The Physiology of Flowering

Volume II: Transition to Reproductive Growth

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OBJECTIVES AND ORGANIZATION OF THE WORK

Scientific advances often come from uncovering
a hitherto unseen aspect of things as a result,
not so much of using some new instrument, but
rather of looking at objects from a different
angle.

F. Jacob*

From the point of view of basic biology, plant scientists have given much attention to flowering because this is the first step towards sexual reproduction in plants. Also, since the onset of flowering is under absolute environmental control in many species, some biologists with a deep interest in morphogenesis chose the flowering process as their field of research. Interest in this developmental step has a strong economical basis, too, since many aspects of agronomic and horticultural crop production are intimately associated with flowering. Fundamental investigations provide a conceptual framework for the development of practical applications, particularly following the discoveries of photoperiodism and vernalization early in this century. From this period to the present, research has expanded rapidly. Just before World War II, the concept of the floral hormone or florigen was defined; this idea has received experimental support and is apparently the most widely accepted theory for the control of floral initiation. Despite numerous attempts to isolate and identify the hypothetical hormone, we still have no idea of its chemical nature.

With the introduction of more refined investigation techniques in the field, it is apparent that the flowering process is extraordinarily complex. It includes several interrelated steps, each of which is influenced by several factors of both internal and external origin. The simple florigen concept seems no longer commensurate with the complexity of the phenomenon it was supposed to explain. Possibly because of its simplicity it has remained the favorite theory. The physiology of flowering is thus in the uncomfortable situation, perhaps unique in biology, that it is still dominated by a concept proposed more than 40 years ago. It is recognized more and more that this concept puts severe constraints on further developments in both fundamental and applied research programs in which the onset of flowering is the key process.

In all experimental sciences, the appearance of new evidence that is irreconcilable with a theory, no matter how well established the theory may be, requires consideration of alternate hypotheses. Following this rule we suggest that a reconsideration of accepted notions of the physiology of flowering is absolutely and urgently required.

The present work is organized such that the whole phenomenon of flowering is divided into two major steps: (1) the initiation of flower primordia and (2) the development of these primordia into mature flowers until anthesis. Despite the fact that a separation of these two stages does not appear possible on theoretical or evolutionary grounds, the absence of clear distinction between the various stages of the flowering process in several investigations makes interpretation difficult or impossible, and therefore, the results are of little value. These stages are not all alike and do not always react similarly to external and internal variables. They should thus be considered independently to avoid confusion.

The first two volumes are devoted to "flower initiation" which includes not only the production by meristems of clearly recognizable flower primordia, but also all preceding reactions that are required if flowers are to be initiated. This has been by

far the most studied stage because many physiologists view it as the critical turning-point from vegetative to reproductive growth.

Volume I is concerned essentially with a review and critical analysis of the classical data and concepts. The aim here is to pinpoint the firmly established facts and contro-versial issues as well as to stress the shortcomings of classical work and interpretations. Research has indeed focused very heavily throughout the past 60 years on the effects of physical and chemical factors of the environment, while unfortunately little atten-tion has been paid until quite recently towards gaining an understanding of the basic internal mechanisms that underlie the floral transition.

The first section of Volume II deals with these more intimate aspects of the onset of reproductive growth. The basic role of correlative influences in flower initiation, even in the simplest experimental systems, is demonstrated. Then, we proceed by a description of the floral transformation of shoot apices at levels ranging from the macromorphological to the molecular. The idea is that a rather complete description is central to an understanding of the process of flower initiation and that it may further give some insights into the controlling agents of this process.

The second section of Volume II begins with a search for exogenous chemicals that control in part, or totally, the events of the floral transition. This is followed by a review of the work on endogenous substances that are considered as possible promoters or inhibitors of flower initiation. An attempt is made to see how far we have come in the understanding of the ultimate processes whereby a meristem begins to initiate flow-ers instead of leaves. The not surprising conclusion is that we are still a long way from the goal, but despite the fragmentary nature of the available evidence, the analysis developed in Volume II may provide a useful conceptual framework for future inves-tigations in this important area of plant science. Also, it is anticipated that this new approach will result in development of more rational and efficient controls of flower-ing for agricultural and horticultural purposes. These applied aspects will be discussed in the third volume of this series.

The treatment of the different chapters is neither simplistic nor exhaustive. Our gen-eral philosophy has been to avoid extreme positions, either abusive generalizations that mask the real complexity of the problem and the diversity of plant behaviors or com-plete descriptions of all possible types of plant responses that create confusion and discourage the readers. Evidently, when one attempts to cover such an extensive sub-ject in a limited number of pages there is inevitably a problem of topic selection. While our aim was to provide a balanced account of the most important and recent contri-butions in all aspects of the subject, some topics have wittingly received special treat-ment. Their selection reflects essentially our personal interest; other writers would have certainly made other choices and presented a differently balanced book. We like to think, however, that it will be recognized as timely and essential for the field to be reexamined from widely divergent points of view.

