THE

LIFE PROCESSES

OF SEEDS
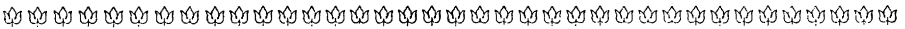
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Light, Flowering, and the Production of Seed

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ONE MINUTE of artificial light each night holds back the flowering of certain plants, promotes the flowering of some, and has no measurable effect on others.

Our understanding of how we can use light to control flowering—a matter that has practical applications and a direct bearing on the organs in which seeds are produced—has grown in the past few years.

For countless generations people have known that external conditions influence the flowering of plants.

They knew, for example, that the flowering dates of fruit trees vary somewhat from year to year. They attrib-
uted this variation largely to seasonal differences in temperature.

They also knew, however, that despite these relatively small differences in time of flowering, their fruit trees always bloomed in early spring, their cereals in early summer, and many of their ornamentals, such as chrysanthemums and cosmos, in late summer or early autumn. These plants thus were able to recognize the onset of the various seasons and to synchronize their development with the change of season.

The mechanism by which certain plants are able to time the events of their lives in such a way that they always bloom at a particular time of year remained undetected until 1919.

In that year Dr. W. W. Garner and Dr. H. A. Allard, plant physiologists in the Department of Agriculture, discovered that Maryland Mammoth tobacco and Biloxi and other varieties of soybean, which normally bloom in autumn, could be made to flower in

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June or July by subjecting them to artificially shortened days and lengthened nights.

This discovery was one of the most significant advances in botanical science in this century. It marked the recognition of a hitherto unsuspected feature of the environment, the daily duration of light and darkness, as a most important factor regulating plant growth and development.

Dr. Garner and Dr. Allard called the phenomenon photoperiodism, an appropriate name because it recognized the importance of both a light-requiring (*photo-*) reaction and a time-measuring one (*period*) in the response.

Their discovery was confirmed promptly by countless investigators. The phenomenon was found to occur among many flowering plants.

Many kinds, the short-day ones, flower only when the daily light periods are short and the dark periods are long. Examples include numerous fall-flowering plants, such as cosmos, chrysanthemum, and cocklebur.

Others, the long-day ones, flower only when days are long and the dark periods are short. Sugarbeet, garden beet, spinach, wheat, oats, and barley are examples.

In still others, the day-neutral or indeterminate ones, flowering apparently is not influenced by daylength over a wide range of daylengths. Among such plants are tomato and many kinds of garden beans.

THE PROCESS by which plants form flowers is complex and takes time. One can measure the time in some plants by transferring them at a given moment from daylength conditions that prevent flowering to daylength conditions that lead to flowering.

The date of transfer thus gives us a known starting point, which is important because we cannot see the first steps of flowering. Under natural conditions, therefore, one never knows when the flowering process really starts.

We should keep in mind that before we can see any microscopic evidence of flower formation, some most important biochemical changes must occur to cause this shift from vegetative development to floral development.

It is to the initial causal reactions of this change that we give special attention. When we learn more about these first reactions, we should be in a better position to study the remaining steps in flowering.

The entire flowering process is completed in some species in several days. In others it may require many weeks or even months. We can recognize the earliest visible stages of flower formation in a soybean or cocklebur 3 or 4 days after the plants receive short-day treatment if we examine the growing points with a microscope. In another week or more we might be able to see them without a microscope.

The start of flower formation in the chrysanthemum can be seen microscopically a week or 10 days after the beginning of short-day treatment. The flowers are not ready for harvest, however, until 8 to 10 weeks later, or more, depending on the variety.

In some plants, such as apple, the flower primordia are formed during the summer. They develop throughout the rest of the growing season, remain in the bud over winter, and open during the following spring. The entire process takes 8 or 9 months.

We know that the entire process of flower formation and in some instances the formation of fruit and seed are influenced profoundly by daylength.

