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CURRENT PROBLEMS IN RESEARCH

Photoperiodism in Plants

Growth is controlled by light and the measurement of night length through reversible reactions of a pigment.

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Flowering of plants depends upon the length of the night. Barley, wheat, and many other small grains bloom in early summer in response to short nights, while the later-maturing maize, soybeans, and chrysanthemums are induced to bloom by the longer nights of midsummer and autumn. This control of flowering is one of the methods of adaptation of species by which an unfavorable season is anticipated. It implies a time-measuring system that distinguishes between light and darkness through mediation of a pigment. Ways of finding the pigment and explanations of some of the features of seasonal response are described in this article.

First we give a partial explanation of the control mechanism, as a guide to understanding the seemingly odd methods used to find the explanation of seasonal response. The pigment, now called phytochrome, is a blue or a bluish-green protein that exists in two forms interconvertible by light, thus,

$$P_{660} \underset{730\ m\mu}{\overset{660\ m\mu}{\rightleftharpoons}} P_{730}$$

with 660 and 730 mμ the absorption maxima of the two forms. Form P_{730}, which is enzymatically active, changes in darkness to the inactive form P_{660} in the course of some hours, and the rates of the change and of the enzymatic action are essential factors in the plant's measurement of night length. The enzymatic reaction controlled by P_{730} also affects many aspects of plant growth besides flowering and results in a general control of growth by light. Phytochrome is present to the extent of about 1 part in 10 million in many plant tissues—an amount too little to give a noticeable color to leaves or stems of albino plants.

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Discovery

Photoperiodism as a control of flowering was discovered in 1918 by Garner and Allard (*1*). Their first observations were on a variety of tobacco induced to flower by the combination of a long night and a short day. Garner and Allard soon found the control in many kinds of seed plants and discovered that some varieties are responsive to long nights, others to short nights. At the time, these findings had a very great impact upon students of plant growth, who had widely held, without serious questioning, that the seasonal controls must depend upon the obvious changes in temperature. Garner and Allard also pointed out the close similarity in seasonal responses of animals and plants and suggested, on the basis of its general features, that bird migration, too, is photoperiodically determined, as was later shown to be the case for several species.

Germination of many kinds of seeds also depends upon light through the mediation of phytochrome (*2*). The need for light was recorded by Caspary in 1860 for seeds of *Bulliarda aquatica* (L.) DC. (= *Tillaea aquatica* L.) and was widely studied for many kinds of seeds in the ensuing century. In nature, the light requirement, which can be just a fraction of a second of sunlight, aids in preserving the species by insuring the prolonged dormancy of a store of seeds held in darkness through accidental covering with soil. This retention of viability by seeds is a plague to farmers and gardeners who expose them to light in cultivating, to germinate and grow as weeds.

The changes in length of stems, leaves, and other plant parts which occur in plants grown in subdued light or darkness, which are other manifestations of the action of phytochrome, must have been known to primitive man. In nature, the shoot from a deeply planted seed elongates until the food reserves are exhausted or until it reaches the surface and is exposed to light, which inhibits further lengthening. Plants growing in darkness are long and limber, but given just a little light, as from a 50-watt lamp at 1 meter for 1 second, they will be shorter and will stand upright. In 1929 Robert Bridges, the poet laureate, was moved to write in his *Testament of Beauty* (*3*):

> and haply, if the seed be faln in a place of darkness
> roof'd in by men—if ther should be any ray or gleam
> how faint soe'er, 'twil crane and reach its pallid stalk
> into the crevice, pushing ev'n to disrupt the stones.