It is important to underline that constant reference to source material and use of a rich illustration should assist the unspecialized reader to obtain a full understanding of the discussed topics. Concluding sections are also inserted in many places and hope-fully will be considered as resting spots. The busy reader may begin with these sections and the short Chapter 9, Volume II, and return to the main text for examination of important details. A glossary is also included for the reader who is unfamiliar with the scientific jargon of the field. In a work like this, there is some unavoidable repeti-tion of material, but this has been reduced by frequent use of cross references. The species most commonly used in flowering studies will be usually referred to by their generic names alone.

The third volume will be concerned with the stages of flowering that follow initia-

tion, essentially flower organization and maturation until anthesis. While the necessity of considering separately these successive stages was stressed above, it is also obvious that flowering is a unitary phenomenon and that its component steps are necessarily related to one another. After all flowering is about sexual reproduction in plants and we must expect that in evolution the entire physiological process is designed to expedite recombination of genetic characters and reproduction of the organism.

In this work we deal mainly with angiosperms, although gymnosperms are occasionally considered.

We hope that these three volumes will convey some of the excitement that we have felt during their preparation as well as during our investigations on flowering.

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SECTION I — CLASSICAL EXPERIMENTAL SYSTEMS AND DATA; ANALYSIS OF COMMON CONCEPTS

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SECTION II
Basic Mechanisms of Flower Initiation

There can be no true understanding of
morphogenesis unless there is precise
observation of the size, shape and relationships
in time and space, of nascent organs in
embryonic regions . . . Without this exact
preliminary information, there can be no proper
formulation of the relevant physiological
problems nor can an adequate experimental or
analytical programme be designed.

C. W. Wardlaw

INTRODUCTION TO SECTION II

Despite the enormous amount of physiological work, reviewed in Chapters 2 to 7 of Volume I, we are still far from understanding the basic internal mechanisms that underlie the floral transition. As pointed out by Zeevaart, this is partly because these mechanisms turned out to be exceedingly complex.[92] Another equally important reason we believe is that priority has been given to the study of the environmental control of this developmental step and perception of external variables by plants rather than to the study of the internal mechanisms underlying the initiation of flowers.

The aim in Section II is to analyze these more intimate aspects of floral evocation. The term "evocation" was introduced by Evans for the events at the shoot apex following induction of flowering which commit the meristem to formation of flower primordia.[15] This term will be used here by preference to "apex induction", widely used in earlier days, because induction refers, on priority grounds, to events occurring in the leaf and which are totally different from those occurring in the apex. At this point, it must be quite clear that evocation "precedes" the initiation of flower primordia.

Chapter 1

THE BASIC ROLE OF CORRELATIVE INFLUENCES

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I. INTRODUCTION

Many classical studies, as we have seen in Volume I, presented flower initiation in photoperiodic plants as a simple, one-way chain of processes consisting of leaf photoinduction, transport of a floral stimulus, and meristem reaction (evocation). In cold-requiring plants, the situation appeared even simpler since the site of cold perception and of floral evocation is the same, i.e., the shoot apex. On this basis, flower production was seen apart from major correlative influences.

Because of its simplicity this view is very attractive, but it is undoubtedly an oversimplification. Perennial species, either herbaceous or woody, in which only a fraction of the existing and active meristems is allowed to flower at any time, must possess systems that counter the promotion of flowering at some specific meristems. Correlative influences are not restricted to perennials, however, and have also been found in several monocarpic plants. Evidence for the existence of these influences in intact plants of all sorts will be reviewed in the first part of this chapter.

Intact plants possessing leaves of different physiological ages, meristems in different states of activity, and a root system are certainly not ideal for determining whether or not correlative influences are basic to flower initiation. In an effort to simplify the experimental units, flower formation in excised and cultured plant parts has been studied. First, the behavior of excised shoot apices when grown in various physical and chemical environments was explored, but interest expanded later to the flowering of tissue pieces taken from various locations in the plant and initially devoid of primary meristems. These in vitro investigations will be discussed in the second part of this chapter.

II. CORRELATIVE INFLUENCES IN INTACT PLANTS

The easiest way to determine the role played by a given organ is to compare the behavior of the intact plant with that of the plant when this organ is surgically removed or destroyed. Such organ removal tests should ideally be associated with transplantations, i.e., replacement of the removed part by another one of similar or different initial location in the plant. Unfortunately, transplantations are difficult to perform and have almost never been made. It must also be borne in mind that in some of these experiments the plant is more or less severely wounded and that considerable metabolic disturbance might ensue.

A. Perennial Plants

Investigations with several herbaceous perennials requiring a chilling treatment for flower initiation show convincingly a role for apical dominance in the control of flowering. In *Geum urbanum,* for instance, the perennial habit is preserved because very young undifferentiated axillary meristems, located quite close to the terminal meristem of the rosette, and old axillary meristems (those in the axils of mature leaves) as well as the terminal meristem itself are all insensitive to low temperature. Relatively few intermediate axillary meristems, those bearing two leaf primordia, with little zonation, and an activated central corpus at the time the cold treatment is started, respond to this treatment.[31]