In some plants, however, the initiation of flowering is so clearly under control of the daylength reaction that we can advantageously restrict the observations to that step in the flowering process. For example, a cocklebur that receives only one short day in its entire life may flower.

The chain of reactions leading to flowering, once they are started by a short-day treatment, can proceed to completion in long days. This does not mean that these reactions might not go faster if more short days were given.

It permits us, for experimental convenience, however, to deal with this initial effect of light without becoming involved with the complexities of the many reactions that make up the flowering process itself.

THE MECHANISM by which light acts to control flowering received attention immediately after Garner's and Allard's discovery of photoperiodism.

Scientists soon learned that the day-length stimulus is received by the leaves and that its controlling action on flowering is transmitted in some way through the leafstalks to the growing points of the stem where the flowers are formed. They found they could bring about flowering in some short-day plants by subjecting a single leaf to short days even though all other leaves receive long days. That fact indicates that a flower-promoting stimulus is produced in the short-day leaf—not a flower-inhibiting one in the long-day ones.

Many workers have searched without success for a flower-inducing hormone in leaves of photoperiodically induced plants.

Because plants flowered on some photoperiods and not on others, it was evident that plants were able to measure time. Whether they measured duration of darkness or light, however, was not apparent until experiments were performed in which each long dark period of daily short-day cycles was broken into two short dark periods by insertion of a few minutes of light near the middle. The effect, which was equivalent to that of a treatment with long days and short nights, inhibited the flowering of short-day plants and promoted that of long-day ones.

The opposite kind of experiment, in which a short period of darkness was placed in the middle of a long light period, resulted in no detectable difference in plant response.

These experiments showed that the time measured was the dark period.

The effectiveness of the brief period of light during a long dark period depends markedly on whether the light period is placed in the middle of the dark period or elsewhere.

It has maximum effect if it is placed near the middle. It may have no observable influence on flowering if it comes near the beginning or end of the dark period.

Flowering of many short-day plants, such as soybean, chrysanthemum, and Japanese morning-glory, can be completely inhibited—checked and held up—by less than a minute of light of 25 to 50 foot-candles in the middle of dark periods at least 12 hours long. Long-day plants, such as barley and other small grains, are induced to flower by similar light treatments in the middle of 12-hour dark periods.

The discovery of this remarkable responsiveness of plants to irradiances of such brief duration and low energy suggested that a further way to investigate the nature of the light reaction would be to interrupt the dark periods with light of narrow wavelength limits and of known energies.

Such experiments done quantitatively show, for example, whether the photoperiod reaction depends on light absorption by chlorophyll or by some other pigmented substance that has light-absorbing characteristics different from those of chlorophyll. The method thus permits one to learn whether responses of plants to light that seem quite different superficially are controlled by the same photoreactions or by different ones.

The procedure involves measuring the minimum light energy required at each wavelength position to cause a particular response, such as promotion of flowering of long-day plants or inhibition of flowering of short-day ones.

Such experiments require special equipment to obtain light that is sufficiently pure and has enough energy to cause the plant to react. This is sometimes done by the use of light filters that permit passage of only the wavelengths desired, or it may be done by passing a strong beam of light through

a spectrograph, an instrument containing a system of mirrors and prisms arranged to produce a spectrum. Such an instrument, when illuminated with very high-intensity light from a carbon-arc or other high-intensity source, produces a spectrum of sufficient size that small plants or whole leaves of larger ones can be irradiated with enough light of the desired wavelength range to cause the plant to exhibit some developmental response that we can measure.

THE EFFECTIVENESS of the different colors of light was measured with such an instrument for several long- and short-day plants. The results were remarkably similar.

Red light of about 6,500 A (A= angstrom, a unit of wavelength of light equivalent to about one hundred-millionth of a centimeter) was found to be far more effective than any other color for inhibiting flowering of short-day plants and promoting flowering of long-day ones. Blue light of less than 4,400 A was slightly effective in some plants but far less than red. Other colors were less effective than blue.

