THE REACTION CONTROLLING FLORAL INITIATION

BY H. A. BORTHWICK, S. B. HENDRICKS AND M. W. PARKER

UNITED STATES DEPARTMENT OF AGRICULTURE, PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND
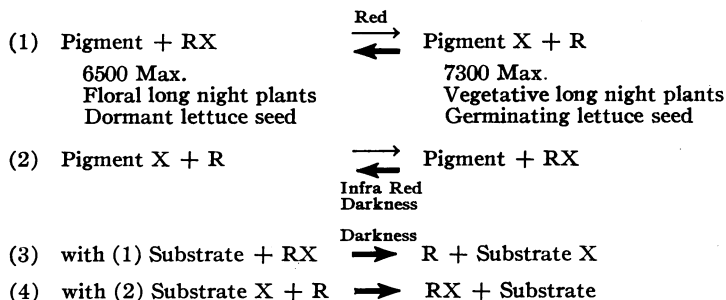
Communicated September 9, 1952

Reproduction in many plants and animals is controlled by the length of the night and the cycle of day and night. A dark reaction affords the measure of time and this reaction can be quickly stopped or reversed by light such as that from the rising sun. The reaction is possibly universal in living forms but its more extreme and evident manifestation is recognized to a rather limited extent. Thus flowering of cosmos is determined by long nights while, to casual examination, growth of petunia and tomato is independent of night length. Similarly, reproduction in some species such as goats, turkeys and snails depends on the length of night while the cow and man seem unresponsive.

Control of morphogenesis serves better as evidence for the photoreactions in plants. Equivalence of the morphogenic and reproductive controls can be tested by the details of the action spectra for the photoreaction. Thus is established the equivalence of the control for the size of a pea leaf, after it has developed somewhat in darkness and then is exposed briefly to light, with the control of floral initiation in soybean and in barley plants.[1] Control of bulbing in the onion or color of coat in the varying hare could be followed in a similar way.

An understanding of the control reaction, in contradistinction to establishing the equivalence of varied phenomena, came from the study of germination response of lettuce seed to red and near infra-red radiation.[2]

The reversible photoreaction and an accompanying dark reaction demonstrated for lettuce seed and extended in this study to control of floral initiation follow:

(1) Pigment + RX $\overset{\text{Red}}{\underset{}{\rightleftarrows}}$ Pigment X + R

      6500 Max.   7300 Max.
      Floral long night plants   Vegetative long night plants
      Dormant lettuce seed   Germinating lettuce seed

(2) Pigment X + R $\overset{}{\underset{\substack{\text{Infra Red}\\\text{Darkness}}}{\rightleftarrows}}$ Pigment + RX

(3) with (1) Substrate + RX $\overset{\text{Darkness}}{\longrightarrow}$ R + Substrate X

(4) with (2) Substrate X + R $\longrightarrow$ RX + Substrate

The nature of the pigments and the reactants RX and R are still to be established.

The purpose here is to present evidence that these reactions also control floral initiation of cocklebur (*Xanthium saccharatum* Wallr.), which is selected as representative of photoperiodically responsive plants. Inadequacies in previous work, chiefly by us, on action spectra for floral initiation have to be examined.

Radiation in the red when used to interrupt a dark period has been found to be the most effective portion of the spectrum for control of flowering for both long- and short-night plants. The maximum effectiveness is in the region of 6300 to 6600 Å which is also the region for maximum promotion of germination of lettuce seed. Radiation in the region of 7000 to 7600 Å hitherto has not been recognized as effective in control of flowering, but this region has been shown to be strongly inhibitory for germination of lettuce. This infra-red effect on seed germination gave us pause to think about the way in which the action spectra were measured for floral initiation. In all cases control was effected by a brief light interruption near the middle of each long dark period.   .

Description of an actual experiment with cocklebur is instructive. All leaves except one are removed from young plants. The plants are then placed on 12-hour dark periods and 12-hour light periods, which are adequate for floral initiation if used for one or more cycles. Usually two or three such cycles are used and the dark periods are interrupted with radiation in various wave-length bands between 4000 and 10,000 Å. The radiation is supplied with a two-prism spectrograph adequate in dispersion to irradiate a single leaf with a wave-length band of about 100 Å in the region of 5000 Å. After the dark-period interruption treatments are completed the plants are returned to 8-hour dark periods and 16-hour light periods to continue development. Whether the terminal meristem is floral or vegetative is ascertained 12 days later by dissection. Under these conditions cocklebur plants remain vegetative if not previously induced by long nights. The minimum energy required various wave-length regions for *suppression of floral initiation* is found by varying the irradiance. These measurements lead to the action spectra.

Radiation at wave-lengths greater than 7200 Å was found to be ineffective in suppression of floral initiation of cocklebur. There are two possible conclusions from such a result—namely: (1) radiation at wave-lengths greater than 7200 Å has no effect on floral initiation, and (2) it has the same effect as darkness. We were aware that the dark-period reaction was the one effective in measurement of time, but the second possibility did not occur to us.