Tran Thanh Van succeeded in obtaining floral evocation of these sensitive axillary meristems in the total absence of cold by: (1) microsurgical destruction of the terminal meristem of the rosette and youngest leaf primordia, (2) repeated applications of kinetin to these axillary meristems, and (3) growth of the plants in conditions of high intensity light and good mineral nutrition.[31] These treatments all have one feature in

common, *viz.* they suppress or reduce apical dominance. Usually, the floriferous stems produced by such treatments remain quite short, and a supply of exogenous GA_3 is required for normal elongation. Thus, low temperatures establish mechanisms for shoot elongation in the released axillary buds, probably by activating GA biosynthetic pathways. Chouard and Tran Thanh Van have repeatedly stressed the fact that competent axillary meristems in cold-requiring perennials apparently flower automatically when released from apical dominance.[383] Accordingly, the effect of the winter chilling in *Geum* is seen primarily as a means of reducing growth of the terminal meristem, thereby weakening its dominance and permitting slow and progressive activation of all axillary meristems. However, only those meristems that are initially at the proper stage of development are sufficiently activated by a 2- to 3-month-cold treatment to reach the reproductive condition.

Clearly in support of this view is work showing that certain axillary meristems of cold-requiring *Chrysanthemum* and *Teucrium scorodonia* are "devernalized" simply by growing the plants after a chilling treatment under conditions that result in strong apical dominance.[55, 383] Also, decapitation of nonvernalized Sunbeam *Chrysanthemum* results in accelerated inflorescence initiation on distal lateral shoots.[384]

The influence of apical dominance on flowering has also been found in several other perennials, including orchids and woody species. In an orchid hybrid, Goh and Seetoh showed that decapitation, i.e., release from apical bud dominance, results in inflorescence initiation by some axillary meristems that remain otherwise vegetative.[385] Apical dominance alone does not account for the clear flowering gradient along the stem axis revealed in this plant: the lowermost buds develop as vegetative branches, the intermediate buds develop into vegetative shoots bearing solitary flowers, whereas the uppermost develop inflorescences.[386] Thus, the stage of development attained by the axillary meristems at the time of release is also critical, as in *Geum*.

Decapitation in *Mangifera indica* and roses also causes flowering of axillary meristems.[387-389] Meristems in roses can initiate leaf primordia and short internodes while subject to apical dominance, but their enlargement is strongly suppressed. Removing apical dominance allows rapid branch extension followed, after a short period of leaf production, by the initiation of the terminal flower of the branch. As pointed out by Cockshull and Horridge, this indicates that axillary meristems of the rose under apical dominance, although active, are not competent to respond to conditions that otherwise promote flowering.[389]

The situation in black currant (*Ribes nigrum*) is similar to that for roses. Nasr and Wareing observed that, during the normal developmental cycle, axillary flower initiation occurs immediately after cessation of extension growth and establishment of a state of dormancy in the apical meristem of the shoot.[390] In other words, flowering occurs after release from apical dominance, as shown by the considerable increase in size of all axillary buds at that time. Despite this enlargement, none of the branches elongate, and only the buds located at upper nodes produce flower primordia. This is due to the fact that the apical meristems of the lower buds are fully dormant (thus unable to grow and flower). The upper buds are partially dormant in the sense that they are unable to develop elongated branches, but are still capable of being transformed into reproductive meristems. There is evidence, however, that flowering is not a simple and automatic consequence of growth cessation. Indeed, exposure to 16 SD induces flowering, although such treatment is insufficient to bring about the complete cessation of extension growth and the formation of terminal resting buds.[391]

B. Monocarpic Plants

1. Are Active Buds Necessary During Photoinduction?

Detached *Perilla* leaves have been photoinduced in the absence of visible bud pri-

mordia.[71] Similarly, carefully disbudded plants of *Perilla* can be photoinduced as demonstrated by the fact that their leaves serve as donors to vegetative plants.[392] Despite the small number of experiments of this sort, it is generally believed that buds do not participate in photoinduction.[21]

This generalization is questionable since Lincoln and co-workers and Carr found that disbudded induced plants of *Xanthium* and *Chenopodium amaranticolor* do not transmit the floral stimulus when grafted to vegetative scions.[88,393] Moreover, induction by a single long night is impossible in plants of *Xanthium* and *Pharbitis* if all active buds are removed just prior to or for a number of days following the SD.[17,88,374] Floral induction is possible only if the long night is given several days after disbudding, when at least one dormant axillary bud has resumed growth. Further experiments with *Xanthium* have indicated that flower initiation in response to 1- to 3-SD cycles is increased in plants with two active buds compared to those having only one such bud (Table 1). Since only the leaves receive the SD treatment, this eliminates a possible direct effect of the photoperiodic regime on the buds. Successful photoinduction in *Xanthium* can be achieved in the absence of active buds, however, provided immature expanding leaves are present.[88] Apparently, active immature tissues from either bud or leaf provide an essential element for induction and/or evocation not produced by mature leaves. Salisbury believes that this element is auxin since he found that auxin can replace the requirement for active buds or young leaves in this species.[17]

The idea that the bud is simply a passive receptor for a floral stimulus generated in mature leaves is unable to explain all these observations. Most investigators postulate that the floral stimulus in *Xanthium* is unstable or inactive at a dormant bud. If the stimulus does not find an active bud (or immature leaves) within a few days, it is dissipated, and flower initiation is prevented. Accordingly, the requirement for immature tissues from either bud or leaf is for "stabilization" of the stimulus. An alternate possibility envisaged by Lincoln and co-workers and by Carr is that "production" of the stimulus or a component of it may depend on an interaction between mature leaf and active immature tissues.[88,393] Inactive tissues of a dormant bud at the time of induction would be incapable of participation, and no stimulus would ensue. Subsequent research is required to settle this question.

