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CURRENT PROBLEMS IN RESEARCH

Physiology of Flowering

Flowering is hormonally controlled, but the nature of the hormone remains to be elucidated.

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The appearance of the first flower is an important event in the life cycle of a plant. After germination of the seed, the apical meristem initially produces only leaves. And then, abruptly, the bud produces, instead of more leaves, a new organ, a flower or an inflorescence. Although flowers, from a morphological point of view, are considered shoots with metamorphosized leaves, their structure and function are quite different from those of normal shoots and leaves. The function of the flowers is, of course, sexual reproduction and thus maintenance of the species.

In the last 50 years it has been firmly established that two factors, which vary with the seasons—namely, temperature and day length—play important roles in determining the flowering time of many plants. As a result, we have a new branch of the plant sciences—the physiology of flowering. The designation is in fact somewhat misleading. In general, only the processes which lead to differentiation of floral primordia are studied. The later stages, such as the differentiation of the individual parts of the flower bud and the growth of the flower, have received much less attention.

This article deals only with flowering processes as they are affected by day length. Many excellent reviews of the subject have appeared in recent years (see, for example, *1–5*). I make no attempt, therefore, to provide a complete survey, but emphasize new experimental developments and concepts.

Photoperiodism and Photoreactions

The response of plants to the relative length of day and night has been called photoperiodism. The term was originally coined for flowering responses, but today it is applied as well to vegetative responses such as effects on shoot growth and on dormancy. Flowering, as controlled by day length, is an inductive process. Exposure to a minimum number of favorable photoperiods results in subsequent flowering even if the plant is returned to a day length which of itself does not cause flowering.

The two best known groups of photoperiodic plants are the long-day and the short-day plants. These flower only under relatively long and short photoperiods, respectively (Fig. 1, *a* and *b*). Since 1950 investigators have become increasingly aware of the fact that certain plants have a dual day-length requirement for flowering. Such plants stay vegetative if grown continuously under long- or short-day conditions. They flower, however, if exposed to long days followed by short days. Such plants are called long-short-day plants (*2*). Their counterparts, short-long-day plants, have also been discovered (*6*).

Photoperiodically sensitive plants do not respond primarily to the length of the daily light period but respond, rather to the length of the dark period. Long nights induce flowering in short-day plants and inhibit flowering in long-day plants. It would be more accurate to call the former "long-night" and the latter "short-night" plants.

Photoperiodically responsive plants, particularly those native to the tropics, can be extremely sensitive to small differences in daily illumination. Njoku (*7*) found that a difference in length of photoperiod of only 15 minutes can determine whether flowering or continued vegetative growth takes place in several short-day plants.

In some plants, flower formation ultimately takes place under any photoperiodic regime, although it is markedly speeded by appropriate day lengths (this is called quantitative response). Many species, however, continue vegetative growth indefinitely under unfavorable day lengths (this is called qualitative response). Obviously, representatives of the latter type have been studied most extensively because their flower formation is fully controlled by one single environmental factor.

Short photoperiods combined with long dark periods induce flowering in short-day plants but cause long-day plants to grow vegetatively (Fig. 1*b*). However, a brief exposure to light near the middle of the long night causes plants to respond as if under short-night conditions: short-day plants remain vegetative and long-day plants flower. By using light of different wavelengths for such night interruption it has been shown that red light is most effective both for flower inhibition in short-day plants and for flower induction in long-day plants (Fig. 1*c*). The effect of red light can be completely reversed if the exposure to red irradiation is immediately followed by exposure to far-red irradiation (Fig. 1*d*). The red–far-red reversible pigment system now known as phytochrome can be obtained and studied in vitro (*8*).

Only more recently has reliable equipment become available for growing plants in narrow spectral regions during the entire photoperiod. This has made it possible to determine the spectral requirements for the principal light period. It has been found (*9, 10*) that

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a long-day effect (flowering in long-day plants, nonflowering in short-day plants) can be obtained in the following two ways.

1) By exposing the plant to long photoperiods of either blue or far-red irradiation (Fig. 1*e*). Long photoperiods of green or red light are ineffective.

2) By exposing the plant to short photoperiods of either blue or far-red light and interrupting the dark period with red light (Fig. 1, *f* and *g*). The latter effect is reversed by far-red irradiation, and therefore it is evident that it operates by way of phytochrome. Short photoperiods of only green or red light always yield the short-day effect, even if the long night is interrupted with red light (Fig. 1, *h* and *i*).

The requirement for blue or far-red irradiation during the principal light period, if a long-day effect upon the plant is to be observed, has been found for all long-day plants so far investigated. This is not true for all short-day plants (*9*).

The requirement for blue or far-red irradiation for the principal light period, dependent as it is on relatively high energies, supposedly acts by way of a pigment system other than phytochrome. The relation between the two photoreactions is, however, not yet clear (*9–11*).

Floral Stimulus

The leaves are the organs which perceive the day length, whereas the floral primordia are differentiated by the apical meristems. The initial perception and the final expression of the photoperiodic stimulus are separated by a petiole and a piece of stem. A signal must move from leaf to bud. This signal has been called floral stimulus, flower hormone, or florigen. The existence of such a floral stimulus has been demonstrated mainly in three different types of experiments.

1) Exposure of one or a few leaves of a plant to an inductive day length results in flowering, even though the rest of the plant is kept under noninductive conditions.

2) In plants which require only one inductive cycle for floral induction it can be shown, by removal of the induced leaf at regular intervals after the end of the inductive dark period, that the stimulus gradually moves to the bud. This is shown in Fig. 2 for the short-day plant *Pharbitis nil*. Only 14 hours of darkness are required for maximal flowering response, so it may be assumed that this is the time required for production of the stimulus in the cotyledons (the two leaves present in the seed). However, if the cotyledons are removed immediately after the 14 hours of darkness, no flower formation takes place. Apparently a certain amount of time is necessary for the stimulus to be translocated from cotyledons to plumule. The lower curve of Fig. 2 can therefore be interpreted as a translocation curve. From it one can conclude that after 18 hours in darkness an amount of stimulus sufficient to induce maximum flowering has left the cotyledons.