These results proved that the photochemical reaction regulating flowering was the same in short- and long-day plants despite the fact that it inhibited flowering of the former and promoted flowering of the latter.

SCIENTISTS investigated also the action of light on some other responses of plants to learn whether the light reaction of photoperiodism might also cause these effects.

One such response was seed germination, the light relations of which are considered in a later chapter. Because some of the studies of seed gave new insight into the flowering reaction, we should consider a few findings here.

It was learned that the action of light in the control of germination is remarkably like its action in control of flowering. Red light promoted germination in seeds. Other colors had little or no promotive effect. This result

alone was strong evidence that the light reaction in photoperiodic control of flowering is the same as that for promotion of germination of seed.

Another important observation, however, which had special significance for studies of flowering, also came from the studies of seed germination.

Short-wavelength infrared, the so-called far red (7,000–7,400 A), was found to inhibit the germination of certain seeds that normally would germinate in the dark. This waveband also reinhibited the germination of seeds that had first been irradiated with red. These reinhibited seeds, moreover, could be immediately repromoted to germinate by another brief red treatment.

The germination results, in brief, showed that the promotive action of red on seed germination was reversed by far red and that the far-red inhibition in turn was reversed by red. Moreover, the results suggested that since opposing actions of red and far red were exhibited in germination they might also be present in photoperiodism.

The presence of red, far-red reversibility in flowering was tested in experiments with cocklebur, soybean, and other short-day plants.

The object of the experiments was to reinduce flowering in the plants by means of a radiation treatment immediately after they had received enough red light in the middle of the night to inhibit flowering.

The experiments were successful. Plants given far red after an inhibitory treatment with red that made control lots of the plants vegetative, flowered nearly as well as plants that received no light treatment during the night.

The discovery that flowering and seed germination were both reversibly controlled by red and far-red light was extremely strong evidence that the same basic photochemical reaction was involved in both responses.

Similar studies of the photoreactions regulating stem growth and leaf expansion (which are vegetative re-

sponses), production of anthocyanin, and several other light responses showed that these also were photoreversible by red and far red.

Photoperiodic control of flowering thus proved to be just one manifestation of a photoreaction that plants use to regulate many features of their growth and development.

Details of the reaction can be studied in the response that best serves a particular experimental purpose because the same light reaction is involved in all of these different phenomena. Thus some of our present knowledge of how light controls flowering has come from studies of how it controls seed germination and stem elongation.

DETAILS of the flowering reaction for which there had been no apparent experimental approach could now be investigated by making use of this characteristic.

For example, one could learn how quickly the flower-inhibiting action of red light actually takes place by starting it with a red treatment and then finding how long a far-red treatment could be delayed without loss of reversibility.

Such experiments required two light sources, one of red light and one of far red. A satisfactory red source consists of a fluorescent lamp equipped with a red cellophane filter to remove the light of wavelength shorter than 6,000 A. The fluorescent lamp is used instead of an incandescent-filament one because it emits very little far red. The resulting filtered light is, therefore, reasonably pure red.

A suitable far-red source is an incandescent-filament lamp filtered with blue and red cellophane. The cellophane filters remove almost all of the visible light but are transparent to far red, which is abundant in the radiation from such lamps.

One notes that a 1-minute treatment with red light in the middle of the night prevents flowering of a cocklebur plant, but that a far-red treatment after the red one is completed stops the flower-inhibiting reaction before it has actually gone far enough to interfere seriously with flowering.

If one delays giving the far-red treatment after the red treatment, however, he finds that after 30 minutes to an hour the far red no longer reinduces flowering. This shows that the flower-inhibiting reaction started by the red light does not go to completion immediately, but continues to operate for at least an hour after the light is turned off: Some product made by the red light persists therefore after the light is turned off and functions in some way to interfere with the reactions leading to flowering.

Flowering is much more completely reversible by red and far red in some plants than in others.

Flowerbuds reinduced in cocklebur by far red after an inhibitory treatment with red, for example, are often almost as large as those of untreated control plants.