2. The Role of the Root System

As a rule, roots do not seem to play a role in leaf photoinduction since the SDP *Perilla*,[71,394] the LDP *Brassica crenata*,[394] *Lolium*,[7] and *Sinapis*,[13] etc. can be photoinduced in the absence of any visible root or root primordium. Of course this fact does not demonstrate that these organs, when present, have no influence in other steps of the floral transition.

The rosette LDP *Rudbeckia* does not flower when deprived of its root system. Chailakhyan and Khlopenkova demonstrated that leaves of derooted *Rudbeckia* plants can be photoinduced but that the absence of roots prevents a critical step of flower initiation other than photoinduction.[395] They believe that this step is bolting, but it is well established that bolting and flower formation are basically distinct processes, experimentally separable in *Rudbeckia* and other rosette species (see Volume II, Chapter 2, Section II.). Possibly, presence of roots is required in these plants for floral evocation itself.

Another interesting case is that of *Silene armeria*, another rosette plant. Flowering of this LDP is promoted in SD simply by removal of the roots.[91] Flowering in SD is also promoted by raising the temperature from 20 to 32°C (Volume I, Chapter 3, Section VIII.A.), a change reported by Wellensiek to be perceived by the roots.[116] Apparently, roots have a detrimental effect on flower initiation in *Silene* and this effect

Table 1
INFLUENCE OF THE NUMBER OF ACTIVE BUDS LEFT ON THE PLANTS[a] ON FLOWER INITIATION IN THE SDP *XANTHIUM STRUMARIUM*

Number of inductive SD cycles given	Flowering response[b] in plants with	
	One bud	Two buds
1	0.0	0.0
2	0.0	1.0
3	1.5	4.1

[a] Plant bearing only the primary leaves and one or two cotyledonary buds. Sufficient time elapsed between the combined defoliation and disbudding and the first inductive cycle as to allow resumption of growth of the cotyledonary buds. Only the leaves received the SD treatment by means of paper covers.

[b] Flowering response measured by the same score system as in Volume I, Chapter 7, Figure 5.

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is inactivated at high temperatures. Derooting also promotes flowering in several other species, e.g., the SDP *Chenopodium rubrum* and *C. polyspermum*.[396,397]

Conversely, stem cuttings generally have reduced rooting capacity during initiation of flowers.[398] Not only root formation, but also root elongation is markedly decreased when plants enter the reproductive state, as exemplified by the SDP *Perilla* in which the rate of root growth decreases as a result of flower formation.[399] In *Anagallis* cuttings, sensitivity to a single inductive LD strongly decreases with time after cutting, and this drop correlates with start of root growth (Figure 1). Flower initiation and root formation and/or growth are thus very often antagonistic events, a situation well-known to horticulturists.

3. The Role of Apical Dominance

The problem of the influence of various plant parts, including the apical bud and also the roots, has been thoroughly studied by Miginiac using the facultative LDP, *Scrofularia arguta*.[400] The cotyledonary axillary buds of this plant generate, regardless of the photoperiod, stoloniferous shoots bearing very small leaves and flowers ("microphyll" shoots). Axillary buds of the upper nodes of the shoot produce, on the contrary, "macrophyll" shoots bearing large leaves. Flowering of these upper axillary shoots is promoted by LD. In vitro culture of cotyledonary or upper nodes always causes production of microphyll axillary shoots that flower rapidly in SD, provided the axillary buds are young at the time of isolation. This indicates that all axillary buds have a high and equal potential for flower initiation in this plant and that retardation of flowering in macrophyll shoots in SD can be ascribed to correlative influences. When cotyledonary nodes are grown in vitro with various plant parts attached or removed (Table 2), it is found that: (1) presence of epicotyl in the absence of roots leads to early flower formation and (2) presence of roots in the absence of the epicotyl leads to vegetative growth (macrophyll shoots).[401] Since in intact plants, only microphyll flowering shoots arise at the cotyledonary node, it appears that the influence of the
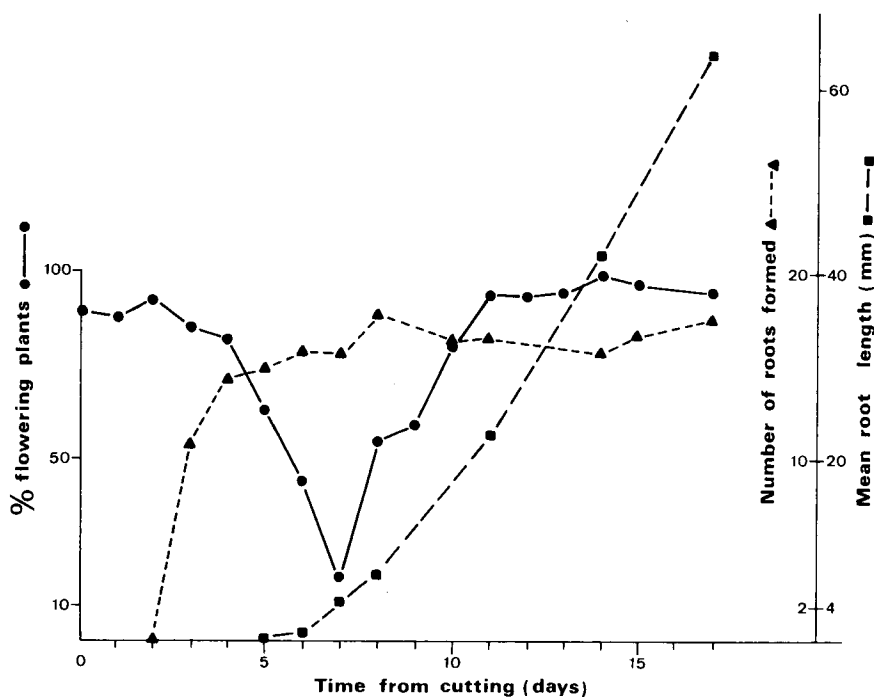
FIGURE 1.   Flower initiation, root formation, and growth in cuttings of the LDP *Anagallis arvensis* as a function of time after cutting. Flower initiation is induced by a single 24-hr LD. (From Bismuth, F., Brulfert, J., and Miginiac, E., *Physiol. Veg.*, 17, 477, 1979. Reproduced by permission of Gauthier-Villars, Editeur, Paris.)