3) Grafting of an induced leaf or branch (donor) to a noninduced plant (receptor) causes flowering of the latter. This demonstrates transmission of a flower-inducing material (Fig. 3). Furthermore, an induced short-day plant (donor) can induce flowering in a noninduced long-day plant (receptor) (Fig. 4), a finding which suggests that the floral stimuli of the two reaction types are identical.

If it is assumed, as it is by most investigators, that the floral stimuli are specific substances of a hormone-like nature, one can formulate a number of questions concerning them: What is the chemical nature of the flower hormone? How does day length control its production? Is its biogenesis blocked at the same step or at different steps in the different photoperiodic reaction types? Are there two different blocks in long-short-day and short-long-day plants? How does the hormone bring about floral differentiation in the apex; what is the mode of its action?

The most direct approach to these problems would be extraction and identification of the hormone, but attempts to achieve this, with the exception of one attempt recently reported (*13*), have been unsuccessful or irreproducible.

Studies of changes in metabolism during floral induction have remained fruitless. Numerous metabolic differences between flowering and vegetative plants have been found, but these seem to be merely symptoms of flowering rather than its primary cause.

Another approach has been the application of antimetabolites and metabolic inhibitors, in the hope that, in the best case, one might find a specific inhibitor of hormone synthesis. Such studies have been principally carried out with the short-day plant *Xanthium pennsylvanicum* and more recently also with *Pharbitis nil*, another short-day plant. Both of these plants require but one inductive dark period for subsequent flower formation, and with them it has been possible to subdivide the induction of flowering into a number of so-called partial reactions. This concept has proven to be very useful in the interpretation of the work with metabolic inhibitors.

Partial Reactions in Short-Day Plants

The discussion that follows deals primarily with *Xanthium*, since most results have been obtained with this plant. *Xanthium* requires one dark period longer than 8½ hours for floral induction. Low temperature during the dark period prevents flowering. Thus, it seems at first sight that only darkness is needed, but this is not true. If the plant is depleted of carbohydrates by darkness, interrupted every 3 hours with a few minutes of light to prevent flowering, and is then exposed to an uninterrupted night, no flowering occurs. If, however, this long night is preceded by a short period of light of high intensity, the ability to flower is reestablished. This is called the first high-intensity light process. Since sucrose and acids of the Krebs cycle can replace the exposure to light, it seems that the function of the light is to produce respiratory substrate, although this may not be the whole story (*14*).

During the dark period the plant measures time. The first event during darkness is the conversion of phytochrome P_{730} to the red-absorbing form P_{660} (*5, 8*), thus,

$$P_{660} \underset{\text{Far red (730 m}\mu\text{) (more slowly in darkness)}}{\overset{\text{Red (660 m}\mu\text{)}}{\rightleftharpoons}} P_{730}$$

Results of various experiments indicate that this conversion probably does not take more than 2 to 3 hours, whereas the critical dark period for flowering is 8½ hours. Thus, there still remain 6 to 7 hours for measuring time. It has been suggested that some special preparatory process goes on during this period. In any case, it may well be that phytochrome must be present in the red-absorbing form during this period to permit the processes leading toward flowering to proceed. Brief irradiation with red light after 8 hours

of darkness, converting P_{660} to P_{730}, still completely inhibits flowering. However, if the flash of red light is immediately followed by one of far-red irradiation, switching P_{730} back to P_{660}, flowering proceeds normally. This strongly suggests that even after 8 hours in darkness no reactions beyond pigment conversion have taken place.

At the present time the cobaltous ion is the only known chemical that affects the timing mechanism. Application of the ion extends the critical dark period by about 2 hours. The effect can be reversed by application of cysteine or of glutathione (*5*).

As soon as the time-measuring mechanism has registered a period equal to the critical dark period, synthesis of the floral stimulus appears to start. The flowering response increases with increasing length of the dark period up to a length of about 15 hours. Interruption by red light during the second half of the dark period does not nullify the effect of the preceding dark period but presumably merely stops hormone synthesis by conversion of P_{660} to P_{730}. Application of 2,4-dinitrophenol during the dark period strongly suppresses flowering. This result indicates that high-energy phosphates are required for synthetic reactions, but it reveals nothing about the biochemical nature of these reactions.

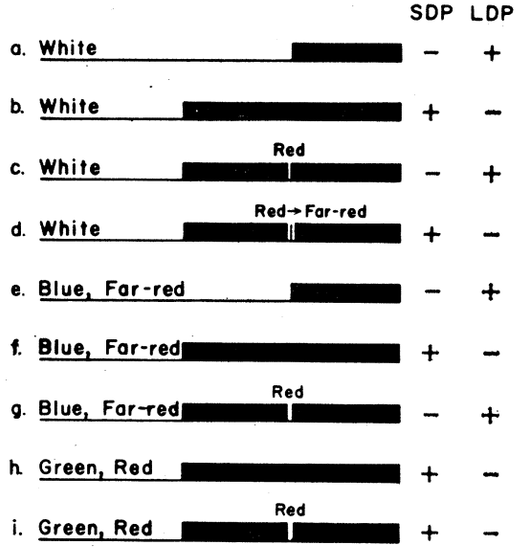
The effect of two other antimetabolites, ethionine and 5-fluorouracil, during the dark period is of interest. Ethionine is an effective inhibitor of flowering, and the inhibition is reversed by methionine. As all other amino acid analogues are ineffective as inhibitors of flowering, it seems that protein synthesis is not involved in hormone synthesis. Ethionine may antagonize the donation of methyl groups by methionine (*5*).