In soybean the reinduced buds are less numerous and usually smaller than in the controls. In chrysanthemum the buds of reinduced plants frequently are only one-half to two-thirds the size of those of controls.

In *Pharbitis nil*, the Japanese morning-glory, reinduction of flowering by far red in the middle of the dark period fails completely. Seedlings of this plant only a few days old initiate flowerbuds readily when subjected to only 2 or 3 long nights. If irradiated with red in the middle of each dark period, however, flowering is prevented and is not reinduced by far red. However, weak reinduction of flowering by far red in older seedlings of *Pharbitis* that have developed true leaves has been observed.

The reasons for this variation in response to far red from one species to another or within the same species are not yet fully understood.

Repeated reversals of flowering response have been made in cocklebur, chrysanthemum, and a few other plants. In these experiments, a group of plants is subjected alternately in the

middle of each night to a brief treatment with red light followed by a brief far-red one, and so on for as many as four alternating treatments with each kind of light. At each step in the treatment one lot is withdrawn to darkness until finally the last remaining lot received its final far-red treatment. In these experiments, half of the lots flower and half remain vegetative. The flowering ones in all instances are the ones that receive far red last.

SCIENTISTS deduce the nature of the light reaction from results of these various experiments on flowering and of others on germination of seed, elongation of stems, and other phenomena that are controlled by the red, far-red reaction.

The response to red or far-red light occurs because the plant preferentially absorbs the energy of those wavelengths and transfers this absorbed energy to some chemical reaction. The response of a plant to red therefore requires the presence in the plant of a red-absorbing compound. Thus, without actually seeing it, one knows that a special pigment is present, because the plant responds to red light. He also knows the color of the pigment, because red-absorbing compounds are necessarily blue or green.

The amount of these pigmented compounds is very low, however, as can be shown in albino seedlings. The elongation of the stems of albino barley seedlings is regulated by the red, far-red reaction and is as effectively controlled in them as in the normal green ones. Concentrations of the pigment in the albino plants thus are adequate to control lengthening response but so low that the eye detects no color.

THE PIGMENT exists in two interconvertible forms, as shown by experiments on photoreversible control of flowering, germination of seed, and other plant responses.

When the plant is irradiated with red light, for example, the pigment molecules absorb some of the light and thereby are changed in structure. The changed pigment molecules have the property of absorbing far red very effectively. They are changed back to the original red-absorbing form when they are irradiated with far red. The pigment thus can be converted repeatedly from one form to the other.

One pigment form or the other apparently is a necessary link in the chain of chemical reactions leading to flowering, germination, and so on.

Evidence from experiments strongly suggests—but does not prove—that the active form is the far-red-absorbing one.

The active form, irrespective of which one it is, however, functions as an enzyme in a reaction that is still unidentified. Red light thus either activates or inactivates this enzyme.

If the radiation treatment activates the enzyme, the latter continues in the active form even though the light is turned off. Thus, in the prevention of flowering of such short-day plants as cocklebur or soybean, interrupting the dark period briefly with light completely inhibits flowering if the plants are allowed to remain in the dark for 30 minutes or more before they are reirradiated with far red.

DIRECT DETECTION of the pigment in plant material by chemical methods was not possible because of lack of knowledge of its biochemical reactions. The extremely low concentration of the pigment in the plant, moreover, made its detection by ordinary laboratory spectrophotometers improbable. Physiological experiments, which gave detailed knowledge of the pigment, however, indicated that a spectrophotometer of special design should detect its presence. Such an instrument, constructed at the Plant Industry Station for a purpose unrelated to this problem, was used successfully.

The pigment was found in several kinds of dark-grown seedlings. It was detected, in fact, in a single intact corn seedling, in which it proved to be most abundant in the uppermost part

of the elongating first internode and in the coleoptile. The reversibility of the pigment was still present after the seedling was fragmented and ground under proper conditions, and it remained in the liquid portion upon filtration. This meant that the scientists could now study the biochemistry of the reversible photoperiodic pigment.