epicotyl predominates over the influence of the root system at this node. Further work has shown that the apical bud is the active component of the epicotyl.[402] Flower initiation of upper axillary buds is also entirely controlled by antagonistic correlative influences: (1) apical dominance that promotes flowering and inhibits vegetative growth and (2) influences from the root system and the axillant leaf that both inhibit flowering while promoting vegetative growth.[400] The axillary dimorphism exhibited by intact plants can then be explained by a change in the balance between these different interactions during development.

Thus, contrary to what is observed in several perennials, apical dominance has in *Scrofularia* a promotive effect on axillary flowering. A situation similar to that of *Scrofularia* apparently exists in *Acalypha* and pea, two species in which removal of the apex, leading presumably to release of axillary meristems from apical dominance, inhibits the reproductive development of these meristems.[403,404] Specific microsurgical removal of various apical parts indicates that in pea, the leaf primordia are more influential than the apical meristem.

Apical dominance in several biennials inhibits flowering of axillary buds. Dormant axillary buds located at the base of the inflorescence in beet and rape develop reproductively when the apical part of this inflorescence is removed. Interestingly enough these buds stay strictly vegetative if completely separated from the mother-plant and grown in vitro.[405] In this case, apical dominance is thus only one of the several correlative influences which are involved in the control of flowering of axillary meristems.

4. Is the Apical Meristem of Plants with Axillary Flowering Unresponsive?

In several species, terminal inflorescences are apparently never produced even with

Table 2
INFLUENCE OF EPICOTYL AND ROOTS ON GROWTH AND
FLOWER INITIATION OF COTYLEDONARY BUDS IN THE LDP
SCROFULARIA ARGUTA

Experimental system[a]	Length (cm)	Leaf morphology	Cotyledonary shoot	
			% Flowering shoots	First floral node
Intact	0.5	Microphyll	100	2.0
Roots removed	0.6	Microphyll	100	2.0
Epicotyl removed	4.1	Macrophyll	0	—
Roots and epicotyl removed	1.8	Macrophyll and then microphyll	93	3.8

[a] All cultures are grown in SD.

Reproduced by permission of the National Research Council of Canada from Miginiac, E. and Lacombe, N., in the *Can. J. Bot.,* 51, 465, 1973.

strong floral induction. Examples are the SDP *Impatiens,*[406] the LDP *Fuchsia,*[407] *Anagallis,*[82] etc. In other species, such as the SDP Biloxi soybean,[377] *Pharbitis,*[59] *Salvia occidentalis,*[83] and the cold-requiring *Lunaria,*[367] and *Geum,*[31] terminal inflorescences are found only following maximal induction. Generally morphologists note whether species are terminal or axillary flowering as if this were a fixed species character, and geneticists are interested in whether cultivars have determinate (terminal) or indeterminate (axillary) flowering branches. These are important features in many agricultural crops since flower position is rather strongly correlated with plant stature. Thus, the question of terminal vs. axillary flowering is by all means an important one. If this were purely an inherent gene-controlled feature, it is unlikely that we would find so many cases in which suboptimal inductive conditions produce primarily or only axillary reproductive structures.

Since in *Impatiens* and *Anagallis,*[406,408] terminal flowering occurs with continuous removal of axillary flowers, there is some support for the idea of diversion of assimilates and accompanying floral stimuli as the controlling factor. That is, removal of axillary flowers may remove sinks competing with the terminal meristem, thereby, making assimilates and floral stimuli more available to the latter.[406] It is hard to believe, however, that in the presence of axillary flowers the terminal meristem which is quite active is not supplied with assimilates and floral stimuli.