5-Fluorouracil also strongly inhibits flowering if it is applied during the earlier part of the dark period. Since the site of this inhibition is the apical meristem, I will discuss this effect later, under differentiation.

Analysis of the reactions of the inductive dark period, as already described for *Xanthium,* indicates that the reactions of the earlier part of the night are qualitatively different from those of the later part. This conclusion is supported also by results obtained by exposing other short-day plants to a temperature of 35°C during different parts of the dark period. In all species high temperature is inhibitory only when it is applied toward the end of the dark period (*4*).

Translocation of floral stimulus from an induced *Xanthium* leaf starts 20 to 24 hours after the beginning of darkness. Until recently it was believed that light of high intensity was necessary for conversion of precursor to hormone or for hormone stabilization (this is called the second high-intensity light process). Furthermore, high light intensity was assumed to promote hormone translocation from the leaf. Results of detailed experiments under controlled conditions by Searle (*15*) have now shown clearly that most of the stimulus is produced between the 9th and 16th hours of darkness. Once produced, the stimulus is stable under both strong and weak light and in darkness; transport out of the leaf takes place equally well under all of these conditions. Translocation of floral stimulus out of leaves in darkness has previously been shown in *Pharbitis* (*16*) and in *Perilla* (*17*). In young seedlings of *Pharbitis,* transport from the cotyledons starts after 15 to 16 hours of darkness, and about 4 hours later the response is completed (Fig. 2). Again, translocation proceeds equally well in light and darkness.

The rate of hormone translocation has been determined most accurately in *Pharbitis* (*18*). The rate is 6.2 to 9.1 centimeters per 24 hours. In a simple experiment such as that shown in Fig. 2 one can also estimate the rate of translocation by determining the time interval between maximum hormone production (after 14 hours) and maximum flowering after removal of the cotyledons (18 hours). Evidently it takes the hormone 4 hours to travel from the cotyledon, by way of a petiole 14 millimeters long, to the stem tip. This finding yields a rate of 8.4 centimeters per 24 hours. Although all



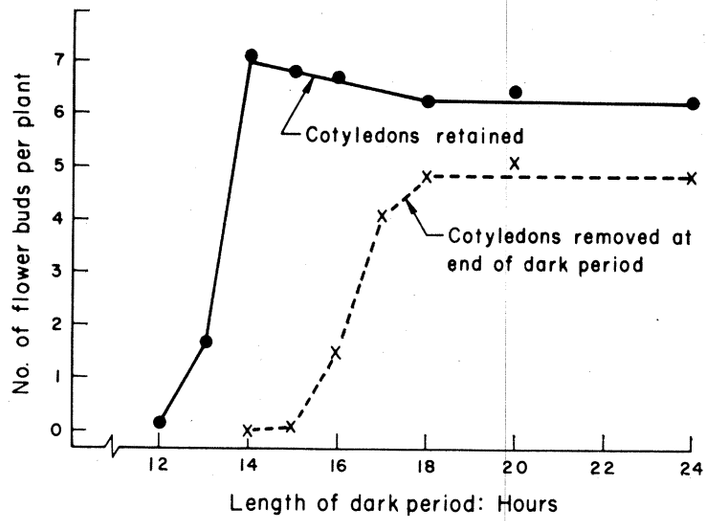


Fig. 1 (left). Diagrams showing the effects on short-day plants (SDP) and long-day plants (LDP) of day length, night interruption, and light quality during the main light period. Light plus dark period always equals 24 hours. (Solid line) Light period; (solid block) dark period; (+) flowering; (—) vegetative. [Diagrams *e–i*, after Meijer (*9*)] Fig. 2 (right). Translocation of floral stimulus from cotyledons of *Pharbitis nil*. Four-day-old seedlings were exposed to one dark period, varying from 12 to 24 hours, at 28°C. Upon transfer to light, either the seedlings were left intact [upper curve (production curve)] or the cotyledons were cut off immediately [lower curve (translocation curve)] (*12*).

Fig. 3. Transmission of floral stimulus across a graft union in the short-day plant *Perilla crispa*. The shoots on the left are on a plant under long-day conditions and have been induced to flower after grafting of a leaf taken from a plant which had been exposed to 29 short days. The control, on the right, grafted with a leaf from a plant under long-day conditions has remained vegetative. The photograph was taken 34 days after grafting. [After Zeevaart (*17*)]

evidence indicates that the hormone moves in the phloem, these rates are much lower than those found for phloem conduction of organic substances. Some possible reasons for this discrepancy have been discussed elsewhere (*17*).

In *Xanthium* the translocation of floral stimulus continues for approximately 24 hours, much longer than it does in *Pharbitis*. Once the apex has been transformed by a sufficient amount of floral stimulus, differentiation of the inflorescence begins. After about 3 days from the beginning of darkness, the first changes in the apex can be detected. The further development of the inflorescence can be inhibited only by general growth inhibitors.

Persistence of Induction

When a plant is exposed to a day length which favors flowering and is subsequently returned to noninductive conditions, the floral stimulus nonetheless causes morphological changes in the apical meristem, and flowering continues for a certain period. Concerning *production* of the floral stimulus, one can visualize either that this is discontinued upon the shift to a noninductive day length or, that, once it is started, it may continue under any day length. This problem has been studied principally with the short-day plants *Perilla* and *Xanthium* (*17, 19*).

If an induced *Perilla* leaf is grafted to a vegetative receptor stock, the receptor flowers. The donor leaf, now under noninductive conditions, can be regrafted to a second receptor and will again induce flowering, and so on. In one experiment, induced *Perilla* leaves were consecutively grafted to seven different groups of receptors. Although by the time of the last grafting the donor leaves had been exposed to long-day conditions for more than 3 months, the flowering response which they elicited was still undiminished at the last grafting. Consequently, it may be concluded that after a sufficient number of short-day cycles the leaf continues to produce floral stimulus under subsequent long-day conditions until it dies. Indirectly induced flowering shoots grafted to receptor stocks were, however, never effective as donors. Therefore, two different phenomena may be distinguished in the photoperiodic induction of *Perilla* (*17*):

1) The induced state [called the photoperiodic impression by Lona (*19*)]—that is, the ability to produce the floral stimulus. This is gradually built up under short-day conditions; it is irreversible and strictly localized.