Possible interrelations of these several responses and of autumnal leaf drop, orientation of leaves in darkness, and root enlargement and bulb formation were not generally suspected, although the possibility of such interrelationships was apparent to Garner and Allard, who were also aware of many varied displays of photoperiodism in both plants and animals. Knowledge of each response was at first restricted to its occurrence among plant species

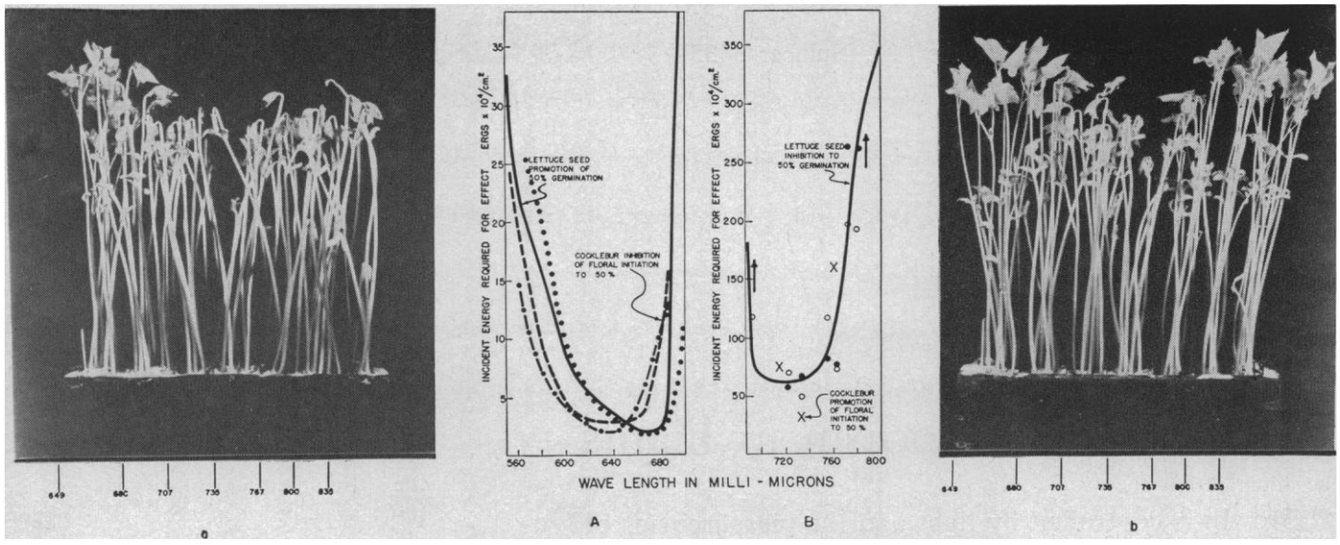

Fig. 1. Effects of radiation in the region of 560 to 850 mμ on plant growth. (*a*) Effects of a short period of irradiation in the region of 650 to 850 mμ on stem elongation, plumular hook unfolding, and leaf expansion in dark-grown red kidney bean seedlings. (*A*) Action spectra for a short period of irradiation to promote germination of lettuce seeds and inhibition of floral initiation in cockleburs: (dotted curve) enhancement of elongation of a pea leaf (by 45 percent); (dot-dash curve) the promotion of flowering in barley. (*B*) Action spectra for inhibition of germination of lettuce seeds and enhancement of floral initiation in cockleburs. (b) Effects of irradiation on dark-grown red kidney bean seedlings under conditions similar to those of (*a*) except that plants were first exposed to radiation in the region of 660 mμ prior to the short exposure across the spectrum.

and was systematized in works restricted to one type of response. Interest in possible causes for the control of growth by light was almost entirely speculative because of lack of experimental leads and of the rather natural tendency of observers to concern themselves more with the display—the flowering or the germination, for example—than with the causes. The first stirrings toward understanding actually came quite early, from findings on the dependence of etiolation upon light intensity (Batalin, 1871) and color (Vogt, 1915). By 1952, red light was known to be most effective in influencing most of the responses (*4*).