Cytohistological studies in plants with axillary flowers clearly demonstrate that far from being nonresponsive to induction their apical meristems, including the central zone, react much the same as the apical meristems of species with terminal flowering (Volume II, Chapter 3); thus, these meristems are well supplied with floral stimuli. It
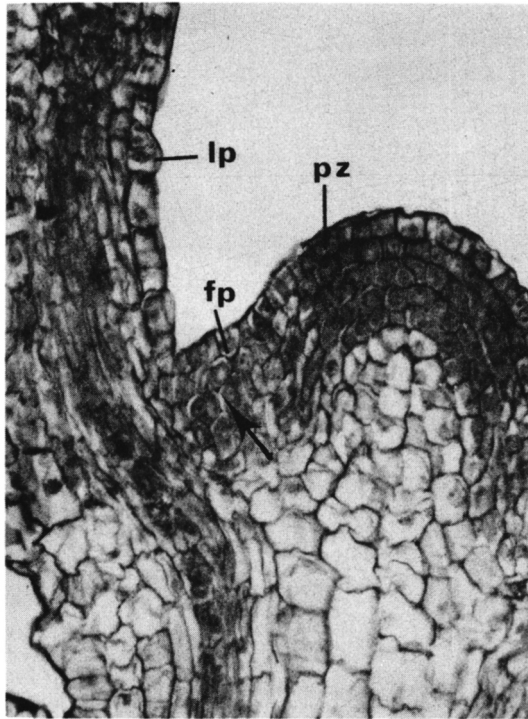
FIGURE 2.   Apical meristem of the LDP *Anagallis arvensis* in longisection. Meristem collected 55 hr after start of the inductive LD. Start of initiation of a flower primordium (fp) by periclinal divisions (arrow) in the third and fourth cell layers in the axil of a leaf primordium (lp). The cells involved in this morphogenetic process were in the peripheral zone (pz) of the meristem during the LD. (From Taillandier, J., *Z. Pflanzenphysiol.*, 87, 395, 1978. With permission.)

is important to realize that at the time of induction the cells that will generate the axillary flowers in species such as soybean,[409] *Pharbitis,*[1] *Anagallis,*[82] etc. are in the axils of the last-formed pair of leaf primordia, i.e., are within the apical meristem itself (Figure 2).

On the other hand, terminal flowering in species such as *Sinapis, Perilla, Amaranthus,* etc. with indeterminate racemose inflorescences may be more apparent than real. In these plants, florets are also initiated in axillary (lateral) positions only, and the central apical part of their meristems never produces a flower primordium. The situation in these plants is thus very similar to that existing in species with axillary flowers, such as soybean and *Anagallis,* except that the latter species continue to produce leaves whereas the former species initiate reduced bracts (*Perilla, Amaranthus*) or no subtending appendages (*Sinapis*).

The important finding emphasized here is that all apical meristems are responsive to floral stimuli and react much the same whether flowering is of the terminal or axillary type. The main difference between the two types lies in the kind of axillant (subtending) appendage produced by the meristem flanks.

Species with axillary flowers are perhaps the most interesting for determining the changes in sensitivity of meristematic tissues towards flower stimuli as a function of
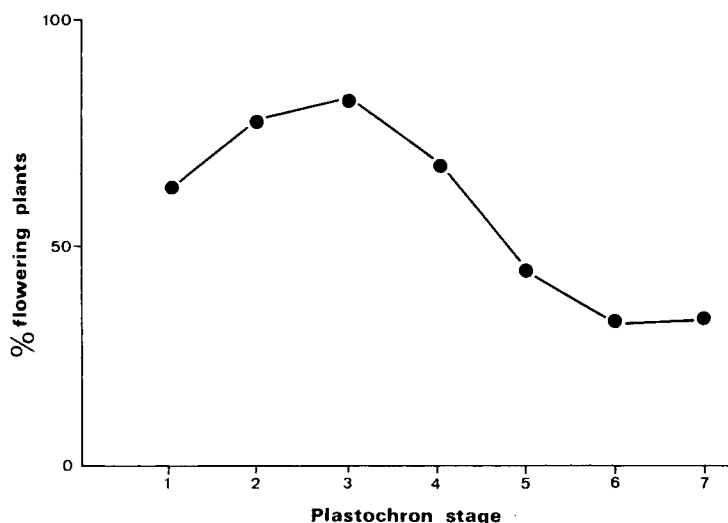
FIGURE 3. Flowering in the axils of the last-formed leaf pair as a function of the plastochron stage in *Anagallis arvensis* induced by one LD. Arbitrary plastochron stages of the apical meristem at the start of the LD are shown on the abscissa. Initiation of the next pair of leaf primordia occurs at Stage 6. Leaf primordia have a height of about 50 and 100 μm at Stages 2 and 4, respectively. (From Brulfert, J., Imhoff, C., and Fontaine, D., *Etudes de Biologie Végétale. Hommage au Professeur Pierre Chouard*, Jacques, R., Ed., 1976, 443. With permission.)