2) The floral stimulus, produced in induced leaves and transmissible to apical meristems.

The strict localization of induction in *Perilla* has also been demonstrated by exposing parts of a leaf to long- or short-day conditions. Only those parts that were exposed to a sufficient number of short days could function as donors for long-day stocks, even when the apical half had been exposed to short days and the basal half to long days. Apparently, only those cells that had been exposed to a sufficient number of short days could produce the floral stimulus. The induced and noninduced state coexist in the same leaf, without affecting one another.

In *Xanthium* the situation is different. Mature leaves lose their power of induction when exposed to long-day conditions (*19*). But young leaves formed on a plant with induced leaves can function as donors; thus, unlike *Perilla*, *Xanthium* is capable of indirect induction. Induced shoots also cause flowering in receptors, and the resulting induced shoots themselves can function as donors. Through repeated grafting of such shoots it was possible to carry the flowering effect with undiminished vigor through five to seven graft transfers (*20*). Flowering in *Xanthium* thus resembles a virus disease. These results can best be explained by assuming that the stimulus is not merely stored but continues to be produced in young leaves and buds after short-day treatment is discontinued. Consequently, removal of young leaves and buds should cause reversion to vegetative growth; this, in fact, has been demonstrated (*21*). Thus, continuation of production of the floral stimulus under long-day conditions is achieved in different ways in *Perilla* and *Xanthium*.

It is well known that after transfer to noninductive conditions certain plants revert more readily to vegetative growth than others. *Xanthium* continues to flower under long-day conditions, as one might expect, but *Perilla* reverses rapidly, probably because the newly formed leaves are not induced.

Although *Xanthium* will flower after one long night, additional long nights considerably increase the magnitude of the response. Exposure of one leaf to four short-day cycles is more effective than division of exposure to the long nights between two leaves (*22*), indicating that the effect of several short days is not merely additive and that the short days become increasingly effective if the same leaf is exposed throughout.

Why certain plants flower after one inductive period while others need sev-

eral is an interesting problem. One might expect that in the latter case an insufficient amount of floral stimulus is produced during the first inductive cycle. This was shown to be true in a *Xanthium* strain which requires more than one long night for flowering, by grafting leaves of the sensitive strain to the less sensitive one and vice versa (*23*).

Gibberellins and Flowering

As reviewed in an earlier report in *Science* (*24*), applications of gibberellins induce flowering in many cold-requiring plants and also in long-day plants. In certain short-day plants such application promotes but does not induce flowering (*25*).

The gibberellins are now well established as a class of plant hormones. The question is often asked, Is the floral stimulus identical with a naturally occurring gibberellin? Since the floral stimuli of short-day and long-day plants seem to be identical, and since gibberellin induces flowering only in the latter, this does not seem to be the case (*26*). Chailakhyan (*27*) suggests that florigen consists of two substances: anthesin and gibberellin. Anthesin would limit flowering in short-day plants; gibberellin, in long-day plants. Attractive as this idea may be, it is not supported by conclusive evidence. For example, grafting of a vegetative short-day plant to a vegetative long-day plant should result in flowering, since each partner should supply the complementary factor to the other. Such grafts have, however, consistently yielded negative results (*17*).

In view of the many unsuccessful attempts to extract the flower hormone, increasing attention has been paid to flower-inhibiting effects. Unfortunately, the term *flower inhibition* has been used quite indiscriminately, without further specification of what kind of inhibition is meant. One might suppose, for example, that vegetative growth under noninductive conditions is due either to absence of the floral stimulus or to a specific inhibitor of flower formation. It has now become clear that a number of qualitatively different flower-inhibiting effects should be distinguished.

Table 1. The effect on flowering response in *Kalanchoë blossfeldiana* of intercalating single long-day (LD) cycles between 12 short days (SD). [After Schwabe (*29*)]

Treatment	Long days (total No.)	Flowers per plant (No.)
12 SD	0	65
6 SD, 1 LD, 6 SD	1	33
4 SD, 1 LD, 4 SD	2	8
3 SD, 1 LD, 3 SD	3	2
2 SD, 1 LD, 2 SD	5	2
1 SD, 1 LD, 1 SD	11	0

Table 2. The effect on the flowering response of *Kalanchoë blossfeldiana* of 24-hour dark periods (D) preceding or following long days (LD), which themselves are intercalated between short days (SD). [After Schwabe (*29*)]

Order of cycle*	Flower buds per plant (No.)
SD, LD, 24 D	158
SD, 24 D, LD	3
SD, LD	1
SD, 24 D	175

*SD, 8 hours of light, 16 hours of darkness; LD, 16 hours of light, 8 hours of darkness. Each cycle was repeated 12 times.

Inhibition of Translocation

In many plants, particularly the short-day ones, the presence of long-day leaves *between* induced leaves and receptor buds prevents or delays flowering. Therefore, receptor shoots in grafting experiments must be defoliated if successful transmission of floral stimulus is to occur. In two-branched short-day plants the response may be strictly localized if one branch is under short-day and the other is under long-day conditions. Defoliation of the long-day branch results, however, in subsequent flower formation upon it. This has been taken as evidence for the production of flower inhibitors under noninducing conditions. Lang (*1*) has suggested that, alternatively, the inhibition may be due to interference with hormone translocation. Most probably the floral stimulus moves in the phloem with the assimilates, but if noninduced leaves are closest to the receptor buds, they will supply their assimilates to these buds and the hormone will be diluted or will even fail to reach the bud.