Action Spectra

Knowledge and understanding of the causes of the several responses have come from measurements of action spectra. The direct result is the finding that all the responses depend upon radiant energy and wavelength in the same way. Barley and cocklebur, representatives of plants requiring short or long nights, respectively, for flowering, have identical action spectra for the opposite responses of flowering in the former and inhibition of flowering in the latter. Although the control of flowering is not fully understood, these action spectra indicate that the mechanism is identical for the two types of plants. An even more surprising result is that this identity of action spectra is found in studies of control of stem lengthening and seed germination (Fig. 1) (*4*).

The action spectra have two parts, one for potentiating a response and the second for nullifying it (*2, 5*). Radiation in the region of 540 to 695 mμ, with a maximum near 660 mμ, potentiates the flowering of barley, the germination of lettuce seeds, the enlargement of a pea leaf, the suppression of flowering in cocklebur, and the inhibition of lengthening of the pea stem. These potentiated responses are reversed before actual responses can occur by radiation in the region of 695 to 800 mμ. The reversals, which can be repeated many times, and the near identities of the action spectra for both promotion and reversal of the various responses on an *absolute* scale of incident energy, indicate an action of light that affects many aspects of plant growth.

The identity of the action spectra has several interesting corollaries. The action depends upon the extent of the interconversion of the phytochrome forms, and, in fact, the length of a bean stem can be controlled by applications of radiant energy (Fig. 2). If the light is intense and of mixed wavelength (white), like sunlight, an equilibrium at an intermediate pigment conversion is soon attained, the position depending upon the energy distribution in various spectral regions of the source but not upon the intensity. The pigment system can be driven to the same position by light from a flashlight or by full sunlight, with similar effects upon growth. Because the pigment changes form, conversion cannot be more than complete. Radiation from a red or a far-red source, which drives the interconversion toward completeness in one direction, can be counteracted by a low reversing irradiance.

At this stage of understanding, which was reached by 1952 (*2, 5*), the objectives of isolating the pigment and of finding its mode of action seemed attainable. Progress toward objectives of this type usually depends upon development of a bioassay. But the probability that the pigment was a protein made reintroduction of extracts into living plant tissue seem unpromising. A more promising approach was to attempt to detect the pigment in vivo by physical methods which might be used for assay. The first approach was an attempt to obtain fluorescence of light, which can be detected at extremely low intensities, but none was found. Another approach was to search for plants with a high concentration of phytochrome, as might be evident from a blue or a bluish-green color of albino or etiolated tissue in which only small amounts of obscuring chlorophyll are present. Results again were negative, as were the re-

sults in studies to determine whether the pigment might be related to some known type of biologically active compound such as the bile pigments and the pigments of blue-green algae.

Physiological Characteristics of Phytochrome Action

Continued physiological studies were more encouraging. These indicated that the absorption coefficients of both forms of phytochrome and the relationship between their degree of interconversion and their physiological response could be found from the reversibility effect of light (*6*). In a first-order reaction—indicated by a measured temperature coefficient of 1.0—the rate of change of pigment concentration [*P*] with incident energy *E* (einstein units/cm^2 or 6.02×10^{23} quanta/cm^2) is

$$\frac{d[P]}{dE} = -k\ [P_o]\ (1 - F)$$

where *F* is the fraction of the pigment converted and is given by $F = \alpha\ \phi\ \chi \cdot E_{\text{incident}}$.

In the last expression P_o is the amount of phytochrome in a square centimeter of the test object, α is the molar absorption coefficient, ϕ is the quantum efficiency of the change, and χ is the fraction of incident light reaching the pigment. If the change is reversible, a similar first-order differential equation expresses the reverse change, and the two equations can be solved to give the degree of pigment conversion that corresponds to various degrees of physiological response and the value of $\alpha\ \phi\ \chi$ for the two pigment forms. In this way, α was found to be of the order of 10^7 square centimeters per gram molecule for both forms of phytochrome; this means that both forms are as intensely colored as chlorophyll and most dyes.