the plastochron stage. In *Anagallis*, a species with a decussate phyllotaxis, Fontaine has shown that following induction with one LD flowers are formed at one node only, that corresponding to the last pair of leaves initiated. Sensitivity of the cells lying in the axils of this leaf pair (these are the cells that generate the flowers, see Figure 2) is limited to a certain period during the course of the plastochron (Figure 3). Greatest sensitivity to LD induction is when leaf primordia are about 50-μm high.[410] This observation makes it likely that the timing of critical events in the key axillary cells is determined by correlative influences within the meristem and/or recently initiated leaf primordia. A similar situation seems to prevail in several other species, e.g., *Geum*, *Fuchsia*, and *Pharbitis*, since the meristematic cells that generate the axillary flowers in these plants respond to floral stimuli only during brief periods (See also Volume II, Chapter 6, Figure 6).[31,407,411]

5. Flowering Gradients

The expression of the reproductive condition clearly changes along the axis of many plants. A basipetally decreasing potential to flower at axillary positions is commonly observed. Explanations for this flowering behavior have probably been sought most in day-neutral tobaccos where decapitation readily reveals this gradient (Figure 4A). Axillary buds located in the apical part of the stem flower rapidly; whereas, those located in the basal part of the stem do so far more slowly, only after production of a much greater number of leaves. Chailakhyan and Khazhakyan have shown that apical axillary shoots grafted into apical nodes flower rapidly, whereas when grafted into basal nodes, they flower slowly (Table 3).[413] Basal shoots grafted into basal nodes remain extremely slow to flower whereas if grafted into apical nodes, they flower relatively rapidly. Similar results have been reported by McDaniel and Hsu for the Wisconsin(W) 38 cultivar, another day-neutral tobacco.[412] Thus, the ability of an axillary

bud to flower is altered by moving it up or down the stem. Flowering is a function of the bud position on the stem rather than of its inherent "ontogenetic rank" (or "physiological age"). Further support for the primacy of position is that apical and basal axillary buds flower at approximately the same time and after having produced the same number of leaves when totally isolated from the mother-plant.[412,413] Released from the plant influence, the buds apparently have no "memory" of their original location. According to the experimental data of Chailakhyan and Khazhakyan, this influence does not come from the roots or leaves but from the stem, and is perhaps due to interaction among the nodes.[414-416] Indeed, disruption of phloem transport at various places along the stem by girdling considerably disturbs the flowering gradient in these plants and may even result in the establishment of a gradient opposite to the normal one (Figure 4B).

The outcome of all these observations is that flowering gradients, as many other aspects of the process of flower initiation, appear to be generated by correlative influences. Thus, axillary meristems respond to information from the rest of the plants. This information might be one or more chemical gradients which change as the number of nodes increases (to be discussed in Volume II, Chapters 4 and 7).

Whether or not these conclusions can be extended to species other than day-neutral tobaccos is a problem left to future investigators.

CONCLUSIONS

Experiments described in this section have shown beyond any doubt that meristems are not merely passive receptors for the floral stimulus. In *Xanthium* and perhaps other species, buds (or young leaves) might even be required in addition to mature leaves for photoinduction to proceed. The fact that flowers are formed in many perennial and monocarpic plants in some axillary sites only, whereas the terminal meristem of shoot and other axillary meristems may remain vegetative, implicates correlative influences in the control of initiation of flowering. Many other aspects of this developmental process, flowering gradients for instance, are apparently also dependent on this kind of influences. In general, the situation in monocarpic plants does not appear basically different from that in perennials.

The fate of all meristems of a plant is thus under the continuous influence of a network of interactions between each of them and several other plant parts. Both long- and short-distance interactions have been observed. Examples of long-distance interactions are found in the influence of the root system or mature leaves on apical and axillary meristems. Short-distance interactions are within the apex, for instance, between nascent axillary meristems and their subtending leaf primordia and/or the apical meristem. Apical dominance, which has been shown to play such a decisive role in the flowering of many plants, belongs to both kinds of interactions since it may affect flower formation in axillary meristems close to or remote of the apex.