This hypothesis has been tested in *Perilla* (*28*) by feeding $C^{14}O_2$ to leaves and studying the distribution pattern of labeled assimilates in the plant. As shown in Fig. 5, there is excellent agreement between translocation of assimilates and movement of floral stimulus as measured by the flowering response. These results clearly demonstrate why the presence of noninduced leaves between the source of floral stimulus and the receptor buds inhibits flowering. Only the leaves in closest proximity channel assimilates (including the floral stimulus) in large quantity to the bud.

Fractional Induction in Short-Day Plants

Flowering in short-day plants is more or less reduced when long days are intercalated between short days, indicating that long days do not merely stop induction but actively inhibit it. This inhibition has been studied in detail by Schwabe (*29*) in plants which require more than one long night for flower induction. Increasing the number of single long days intercalated between short days reduced flower formation (Table 1), and alternation of 1 short day with 1 long day completely suppressed it. One might suppose that this is either due to the increasing number of long days or, somehow, to the sequence of long and short days. By intercalating an increasing number of long days between two consecutive series of 6 short days, it was shown that the inhibitory effect decreases with each additional long day. Thus, the inhibition by long day is due not so much to the absolute number of long days as to the way in which the long days are intercalated.

The question remains as to whether the long day inhibits the effect of the short days that precede it or of those that follow it. The results of Table 2 show that the intercalated long day

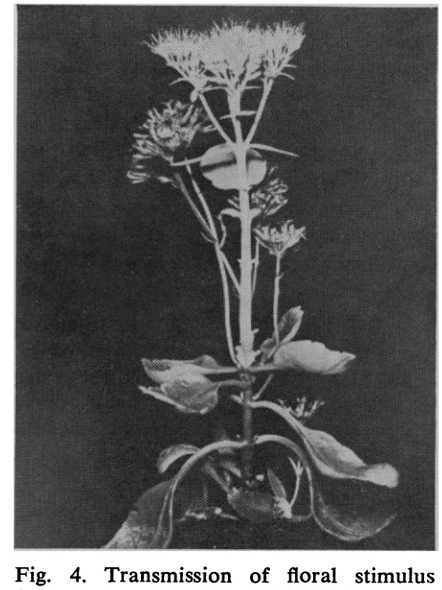


Fig. 4. Transmission of floral stimulus from the short-day plant *Kalanchoë blossfeldiana* (lower part) to the long-day plant *Sedum spectabile* (upper part) under short-day conditions. The axillary shoots of *Kalanchoë* flower under the influence of short days. The *Sedum* (receptor) was induced to flower under short-day conditions by the *Kalanchoë* donor. The photograph was taken 96 days after grafting. [After Zeevaart (*17*)]

affects only the short days that follow it. It may be calculated that 1 long day is capable of annulling the flower-promoting effect of 1.5 to 2 short days. The effects of several consecutive long days are, however, not additive. Thus, the maximum inhibitory effect of a given number of long days is obtained if they are intercalated as a single long day. Schwabe obtained similar results with soybean and *Perilla.* This inhibitory effect explains why soybean needs at least 2 short days for flower formation. It must also be concluded that such inhibition is lacking or at least much weaker in *Xanthium* and *Pharbitis,* which respond to a single long night.

Wellensiek (*30*), working with *Perilla,* found that long days intercalated between short days are inhibitory at 20°C but not at 5°C. In *Perilla,* therefore, flower formation finally occurs in a regimen of 16-hour days at 5°C combined with 8-hour nights at 20°C. *Pharbitis* will even initiate some flower primordia under continuous light, provided the temperature is dropped to 10°C (*31*).

Schwabe has interpreted his results by postulating both a floral stimulus and a flower inhibitor. The latter interferes, according to Schwabe, with the production of the hormone, not with its action.

According to Wellensiek's view, the action of darkness in short-day plants is primarily the destruction of the light inhibitor. He wonders whether the induced state in *Perilla* is formed solely as a result of a dark process or whether it is also built up in light. The latter alternative seems unlikely for several reasons. As mentioned earlier, the inhibitory effect of several consecutive long days is not additive. If the induced state were already formed under long-day conditions, a few short-day cycles should suffice for removal of the long-day inhibition, resulting in a maximal flowering response. It has been shown, however (*17*), that the degree of induction of *Perilla* leaves increases gradually to a maximum over about 4 weeks of short days. In Biloxi soybean (*32*), also, the flowering response to successive long nights is additive, whereas in *Kalanchoë* (*29*) it increases exponentially. This evidence strongly suggests that the effect of darkness in short-day plants must be at least twofold: (i) removal of a light inhibition which interferes with formation of the induced state or production of the floral stimulus, and (ii) causation of the formation of the induced state or production of the floral stimulus.

Flowering in Long-Day Plants

Much less is known about partial reactions in long-day plants, and some of the results obtained with different plants are contradictory. Moreover, many long-day plants are rosette plants in which floral initiation and stem elongation occur almost simultaneously, resulting in additional complications.

Since flowering in long-day plants takes place if the dark period does not exceed some critical value and occurs most rapidly under continuous light, it follows that no darkness is required for flower formation. As is known from grafting experiments, induction of flowering in long-day plants results in the production of a transmissible stimulus. One might suppose that the roles of light and darkness in the production of this stimulus are the reverse of these roles in short-day plants. Darkness should then have an effect antagonistic to flowering. Long photoperiods should remove the inhibition, with resultant production of the photoperiodic stimulus. These suppositions do not hold completely in the most widely studied long-day plant, *Hyoscyamus niger.* Complete defoliation results in flowering under long- and short-day conditions and even in complete darkness. This indicates not only that leaves under noninducing day lengths have an inhibitory effect on flowering but also that the floral stimulus can be formed independently of light. Thus, the role of light in *Hyoscyamus* lies apparently in its counteracting of the dark inhibition. The defoliation of other long-day plants has yielded negative results, but this may be due to the fact that they have no such storage organ as *Hyoscyamus* has.