The small degree of change (of the red-absorbing form P_{660} to P_{730}) required to produce half saturation of the stem-lengthening responses of the pinto bean and leaf lengthening of peas indicated that P_{730} is probably the physiologically active form and is an enzyme and, accordingly, a protein. That P_{730} is an enzyme had first been suspected from the fact that many seeds in which P_{660} is present can lie in the soil for years without germinating and ultimately without respiring. Exposure to light for a few seconds, which changes P_{660} to P_{730}, causes resumption of respiration and leads to germination. The facts that most seeds that require light to germinate are small, implying reserves of fat, and that one of the first evident changes in the germinating seeds is the conversion of fat to starch give additional support to the supposition that P_{730} is an enzyme and possibly one involved in fat conversion.

The requirement for light in the reddening of apples had been known for centuries to some horticulturists, who used light to deepen the color of the fruit and to apply designs with masking stencils. The effective regions of the spectrum, however, were not known even as late of 1956. Upon study, synthesis of anthocyanin, the red pigment in the apple skin and many other plant tissues, was found to take place with light in the region of 550 to 750 mμ (*7*, *8*), although the action spectrum was not limited to this region. The amount of anthocyanin ultimately formed was linearly dependent upon the radiant energy after an induction period of one or two hours. In several objects (milo seedlings, for example) formation of anthocyanin can be reversibly controlled for several hours after being potentiated. The action spectrum for the reversal is identical with that for photoperiodism, and the energy requirements for half conversion of phytochrome and for the reversal are also identical. The high energy requirement for the initial potentiation, however, could not be explained until the action spectrum was found to depend upon a combination of the absorption spectra of the two forms of phytochrome in the spectral region where both forms absorb, as is particularly evident for red cabbage seedlings (Fig. 3) (*7*). From this knowledge the concentration of phytochrome in the seedlings could be estimated; it was found to be of the order of $10^{-7}M$ in a favorable object. It was possible to make the estimate because the anthocyanin formed by the action of a measured amount of incident radiation could be extracted and the amount (in gram molecules) established by analysis.