WHERE DOES this kind of work with light on flowering lead us?

What is its promise in agriculture?

The long-range objective is more complete understanding of the growth and development of plants. This knowledge will help crop specialists develop more efficient methods of production.

We see that light plays a fundamental role in the regulation of flowering and the production of seed. It is important in germination. Light regulates the habit of growth of seedlings and the adult plants.

Moreover, we find that a single light reaction is concerned with each of these expressions of growth and development—probably with others that are still to be studied.

This reaction of plants to light thus gets right to the heart of the regulation of many aspects of plant growth.

IMPROVEMENTS in procedures of plant production came immediately from the discovery of photoperiodism.

One of the first uses made of the information was by cereal agronomists as early as 1922. They used supplemental light over small-grain crops to promote the flowering and fruiting of a winter-grown crop in the greenhouse.

They soon found that they could produce two successive greenhouse crops and still have time to grow a third crop in the field during the normal growing season. This procedure enabled them to complete programs of plant breeding in much shorter time than formerly.

In these first applications, the artificial light was turned on before sundown and continued for several hours to obtain a long-day response. The assumed necessity of following this procedure was based on the feeling that the light, not the dark, was the controlling time period of the daily cycle.

The discovery in 1937 that the reverse was true and that a brief period of light in the middle of the night was about as effective as continuous light from sundown until midnight resulted in modification of lighting procedures.

Agronomists and others found that a few minutes or an hour of light in the middle of winter nights promoted flowering of small grains, for example, as well as did the former method of prolonged lighting.

Florists also were quick to use light to extend the productive period of chrysanthemums. They, too, originally gave the supplemental light in conjunction with the daily period of natural light. Dark-period interruptions proved to be as effective as prolonged lighting, however, and avoided objectionable stem elongation that prolonged lighting often brings about.

Breeders of sugarbeets began almost at once to use supplemental light to induce flowering. They found that incandescent-filament lamps effectively induced flowering. The fluorescent lamps were almost without effect. This difference in response apparently was connected with differences in the wavelength composition of the two kinds of light—incandescent-filament light contains much more far red in proportion to red than does fluorescent light.

We now know that this response of beets is in some way a result of the red, far-red reaction.

The results with sugarbeets emphasize the importance of knowledge of the detailed effects of different wavelengths.

The most extensive commercial application of control of daylength in the United States is made by growers of chrysanthemums. They supply cut flowers in a range of varieties and colors throughout the year.

During periods when natural nights are long enough to induce flowering,

the growers use supplemental light to delay blossoming until the plant attains proper size and to time the harvest for dates of their choice. They bring the plants into bloom by discontinuing the light treatments several weeks before the desired harvest date, thus allowing the natural long nights to induce flower formation. The procedure is practiced widely in greenhouses and out of doors in places where winter temperatures are warm enough for chrysanthemums.

Several hundred acres of chrysanthemums were grown under lights out of doors in the United States in 1961.

During periods of the year when daily night length is too short to promote the flowering of chrysanthemums, the growers cover the plants with black cloth for a few hours morning or evening, or both, to create adequately long daily dark periods. This procedure is practiced in the greenhouse and sometimes out of doors.

The practices of the chrysanthemum growers are applied commercially but less extensively by growers of orchids, asters, tuberous-rooted begonias, *Kalanchoe blossfeldiana*, feverfew, and *Stevia*.

Poinsettia, a short-day plant, usually is grown in the greenhouse in periods of natural long nights. It would seem that no special attention need be paid to its daylength requirements. In practice, however, poinsettias are lighted during the second half of September and the first third of October. Lighting is then discontinued, and often the plants are given artificially lengthened dark periods for a week or two so that the flower-inducing reactions will begin promptly. After that, the natural dark periods are long enough to promote flowering.

Poinsettias are so sensitive to light that special care must be used to avoid low intensities, such as from the watchman's flashlight, a nearby street lamp, or passing automobiles, which would delay or inhibit flowering.