The flower-inhibiting process in long-day plants has a positive temperature coefficient, so that lowering the night temperature favors flowering (*2*) [*Lolium temulentum* (*33*) is an exception]. A nitrogen atmosphere during the dark period also promotes flowering.

There is evidence with other plants that light does not merely remove a dark inhibition but is positively flower-promoting. In *Lolium* and *Trifolium* (*33*), floral induction only takes place if the long-day exposure is given at relatively high temperature—a finding which suggests that a flower-promoting process with a high temperature optimum is suppressed at low temperatures.

The effects of nonconsecutive photoperiodic treatments can be summated in long-day plants, as has been shown most extensively in *Hyoscyamus* (*34*). Alternating 1 long with 1 short day six times results in almost the same flowering response that is obtained after 6 consecutive long days. This indicates that the effect of 1 long day is not destroyed by a preceding or a following

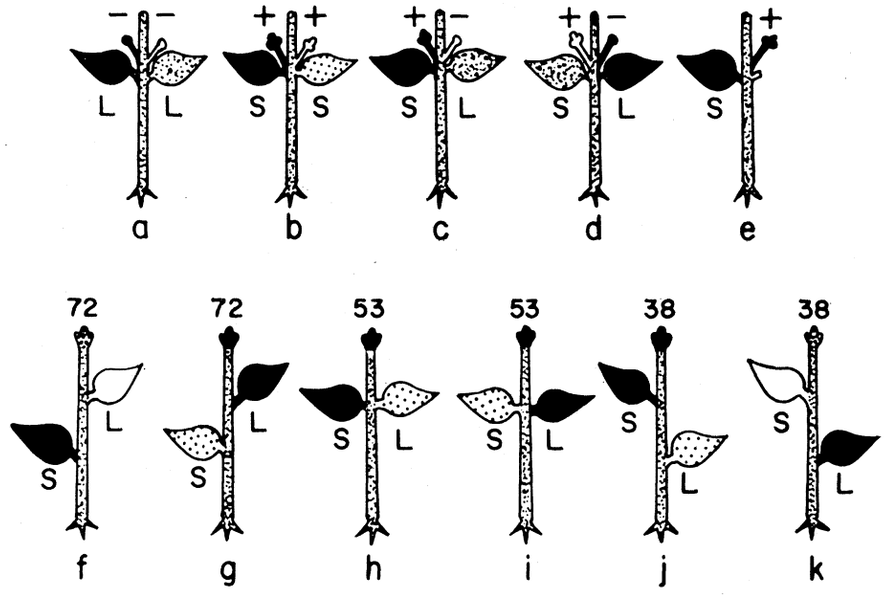


Fig. 5. Translocation of C^{14}-labeled assimilates from leaves to shoots in *Perilla* under various photoperiodic conditions. The degree of shading shows the relative amounts of C^{14}-assimilates in various plant organs 24 hours after exposure to $C^{14}O_2$. (The black leaves were originally exposed to $C^{14}O_2$.) The numbers above the shoots indicate number of days to appearance of flower buds. (*L*) Leaf exposed to long days; (*S*) leaf exposed to short days; (+) flowering; (—) vegetative. [After Chailakhyan and Butenko (*28*)]

short day. If, however, 12 short days are intercalated instead of 1, the flowering response decreases sharply. This may be because the leaves that receive the first long-day exposure die off by the time the last is given. Carr (*34*) has assumed that the results of subcritical photoperiods accumulate in the apical meristems and decay over long periods. This, however, is not the only possible explanation. As mentioned earlier for *Xanthium*, a given number of inductive cycles is most effective if applied to the same leaf, and this may equally well apply to *Hyoscyamus*.

The data on fractional induction strongly support the concept of accumulation of photoperiodic induction. On the other hand, there is no indication of accumulation of an inhibitor, as the sensitivity of long-day plants does not decrease on exposure to short days. By analogy to the mechanism of light inhibition in short-day plants, this would mean that darkness in long-day plants interferes with the production of the floral stimulus.

Translocation of floral stimulus by means of the defoliation technique has been studied only in one long-day plant —namely, *Lolium temulentum* (*33*), which can be induced to flower by a single long day. The most rapid translocation was found to take place 24 to 32 hours after the beginning of the inductive light period. In addition to a stimulus, a transmissible inhibitor was also assumed. The arguments in favor of this latter assumption are, however, not completely convincing. It may still be that inhibition by leaves in the short day has to do with the functioning of the stimulus through interference with its translocation (see Fig. 5) or through its dilution. Transport studies with tracers, as carried out in *Perilla*, might give a decisive answer.

At this stage it appears that darkness antagonizes the production of the floral stimulus in long-day plants. Light removes this darkness inhibition, and it may also be active in the formation of the stimulus in certain plants, although not in others.

Transmissible Inhibition

The flower-inhibiting effects discussed in the preceding sections all appear to be directed against the functioning or production of the floral stimulus. However, the case of cultivated strawberry, a short-day plant, provides good evidence in favor of a transmissible inhibitor (*35*). If the older of a pair of runner plants (connected by a stolon) is exposed to short-day conditions, no evidence is obtained for transmission of a flower-promoting effect via the stolon. When the younger plant of a pair is exposed to short-day conditions it flowers less vigorously than would be the case if it were not connected to a plant under long-day conditions. In translocation experiments, labeled phosphorus also moved preferentially from older to younger plants. These results can best be interpreted by assuming the production under long-day conditions of a transmissible flower inhibitor which at the same time promotes vegetative growth (petiole length, stolon formation). Production of the inhibitor presumably is stopped on exposure to short days. Defoliation causes flowering under long-day conditions, so that removal of the source of the inhibitor is apparently sufficient for flower formation.