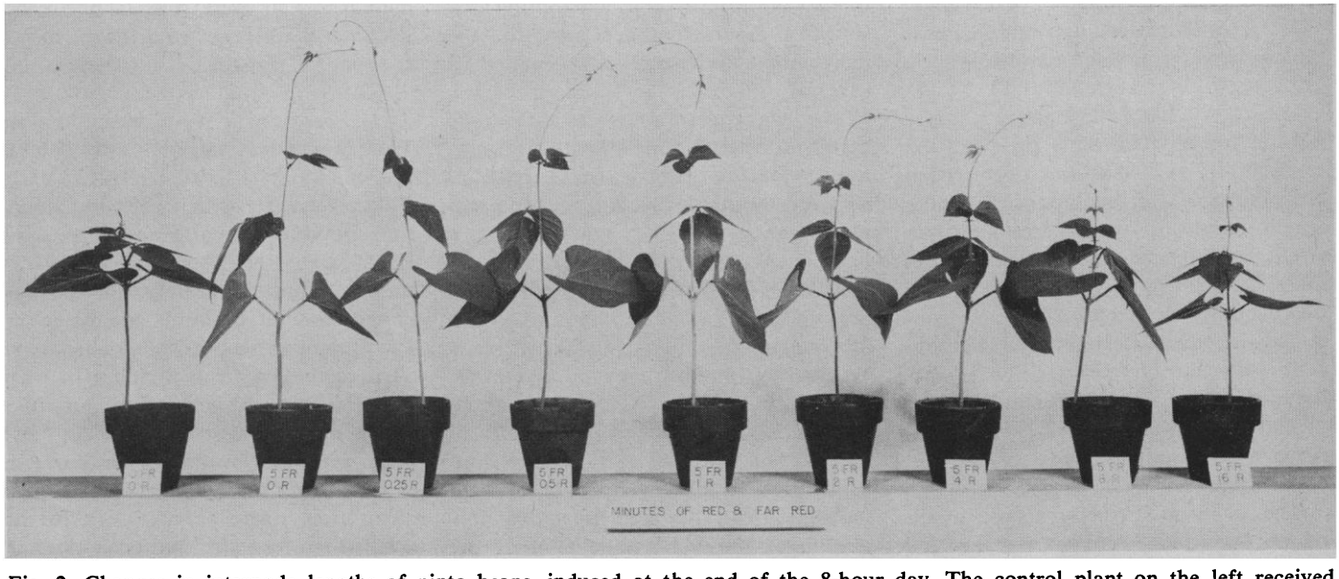

Fig. 2. Changes in internode lengths of pinto beans, induced at the end of the 8-hour day. The control plant on the left received no supplementary radiation. The other plants were exposed to far-red radiation for 5 minutes and then to red radiation for 0, 0.25, 0.5, 1, 2, 4, 8, and 16 minutes, respectively.

Detection of Phytochrome by Differential Spectrophotometry

It was a logical and intuitive conclusion from the experimental observations upon growth, germination, and flowering that phytochrome could be detected in tissue by "adequately" sensitive spectroscopic methods. The difficulty, however, was with "adequately," for the method would have to be sensitive to a change in the light transmitted by the tissue of the order of one part of the incident light in 10^6 parts, and it would have to be applicable to highly scattering media. K. H. Norris and his associates, of the Agricultural Marketing Service, U.S. Department of Agriculture, designed and built a simple differential spectrometer having the required sensitivity, for use in measuring the ripening of fruits (*9*). The instrument is similar in principle to the double-beam differential spectrometer developed by Chance (*10*) for following the changes of respiratory pigments in living tissue. Through use of the instrument of Norris *et al.*, values of Δ in Δ (Δ O.D.) = [(O.D.$_{660}$ − O.D.$_{730}$) after irradiation in the region of 660 mμ] − [(O.D.$_{660}$ − O.D.$_{730}$) after irradiation in the region of 730 mμ] can be obtained as phytochrome is changed from P_{660} to P_{730} or from P_{730} to P_{660} (*11*). An adequately stable single-beam spectrophotometer suitable for use with highly scattering media also was available. The change in optical density in shoots of dark-grown seedlings of maize with wavelength of light as phytochrome is changed in form is shown in Fig. 4*a* (*11*). This curve reflects the features evident in the several action spectra for control of growth (Fig. 1).

We now had the desired assay method for phytochrome, provided the reversibility with light was effective on broken tissue. Fortunately, clear aqueous solutions of extracted nonparticulate cytoplasm of etiolated maize responded reversibly. Protein fractions salted out of the aqueous extracts contained the reversible pigment. The concentration of phytochrome has now been increased to more than 20 times that of the first extract by H. W. Siegelman, who used methods of protein chemistry.

The differential spectroscopic method can be used directly for estimating the concentration of phytochrome in living tissue having low chlorophyll concentrations. Phytochrome is detectable, for example, in cauliflower and artichoke florets, in avocado and Zucchini squash fruits, and in etiolated tissue of many seedlings of grasses and cotyledons of several members of the cabbage family. It has been detected in extracts of spinach leaves, but the reversible change was not detected in extracts of cocklebur and soybean leaves known to be photoperiodically responsive.