III. CORRELATIVE INFLUENCES IN CULTURED PLANT PARTS

A. Studies with Isolated Apices

It was mentioned in Volume I, Chapter 6, Section III.A.4., that excised vegetative apices of the SDP *Perilla* initiate flowers in noninductive as well as inductive conditions provided they only keep extremely young leaves. If these apices have two additional pairs of unfolded leaves, they retain the SD requirement. As already discussed, this finding suggests that leaves in LD actively inhibit flower initiation in this species.
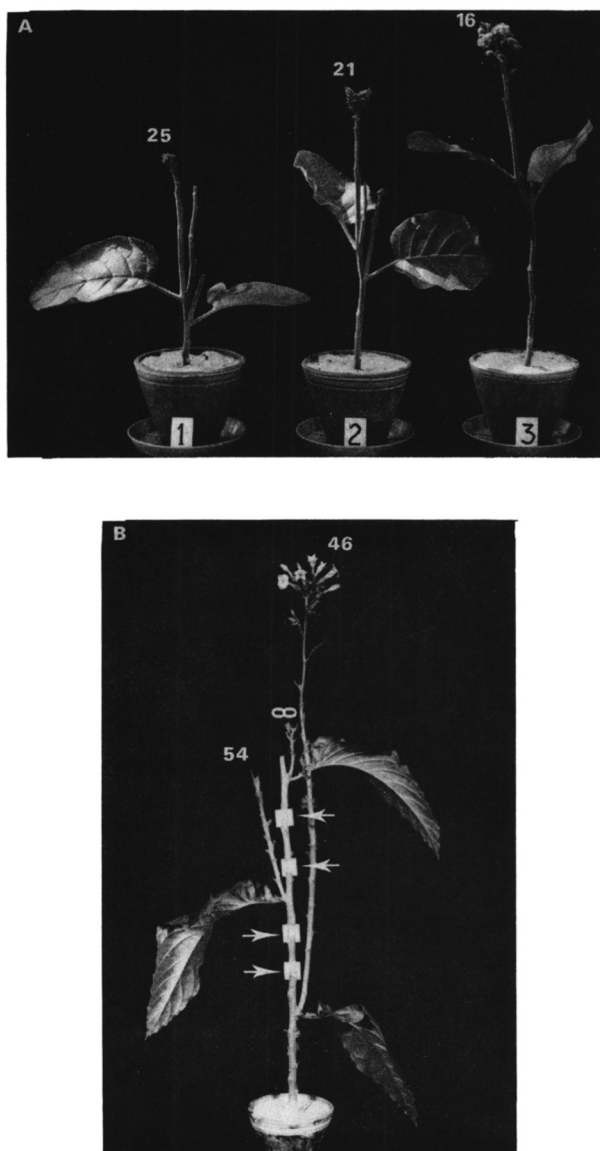
FIGURE 4.   Flowering gradient in day-neutral tobaccos. (A) Normal gradient in *Nicotiana rustica*. After decapitation of the main stem, 2 axillary shoots in the (1) basal; (2) middle; or (3) apical part of the stem are allowed to grow. Two leaves only are left on each plant. (B) Reverse gradient in *Nicotiana tabacum* var. Trapezond obtained after girdling of the stem. Girdlings (arrows) were made below the apical axillary shoot, above and below the middle shoot, and above the basal shoot. (The figures above the axillary shoots are days to appearance of flower buds). (From Chailakhyan, M. Kh., *Fiziol. Rast.*, 22, 1111, 1975. With permission.)

Table 3
FLOWER INITIATION OF AXILLARY BUDS MOVED UP OR DOWN THE STEM IN THE DAY-NEUTRAL TRAPEZOND TOBACCO

Original location of the axillary bud	Node in which the axillary bud is grafted	Days to macroscopic appearance of flower buds	Length of axillary shoot (cm)
Apical	Apical	38	63
	Basal	54	35
Basal	Basal	Vegetative growth	31
	Apical	45	52

Adapted from Chailakhyan, M. Kh. and Khazhakyan, Kh. K., *Dokl. Akad. Nauk SSSR,* 224, 1445, 1975.

The temperature requirements of apices excised from *Iris* bulbs were shown by Rodrigues Pereira to depend on the presence of attached young leaves and/or scales.[417] In order to flower in vitro at 13°C, these apices must be taken from bulbs first stored at 25.5°C and then at 13°C. Remarkably, if the apices are isolated with primordial leaves or scales attached, they can produce flowers without any pretreatment of the bulbs at 13°C. The promotive effect of scales can be demonstrated even when pieces of these organs are implanted in the same medium, but separated from the apices, suggesting the production of one or several diffusible floral promoters.

There is a complex influence of cotyledons on the floral transition of the seedling meristem of *Helianthus*.[418] Flowering occurs in excised apices with small pieces of cotyledonary tissues attached. Apices with larger pieces or totally devoid of attached cotyledons flower infrequently or not at all.

Contrary to expectation, the use of simplified experimental systems consisting of meristems isolated with only few attached small leaves does not result in the total elimination of correlative influences on the floral transformation. The situation with excised apices does not appear very different from that already described in intact plants. It may be argued that excised apices are still relatively complex systems since they possess leaf primordia and young internodes. Also, these apices often regenerate roots and additional leaves so that they finally become identical to miniature whole plants.

B. Studies with Explants Devoid of Meristems
A further attempt to avoid these intricate organ interactions was to grow plant fragments totally devoid of organized primary meristems at the time of excision. This kind of work was initiated by Chouard and Aghion who used stem segments;[419] it was extended later to other tissue pieces, e.g., root or petiole segments, leaf disks, inflorescence fragments, etc.

Generally, the initial size of the explant determines the kind of response that is observed. With segments of inflorescence stalks of W38 tobacco and of *Torenia* stem, flowering capacity decreases sharply if segment length exceeds 1 cm;[101,420] in pieces of flowering axes of *Lunaria* and root explants of *Cichorium*, flowering is promoted with increase in size of the explants.[104,421] These data cannot be explained simply in terms of quantities of reserves initially available in the explants, but suggest that other tissue influences, perhaps mediated by growth regulators, are important in determining the fate of the regenerated meristems.