Floral Initiation in Darkness

Seedlings of both long- and short-day plants grown on nutrient medium in total darkness initiate floral primordia (*36*). If, however, such cultures are exposed to light, each species again exhibits its normal photoperiodic behavior. Similarly, intact green plants ultimately form floral primordia in darkness if they have enough reserve material (*37*). These results all seem to indicate that, in principle, all plants are able to initiate floral primordia in total darkness. Consequently, the photoperiodic responses which are usually observed in green plants must become operative only after exposure to light. This idea has been elaborated by de Lint (*10*). He supposes that floral induction is an autonomous process that is sensitive to light. The light-imposed inhibition can be removed only by a proper ratio of light and darkness.

As shown in some of the preceding sections, photoperiodic induction is due not just to the absence of an inhibitor but also to a transmissible floral stimulus which can accumulate. On the other hand, no cumulative effects of unfavorable day lengths have been observed. Most plants, rather, become more sensitive to induction with increasing exposure to noninductive conditions. If it is accepted that the light-imposed inhibition prevents the formation of the induced state or of the floral stimulus under day-length regimes which do not favor flowering, the question remains, By what mechanism does this light inhibition operate? No direct experimental data are available, but the following considerations may be pertinent.

Before processes leading toward flowering can proceed in short-day plants, P_{730} must be converted to P_{660}. The physiologically active form of phytochrome is known to be P_{730} (*5, 8*). This implies that phytochrome plays only a negative role in the flowering of short-day plants, and that it does this by catalyzing a reaction that prevents production of the floral stimulus. Darkness releases this inhibition. In etiolated seedlings phytochrome is invariably in the P_{660} form (*38*), a fact which may explain flowering in total darkness in short-day plants.

Applying this reasoning to long-day plants leads to a less satisfactory result. A red light flash during a long night leads to flowering. It seems, then, that the P_{730} form of the pigment is necessary for flowering in long-day plants. This is, however, incompatible with the observed flowering in darkness. The picture may well be more complicated. Flowering in long-day plants requires that blue or far-red irradiation be included in the main light period (see the section on photoreactions), although it is during the dark period that phytochrome is known to operate. This suggests either that P_{660} must be present during some part of the cycle or that an additional pigment is involved (*9, 11*). In rosette plants, moreover, floral initiation and stem elongation seem to have different spectral requirements (*5, 10*). Experimental tests of these various suppositions will not be possible until the concentration and ratio of the two forms of phytochrome can be measured directly in leaves of plants grown in different spectral regions.

Differentiation

Differentiation is one of the most basic problems of modern biology. For the topic under discussion, it amounts to the question: Why does an apical meristem of a plant produce only leaves during a certain period and then suddenly turn to the production of flowers? In photoperiodically sensitive plants the onset of differentiation is fully controlled by one external factor, the day length.

Klebs (see *39*) formulated the problem of interaction between plants and

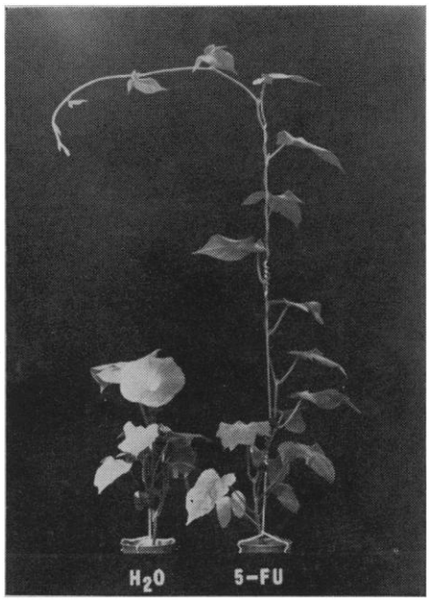


Fig. 6. Flower-inhibiting effect of 5-fluorouracil in *Pharbitis nil.* Seedlings in the cotyledon stage received 0.1 micromole of 5-fluorouracil before a single dark period (right), or received water as a control (left). The photograph was taken 5 weeks after exposure to the dark period. [After Zeevaart (*41*)]

their environment as long as 60 years ago, in concepts which are still valid. Nowadays it can be taken for granted that all cells of a plant have the same genetic information or genotype. All characteristics are potentially present in each cell, although not all of them are expressed in every cell at the same time.

The possibility of a change in genotype directed by the environment can be rigorously excluded. Genetic information remains constant during development. The reaction of an organism to its environment must then be completely determined by its genotype. From this it follows that the amount and kind of genetic information used and expressed during the course of ontogenesis must vary. This sequential turning on and off of genes during differentiation has been called programming (*40*).

From these general considerations it will be clear that the property to flower becomes expressed only as the result of an interaction between the genes for flowering and the environment, in this case the photoperiod. The question stated earlier in this section can now be specified as follows: How does an inductive day length activate the floral genes? The photoperiod has no direct effect on the apical meristem, but the perceiving leaf sends the photoperiodic stimulus to the apex. Upon the arrival of the hormone in the apex, floral differentiation starts. The primary action of the hormone probably consists, then, in causing in some unknown way the genes for flowering to become operative.