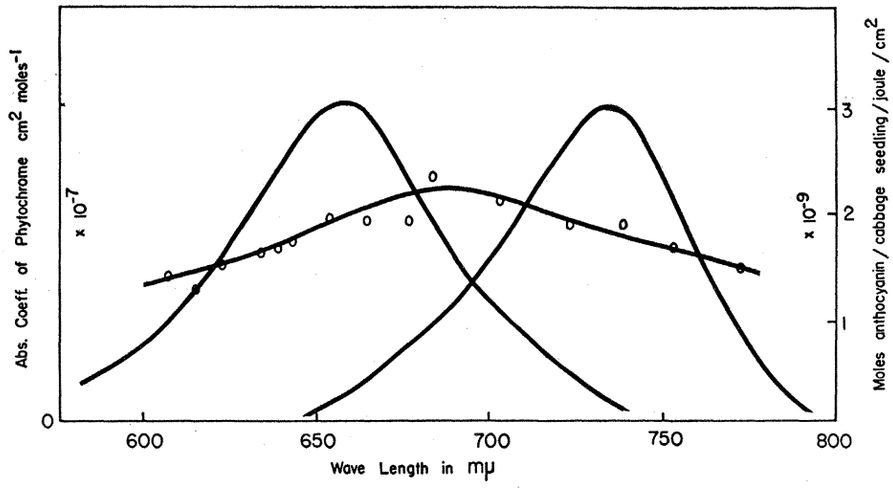

Fig. 3. Variations with wavelength of molecular extinctions of the two forms of phytochrome and the action spectrum for the formation of anthocyanin in red cabbage.

The separated phytochrome is the active principle of photoperiodism and related plant-growth phenomena controlled by light. The properties in solution are those foretold by the physiological responses. In solution, however, neither P_{660} nor P_{730} undergoes spontaneous change to the other form. As would be anticipated, the extracts, even though photoreversible, apparently lack some of the factors necessary for the enzymatic activity of P_{730} with which its reversal in darkness to P_{660} is associated.

The entry of phytochrome into so many aspects of plant growth indicates that P_{730} is an enzyme for a reaction common to many reaction sequences in plants. In a sense, it may control a "bottleneck" through which much of the material for plant growth must pass. Several clues as to the region of action are evident from effects upon seed germination and anthocyanin synthesis, but a further one is most telling. Apple skins produce ethanol in darkness from sucrose as a substrate (*12*). The ethanol production is stopped by light of high intensity, with the accompanying formation of the red anthocyanin of the skin. This indicates that the essential light reaction controls the fate of a two- or three-carbon compound, permitting its passage either to ethanol or, through condensation, to an aromatic compound.

A reasonable, but entirely speculative concept, is that the reaction is closely associated with reactions of acyl coenzyme A compounds which are known to be essential for fat utilization and formation (seed germination), the operation of the Krebs cycle, and anthocyanin and sterol syntheses. In fact, regulation of acetyl coenzyme A levels is an ideal control for growth because more than three-fourths of the carbon of a plant is incorporated in acetyl coenzyme A at some stage of passage (*13*). The purpose of these comments, which are speculative, is to indicate that a single specific type of reaction, not too difficult to demonstrate experimentally, can well lead to the many spectacular displays of growth control by light.

Time Measurement

The way in which plants measure time in darkness can now be outlined. Essential to the discussion is the fact that, in plants irradiated with red light at the onset of darkness to change any P_{660} present to P_{730}, the phytochrome reverts to the P_{660} form within 4 hours of darkness. In the several plants upon which measurements were made, either physiologically by following flowering or physically from the change in light absorption, reversion of P_{730} to P_{660} has a half-life of about 2 hours. If the half-life is constant, P_{730} will be reduced to $(\frac{1}{2})^5$, or to 3.1 percent, of its initial activity after 10 hours in darkness. This

is the approximate critical length of night for control of flowering of plants requiring either long or short nights for induction.

While change of P_{730} to P_{660} in darkness is essential for flowering of photoperiodically responsive plants, it is not the only controlling factor. The metabolic reserves also are time-dependent. These reserves are built up as simple sugars, polysaccharides, starch, and fatty acids through photosynthesis during the day and are utilized at night in systems of reactions, including those controlled by the P_{730} form of phytochrome. In short, the decreasing amount of P_{730} depends for action upon reserves that decrease with time.