The use of artificial light on field crops presents difficulties.

Although it is used commercially to control flowering of chrysanthemums and a few other ornamentals, artificial light is not used in the commercial production of field crops. It is not probable that extensive field use will be made of it in the foreseeable future. The reason is that the cost of providing an extensive lighting installation makes it impractical.

Sugarcane, as grown in Hawaii and Puerto Rico, illustrates the problems. The yield of sugar is less when cane forms flowers because flowering stops the growth of leaves and stems. The further growth of the plant thus is restricted. Sugarcane begins to form flower primordia about the first of September—only at that time of year does the natural daylength become favorable.

Cane, moreover, is unusual in that it is unable to flower when the daylength is longer or shorter than these September days. Therefore it is unnecessary to use light for more than a period of 2 or 3 weeks in September to prevent flowering throughout the entire year. Light applied properly during this period is 100 percent effective in preventing the formation of flowers, and the amount of light needed each night is trivial. Nevertheless, the costs of an adequate lighting installation preclude use of this procedure.

One might therefore reasonably question the wisdom of devoting so much time and money to the study of flower control by light if the knowledge has so little promise of extensive field application.

KNOWLEDGE of the light reactions of plants does have practical applications in other than the direct use of light.

An important one is that it helps us find or breed varieties of crops that are adapted to the natural daylength conditions of an area. If we can do that, we do not have to try to change the daylength conditions of large regions to meet crop requirements.

Soybeans are grown extensively in

the United States, but no one variety is widely grown. Instead, certain varieties are restricted to comparatively narrow latitudes 75 to 100 miles wide. In areas either to the north or south, other varieties are sown because they are better adapted to the slightly different daylength conditions and they are therefore more apt to mature at the proper time and have greater yields.

It seems almost incredible that daylength differences such as occur between points only 100 or so miles apart north and south could cause measurable differences in plant response. An experiment with soybeans at the Agricultural Research Center at Beltsville, Md., however, demonstrated that this was indeed true.

The durations of natural light, including twilight, were calculated for each day of the growing season at Beltsville, and points in southern Virginia and central North Carolina.

Soybeans grown at Beltsville on these three artificially maintained daylength schedules matured at different times. Those of the southernmost schedule were significantly earlier than the middle one. The middle one was earlier than the northern one. The greatest difference in daylength between neighboring lots occurred on the longest day of the season and was only about 15 minutes. Since all of the lots were subjected equally to all other fluctuating environmental variables except daylength, the differences in maturing must be attributed to the effects of daylength.

Daylength influences the further growth and development of flowers after they are initiated, although I did not stress this point in the earlier part of this discussion.

One long night causes floral initiation in cocklebur, but repeated treatments with long night are necessary for more rapid development of the flowers.

Flowers of soybean plants often drop, unless the plants are given long nights until after the pods are set.

Initiation of flowers by red kidney bean occurs regardless of daylength, but at some temperatures daylength markedly influences the yield of beans.

In the blue-mist spirea, the visible flowerbuds form on any daylength but never open on long days. On short days, however, the young buds grow rapidly and the flowers open in about 3 weeks.

The effects of daylength may thus be expressed at any or all stages in the development of flowers.

THE FUTURE of our knowledge and understanding of the action of light in the control of flowering and many other features of plant growth and development is bright.

At the beginning of 1961, when I prepared this chapter, the photoreactive pigment had been extracted from dark-grown corn seedlings and held for several months without loss of photoreversibility. Its presence had also been detected in a dozen or more other kinds of plants, and initial steps in its purification had been made. Its complete purification and identification are expected, and with identification one hopes may come knowledge of the reaction catalyzed by its active form.

This work leads to understanding of a basic reaction controlling growth and development of plants, but the immediate objectives are not the solution of individual problems of plant production.

When the fundamental principles of light action on plants are understood, specialists will apply them intelligently to many production problems peculiar to their individual crops.

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