At the molecular level each gene has the capacity to produce one kind of enzyme via ribonucleic acid (RNA) as an intermediate. It may be that the photoperiodic stimulus interacts with the product of a gene—for example, as a coenzyme—but it is also conceivable that it activates the floral genes directly. Some results obtained recently with *Pharbitis* (*41*) with the antimetabolites 5-fluorouracil and 5-fluorodeoxyuridine (5-FDU) support the latter possibility. Both inhibitors are able to fully suppress flower formation in *Pharbitis* (see Fig. 6). The site of their action is exclusively in the apex, so these compounds interfere with expression of the floral stimulus in the bud, not with production of the stimulus in the cotyledons. The inhibition can be fully reversed by precursors of deoxyribonucleic acid (DNA), such as thymidine, thymidylic acid, and deoxyuridine, indicating that DNA multiplication is the primary process affected by these inhibitors. This has been confirmed directly by the microscopic observation that cell division in the apex is inhibited for more than 24 hours but for less than 48. The inhibitor is most effective when applied shortly before or after the arrival of the hormone (at 0 hours) in the apex. But if 5-FDU is applied 40 hours before the arrival of the hormone in the apex, no flower inhibition is apparent. This is because the inhibitor is metabolized and disposed of within 40 hours, so that DNA multiplication is again able to proceed. Application of 5-FDU about 30 hours or more after the end of the inductive dark period is too late, because the apex has already been transformed by the hormone. These facts show that 5-FDU inhibition of flowering is not due to a general inhibition of growth but is very specific in relation to the arrival of the hormone in the apex.

These observations have been interpreted as follows: The floral stimulus in *Pharbitis* must find multiplying DNA if it is to express itself in the formation of flower buds. Apparently it is during the multiplication of DNA that the floral genes can be activated. This conclusion agrees well with the finding that dormant buds are not able to respond to the floral stimulus.

5-Fluorouracil also inhibits floral induction in *Xanthium* (*42*). As in *Pharbitis,* the site of inhibition is the apex. In order to inhibit flowering effectively, 5-fluorouracil must be applied during the first part of the inductive dark period in *Xanthium.* The effective time of application to the bud is thus even before flower hormone synthesis has started in the leaf. These results indicate that during the first 8 hours of an inductive dark period something is made in a *Xanthium* apex which is necessary for the subsequent successful receipt of the photoperiodic stimulus and for the response of the bud to the stimulus. Further results suggest that this process in the bud is RNA synthesis.

Although the conclusions deduced from the work with nucleic acid antimetabolites are somewhat different in *Xanthium* and *Pharbitis,* they indicate nonetheless that in both species nucleic acid metabolism is an important factor for the transformation of a vegetative apex to an apex in which flowering has been induced. This agrees with the well-known fact that nucleic acids are the carriers or transmitters of genetic information.

Conclusion

Since the discovery of photoperiodism in 1920 an enormous amount of data on the photoperiodic control of flowering has accumulated. Most of the work so far has been descriptive and cannot yet be translated into chemical or physical terms. The isolation of the photoreceptor phytochrome (*8, 38*) is a first step in that direction.

More than 25 years have gone by since the first experimental evidence in support of the flower hormone theory was presented, and such evidence has grown steadily, although flower inhibition theories have been advanced occasionally.

In my opinion extraction and identification of the chemical structure of the hormone are the most urgent problems in this field at the present time. This would result in unlimited possibilities for further research. Therefore I hope that a recent report about a flower-inducing extract from *Xanthium* (*13*) will lead toward further characterization of the active principle. The principal problem is still the design of a simple and reliable bioassay for the flowering substance.

Another approach may be the labeling of the hormone by the use of more specific precursors than $C^{14}O_2$ (*20*). Obviously, such work should be ini-

tiated with the simplest systems available, such as *Xanthium* and *Pharbitis*. It will not be profitable to proceed to more complicated cases until one "simple" case has been well worked out.

Although there are many similarities among the various reaction types, there are also many differences. As pointed out by Lockhart (*4*), the almost universal nature of certain biochemical processes (respiration, photosynthesis) need not necessarily be expected in the flowering process (*43*).

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Recoilless Nuclear Resonance Absorption of Gamma Radiation

A new principle yields gamma lines of extreme narrowness for measurements of unprecedented accuracy.

Rudolf L. Mössbauer

It is a high distinction to be permitted to address you on the subject of recoilless nuclear resonance absorption of gamma radiation. The methods used in this special branch of experimental physics have recently found acceptance in many areas of science. I take the liberty to confine myself essentially to the work which I was able to carry out in the years 1955 to 1958 at the Max Planck Institute in Heidelberg, and which finally led to establishment of the field of recoilless nuclear resonance absorption. Many investigators shared in the preparations of the basis for the research we are concerned with in this lecture. As early as the middle of the last century Stokes observed, in the case of fluorite, the phenomenon now known as fluorescence—namely, that solids, liquids, and gases under certain conditions partially absorb incident electromagnetic radiation which immediately is reradiated. A special case is the so-called resonance fluorescence, a phenomenon in which the re-emitted and the incident radiation both are of the same wavelength. The resonance fluorescence of the yellow D lines of sodium in sodium vapor is a particularly notable and exhaustively studied example. In this optical type of resonance fluorescence, light sources are used in which the atoms undergo transitions from excited states to their ground states (Fig. 1). The light quanta emitted in these transitions ($A \rightarrow B$) are used to initiate the inverse process of resonance absorption in the atoms of an absorber which are identical with the radiating atoms. The atoms of the absorber undergo a transition here from the ground state (B) to the excited state (A), from which they again return to the ground state, after a certain time delay, by emission of fluorescent light.

As early as 1929, Kuhn (*1*) had expressed the opinion that the resonance absorption of gamma rays should constitute the nuclear physics analogue to this optical resonance fluorescence. Here, a radioactive source should replace the optical light source. The gamma rays emitted by this source should be able to initiate the inverse process of nuclear resonance absorption in an absorber composed of nuclei of the same type as those decaying in the source. Again, the scheme of Fig. 1 would hold here, but the radiative transitions would now take place between nuclear states. Nevertheless, all

The author is professor of physics at California Institute of Technology, Pasadena. This article is the English version of the lecture which he delivered in Stockholm, Sweden, on 11 December 1961, when he received the Nobel prize in physics, a prize which he shared with Robert Hofstadter. Dr. Hofstadter's Nobel address has been published [*Science* **136**, 1013 (1962)].