Endogenous rhythms are a third pertinent feature of change in plants and animals during darkness. These rhythmic changes have been studied extensively by E. Bunning, of the University of Tübingen, who has implicated them in the timing of photoperiodism (*14*, *15*). They have been the subject of a recent symposium on "Biological Clocks" (*16*), in which their importance for time sensing by animals was emphasized. A feature of an interconnected system, be it mechanical, hydraulic, electrical, financial, or biochemical, is that a disturbance of input tends to produce oscillations of the output. The frequencies of the oscillations depend upon coupling constants of the system and the degree of entrainment by the disturbance.

The rhythms of biological objects, which are referred to as "circadian" rhythms, generally complete a cycle in about 24 hours. This cycle length is

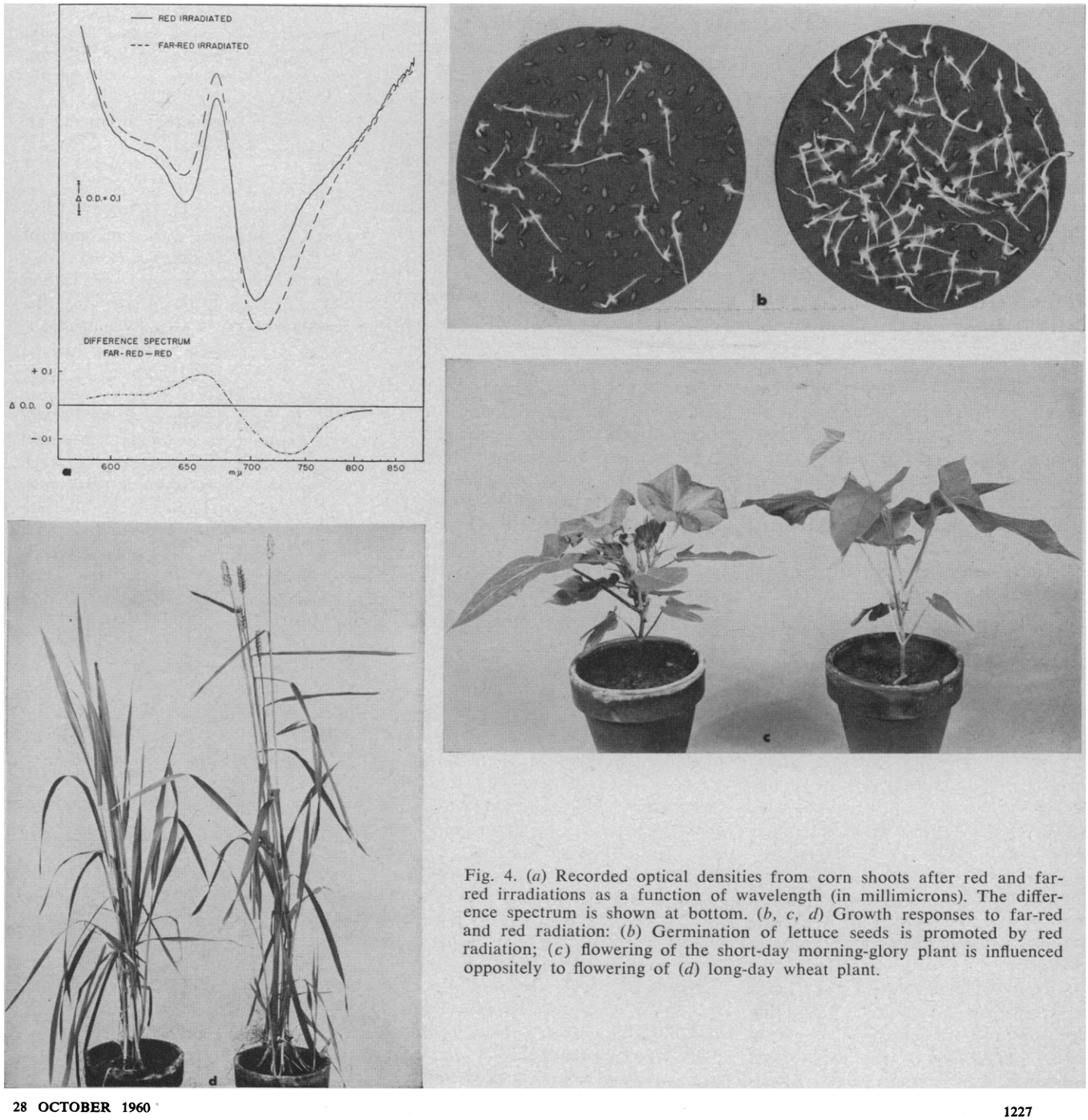

Fig. 4. (*a*) Recorded optical densities from corn shoots after red and far-red irradiations as a function of wavelength (in millimicrons). The difference spectrum is shown at bottom. (*b*, *c*, *d*) Growth responses to far-red and red radiation: (*b*) Germination of lettuce seeds is promoted by red radiation; (*c*) flowering of the short-day morning-glory plant is influenced oppositely to flowering of (*d*) long-day wheat plant.

indicative of the evolutionary origin of the process, locking together the rhythm and the change of day and night. The activity cycles of animals in continuous darkness indicate that the periods of the cycle are endogenous, or "free-running" (*15*). The degree to which the rhythms are "free-running" in plants, however, is difficult to assess.

In plants, the initial disturbances upon entering darkness are so great and the times of marked change are such large fractions of the dark period as to cause entrainment of endogenous periods. Only after the entrainment is lessened can the endogenous rhythm be clearly evident. Data on periodic leaf movement and flowering control in long periods of darkness suggest that this lessening of the initial disturbances reaches critical values only after about 16 hours, a period longer than the natural night in regions where most plants grow.