If the cocklebur plants are irradiated, as described, first with unfiltered radiation from an incandescent-filament lamp adequate to prevent floral initiation as shown by controls and then on the spectrograph with radiation in the region, 7215 to 7450 Å, floral initiation occurs. The results

parallel those for lettuce seed germination.   Radiation in the region of
7300 Å thus is effective in controlling flowering of cocklebur but its action
is opposite to that of radiation at 6500 Å.   Radiation in the region of
7300 Å promotes flowering and thus has the same effect immediately on
the photoreaction as does a prolonged dark period.

    We have now measured the action spectrum in terms of incident energy
for *promotion* of floral initiation of cocklebur as described in the preceding
paragraph.   One set of results is given in table 1.   The maximum response
is in the region of 7215 to 7450 Å, which is closely equivalent to the results
for lettuce seed germination.   The same controlling reaction is operating.

    The previously measured action spectra for regulation of floral initiation

TABLE 1

EFFECT OF VARIOUS WAVE-LENGTHS OF RADIATION ON PROMOTION OF FLOWERING OF
Xanthium saccharatum PLANTS THAT HAD PREVIOUSLY RECEIVED RADIATION IN-
HIBITORY TO FLOWERING

WAVE-LENGTH, Å	INCIDENT POWER (ERGS × 10³/CM.²/ MIN.)	NUMBER OF FLOWERING PLANTS PER LOT OF 4 AFTER IRRADIATION FOR INDICATED TIME			
		16 MIN.	8 MIN.	4 MIN.	2 MIN.
6820–7020	87	0	0	0	0
7020–7215	93	1	3	0	0
7215–7450	99	3	3	3	1
7450–7700	102	1	1	1	0
7700–8010	105	0	0	0	0
8010–8300	111	0	0	0	0

TABLE 2

ENERGY REQUIRED TO CONTROL FLORAL INITIATION OF *Xanthium saccharatum* NEAR
THE MIDDLE OF A 12-HOUR DARK PERIOD

REGION OF IRRADIATION, Å	EFFECT ON FLOWERING	ENERGY (KILOERGS/CM.²)	
		EXPT. 12	EXPT. 16
6330–6560	Prevention	36	400
7020–7450[a]	Promotion	300	100

    [a] Previously irradiated adequately to prevent floral initiation as shown by controls.

thus depend upon the integrated absorptions of the two compounds,
Pigment and Pigment X.   They should be reexamined from this point of
view.   The various spectra while closely similar are not identical.   In
the red portion of the spectrum appreciable displacements from the action
spectrum for cocklebur are evident as might result from a different poising
of the photoreaction: The maximum effectiveness for each action spec-
trum is in the region of 6300 to 6600 Å, which is probably near the absorp-
tion maximum for one pigment.   The curves expressing incident energy
required for a given response of each species are parallel in the region of
5000 to 6000 Å but that for lengthening of a dark grown pea leaf[1] differs
by about 600 Å from that for suppression of floral initiation in soybean.

Effectiveness of red radiation in promoting germination of lettuce seed decreases after they have been imbibed with water in excess of 20 hours while infra-red radiation correspondingly becomes more effective in suppressing germination. This is one of the several evidences for the coupling of the two responses. Similarly for floral initiation of cocklebur, as sensitivity to inhibition by red radiation increases that to promotion by infra-red decreases (table 2). The cause for this variation is still being examined; it might be the age of leaf irradiated or some pecularity of treatment. Previous work with soybeans[3] indicated that the third compound leaf on plants that had expanded from compound leaves was the most effective one for control of floral initiation. Irrespective of cause the reciprocal variation is pronounced in this and other experiments.

TABLE 3

DEPENDENCE OF FLOWERING RESPONSE OF *Xanthium saccharatum* ON THE TYPE OF IRRADIATION DURING THE LAST THIRTY MINUTES OF THE LIGHT PERIOD

LENGTH OF DARK PERIOD, HOURS	NUMBER OF FLOWERING PLANTS[a] PER LOT OF 4 AFTER IRRADIATION AS INDICATED		
	RED	CONTROL	INFRA-RED
7.0	..	..	4
7.5	..	..	4
8.0	0	0	4
8.5	0	4	4
9.0	4	4	..
9.5	4	4	..
10.0	4	4	..

[a] All except two leaves were removed from plants used in this experiment. The fluorescent source for red radiation gave an illumination of 100 f.-c. at the leaf surface before passing through the red cellophane. Infra-red was obtained by filtering direct sunlight through a Corning red purple ultra filter. The control plants received radiation from an a. c. carbon arc supplemented with filament lamps, with a total illumination at the leaf surface of about 200 foot-candles.

Others who might wish to extend these observations and do not have the rather unusual spectrographic equipment can perform many experiments with radiation restricted to broad regions by choice of sources and filters. The effective red region relatively free of infra-red radiation is given by light from a 4500° white fluorescent lamp filtered by several layers of red cellophane. The effective infra-red region is rather precisely separated from the red region by use of a 5.0 mm.-thick Corning red purple ultra filter. An incandescent-filament lamp, the crater of a carbon arc, or the sun can be used as a source of radiation depending upon the intensity required.

