in the preceding part of the dark period and lowered the rate of $C*O_2$ fixation in the following part of the dark period in Xanthium and soybean plants.

5. The fixation of CO_2 in the dark was suggested as a specific physiological factor in the photoperiodic induction of soybean and Xanthium.

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CHANGES IN FREE AUXIN CONTENT DURING THE PHOTOINDUCTION OF SHORT-DAY PLANTS [1,2]

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During the past few years considerable interest
has arisen as to the possible role of growth hormones
or auxins in flower initiation. In 1942 Clark and
Kerns (4) found that low concentrations of the auxin
naphthaleneacetic acid induced formation of an in-
florescence in the pineapple plant when applied as a
foliage spray. The litchi plant (13) has also re-
sponded to auxin treatment. However, other at-
tempts to induce flowering, especially in photo-
periodically controlled plants, have met with little
success.

Although auxin treatment of plants responding to
photoperiod will not in itself bring about flower initi-
ation, such a treatment may materially affect the
process. Leopold and Thimann (10, 11) found that
in barley, treatment with low auxin concentrations
definitely increased the number of flower primordia
while higher concentrations decreased the flowering
response.

In short-day plants, however, treatment with
auxins usually results in an inhibition of flowering.
Thurlow and Bonner (2, 15) inhibited flower initia-
tion in Xanthium by spraying the leaves with solu-
tions of indoleacetic acid and naphthaleneacetic acid.
Auxin treatment has also been shown to inhibit
flower initiation in the short-day plant Kalanchoë
Blossfeldiana (9) and petunia (7).

Considerable work has been done to induce flow-
ering with compounds that possess antiauxin activity.
Bonner and Thurlow (2) were able to suppress the
effect of auxin in inhibition of floral initiation with
2,4-dichloranisole. When this antiauxin compound
was used alone it hastened floral development. In a

later experiment, Bonner (1) found that the applica-
tion of 2,4-dichloranisole and triiodobenzoic acid to
vegetative Xanthium plants resulted in the initiation
of flower-like buds, although true flowers were not
obtained. Galston (5, 6) sprayed vegetative and
photoinduced Peking soybean plants with triiodoben-
zoic acid and noted that the photoinduced plants
showed a tenfold increase in the number of flower
buds formed. However, none of the vegetative
plants were induced to flower.

The results of these and similar experiments have
suggested that auxins may play a major role in the
flower initiation process. It has been suggested (3)
that flower initiation in short-day plants may be due
to a decrease in auxin. In order to test this idea,
the changes in free auxin content were followed dur-
ing the photoinduction period of short-day plants.

MATERIALS AND METHODS

Two short-day plants well known as to their
reaction to photoperiod, were used in the study of
auxin changes during photoinduction. These were
cocklebur (Xanthium italicum Moretti) and Biloxi
soybean (Glycine Soja (L.) Sieb. and Zucc.). Several
other plants were also used to determine the effect
of photoperiod on the auxin content of the plant.
These included Maryland Mammoth tobacco (Nicoti-
ana tabacum L.), stock (Matthiola incana (L). R.
Br.), calendula, zinnia, nasturtium and barley. After
germination the seedlings were transferred to 4-inch
pots. During their vegetative growth the plants were
kept under photoperiods that inhibited flowering. For
short-day plants this was accomplished by giving
supplementary light to provide day lengths of 16
hours. The plants were induced under short-day
conditions by placing them in a small dark room just
off the greenhouse each afternoon and returning them
to the greenhouse each morning. The induced plants
received an 8 hour photoperiod. At the same time a