Another finding, the significance of which is by no means clear, is that photoreversibility of flowering is lost within less than an hour after change of P_{660} to P_{730} near the middle of normal dark periods effective for the control of flowering. In extreme cases, illustrated by the flowering of lamb's-quarters and young Japanese morning-glory plants (*17*), radiation in the region of 660 mμ is effective in producing a response which is not reversed by radiation in the region of 730 mμ. The suggestion is that conversion of P_{730} is quenched, possibly by association with the substrate upon which it acts enzymatically and to which energy is transferred in the course of anthocyanin synthesis.

Temperature changes in the environment also influence many aspects of plant growth, as has been emphasized in particular by F. W. Went of the Missouri Botanical Garden (*18*). That all components of change in an interconnected system should be temperature-dependent is expected. The change in output, however, can be compensated by interconnections of components to achieve an approximate constancy, once the transient of initial change has passed. This is in accord with both the slight change of endogenous rhythms with temperature and the induction of many growth responses by temperature change. An illustration of the control is afforded by the germination of many seeds which require both variation of temperature and exposure to light.

The emphasis placed on the reasons for responses to light has diverted attention from many displays of photoperiodism and striking controls of growth. One of the displays is the dormancy of terminal buds of trees and other woody plants, which affects annual growth and eventual form. The cessation of growth is usually induced by long nights. It can sometimes be broken by a return to short nights but often requires removal of leaves and a period of low temperatures. These features of change within the plant can be understood as synchronization with the seasonal change of the environment in temperature and length of night.

Animals also are photoperiodic for reproduction, migration, and dormancy. Comparisons of descriptive aspects of photoperiodism in plants and animals show many striking similarities. Some workers hold that endogenous rhythms are the basic common causative feature. In keeping with the developments discussed above, however, the working hypothesis is that a common reaction may underlie the endogenous rhythms and the photoperiodic responses. A need exists both for many more studies of plants and for studies of cause rather than display in animals. Even if causes for plants and animals prove to be unrelated, it would be interesting to find in what ways similar ends are achieved.

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