Filtered radiation was used, as mentioned in the preceding paragraph, to perform an experiment in which the critical length of the dark period required for floral initiation of cocklebur was changed. The light period

for a group of vegetative cocklebur plants was extended for 30 minutes with either red or infra-red radiation. Various lots of these plants were then given dark periods of seven different durations from 7 to 10 hours at 30-minute intervals. Control lots were subjected to the same dark periods as those irradiated in the red. Red radiation applied at the end of the light period lengthened the critical dark period from 8.5 hours to 9 hours while infra-red shortened the critical dark period to less than 7 hours (table 3).

The experiment can best be understood in terms of the reaction given earlier in the article. During the light period the reaction is displaced to the right and in darkness it goes thermally to the left. The rate of this thermal reaction determines the effectiveness of the dark period. During the light period plants are usually subjected to both red and infrared radiation. The proposed reversible photoreaction is at neither extreme although closest to that given by red radiation. Thus red light at the start of the dark period displaces the reaction to the right, thereby lengthening the required dark period. Infra-red radiation at the beginning of the dark period immediately displaces the reaction to the left, and thus shortens the dark period required for floral initiation. Again, the experiment is one of the many evidences for coupling of the two reactions.

We have worked much with effects of radiation between 4000 and 5400 Å in the violet, blue, and green on these types of phenomena. Further experiments are required, however, in this region in view of these more recent advances to understand clearly the characteristics of the electronic transitions in the pigment molecules involved, and to untangle the absorptions of the two pigments.

Attention should be called not only to the two pigments but also to the reactants R and RX. All four might be expected to vary in plants as will be observed in time. In this variation is to be found the explanation for the distinctive photoperiodic responses of *Brassica rapa L.* and other Cruciferae[4] to blue and red radiations.[5] Will here also be found part of the explanation for dormancy of buds, biennial character, vernalization, "ripeness to flower," and many other phenomena of plant growth? Where will it lead with animals?

[1] Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Went, F. W., *Amer. J. Bot.*, **36**, 194 (1949).

[2] Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H., and Toole, Vivian K., PROC. NAT. ACAD. SCI., **38**, 662–666 (1952).

[3] Borthwick, H. A., and Parker, M. W., *Bot. Gaz.*, **101**, 806 (1940).

[4] Funke, G. L., *Vernalization and Photoperiodism*, Lotsya 1, Waltham, Mass., 1948, pp. 79–82.

[5] Growth responses to infra-red radiation by *Cosmos bipinnatus* Car., *Spinacia oleracea* L. var. Nobel, *Brassica alba* L. Boiss. and *Pisum sativum* L. have been described to us in private communication by Prof. E. C. Wassink and Dr. J. A. J. Stolwijk (Wagenin-

gen). These results are in course of publication. Some less striking effects of infra-red radiation on growth of Perilla have been reported by B. S. Moskov, *Akad. Nauk.. Doklady* **71**, 391 (1950).

ELECTRON EXCHANGE-POLYMERS*

By Harold G. Cassidy

STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT†

It was proposed in an earlier publication,[1] that it should be possible to prepare polymers which, in partial analogy to the ion exchange resins which are proton-exchangers, would be capable of electron-exchange. Such substances were prepared,[2] and although they were of low molecular weight,[3] nevertheless they showed some of the expected properties. This opened up the concept of a whole new family of polymers, the redox polymers, which can be used to conduct oxidation and reduction reactions by the same methods employed with the familiar ion exchange-resins. High molecular weight substances of this nature have been prepared and studied in electrometric titrations, and in countercurrent applications.

Vinylhydroquinone was prepared by reactions described previously.[2] Since the hydroquinone portion of the molecule inhibited the vinyl polymerization of the other group, leading instead to low-molecular weight products, it was necessary to protect the hydroxyls. This was done satisfactorily by acetylation or benzoylation. The resulting monomers, vinylhydroquinone diacetate and vinylhydroquinone dibenzoate, were also new substances, and their structures were established. When either of these substances was heated in toluene with 1 mol. % benzoyl peroxide smooth polymerization occurred with the production of high polymers. In one preparation, for example, a polyvinylhydroquinone dibenzoate with a number average molecular weight of 52,000 (*ca.* 160 monomer units) was prepared. It showed a saponification equivalent of 180; that calculated for the ester is 172. Copolymers were also prepared. Thus a 1 : 1 molar mixture of vinylhydroquinone dibenzoate with styrene, as 20% solution in toluene, with benzoyl peroxide catalyst yielded, on heating at 85° for 72 hours a polymer with a number average molecular weight of 41,300 (about 200 monomer units on a 1 : 1 basis). It showed a saponification equivalent of 242. That calculated on a 1 : 1 basis is 224.

The ester groups were subsequently removed by saponification with sodium ethylate solution in the absence of oxygen. The resulting free hydroquinone polymers and copolymers were obtained following purification and freeze-drying as fluffy white powders. They tended, with one