THE BOTANICAL REVIEW

VOL. 37 APRIL–JUNE, 1971 NO. 2

THE SHOOT APEX IN SEED PLANTS

ERNEST M. GIFFORD, JR., AND GEORGE E. CORSON, JR.
University of California, Davis, California, and Chico State College Chico, California

I. INTRODUCTION

Within the last ten years there have been several reviews of structure and development of the shoot apex in gymnosperms and angiosperms, each differing to some degree in emphasis. Guttenberg (1960, 1961) provided descriptions of shoot and root apices, leaf development, and embryogeny in both gymnosperms and angiosperms. The entire subject of meristems was reviewed by Clowes (1961a), including newly emerging information on the use of radioisotopes to determine rates of cell division. Romberger's review (1963) was concerned mainly with meristems and growth of woody plants. A very exhaustive review of apical meristems, including development, cytohistology and experimental morphogenesis, was made by Cutter (1965). Nougarède (1965b, 1967) reviewed primarily the information on vegetative and reproductive apices for angiosperms, particularly from the points of view established earlier by Plantefol (1947) and Buvat (1952a, 1955). Wardlaw (1957a,

b,c, 1965a,b, 1968) formulated concepts of organization and structure of the shoot apex in terms of reaction systems and biological fields. Schüepp (1966), in a continuation of his earlier publications, was concerned mainly with analyzing histogenesis and growth in mathematical terms.

The authors do not consider the present review to be a complete analysis of all literature on the subject or even of the literature since the subject was reviewed by one of us (Gifford, 1954). Included in the review are descriptions of specific examples of apical structure, results of certain survey studies of families, cytohistological studies, methods of study, and results obtained by use of labelled isotopes. More extensive discussions of transition to reproductive growth are presented for both gymnosperms and angiosperms. Critical discussions are undertaken where they seem warranted, particularly in reference to certain espoused theories of apical growth.

II. GYMNOSPERMS

A.) Vegetative Shoot Apex

Several investigators have provided descriptions of shoot apices of several genera in the Coniferales since the subject was reviewed in a previous publication (Gifford, 1954). With some individual preferences in terminology, the general results of the more recent publications agree with previous accounts. The shape of the shoot tip and volume of the various cytohistological zones in many conifers may change markedly during the annual growth sequence, but the basic zonation pattern usually remains the same. In an effort to provide a meaningful terminology for this fluctuation, Parke (1959) has distinguished between what should be called the shoot tip and the shoot apex. The term shoot apex was used to include that portion above the youngest definable leaf. The term shoot tip was used to include the shoot apex together with varying amounts of primary meristematic tissues. This distinction has proved to be useful in descriptive accounts. For *Abies concolor* (Gord. & Glend.) Lindl., Parke (1959) recognized four zones (Fig. 1): apical initials, subterminal mother cells, peripheral zone, and zone of central tissue. There is marked seasonal activity in *Abies*, and this annual development can be divided into three growth phases: (1) rest phase, (2) first growth phase (the shoot elongates rapidly and gives rise to numerous cataphylls), (3) 2nd growth phase (a new unelongated axis bearing many needle primordia is formed). Other recent descriptions of conifers with distinct growth phases include *Pinus densiflora* Sieb. & Zucc. (Hanawa, 1966), *Cephalotaxus drupacea* Sieb. & Zucc. (Singh, 1961), *Podocarpus gracilior* Pilger. (Pillai, 1963a), and *Pseudolarix amabilis* Rehd. (Kupila & Gifford, 1963).

Hanawa (1966) recognized three growth phases in the development of the shoot in *Pinus densiflora*: (1) rest phase, (2) bud expansion,

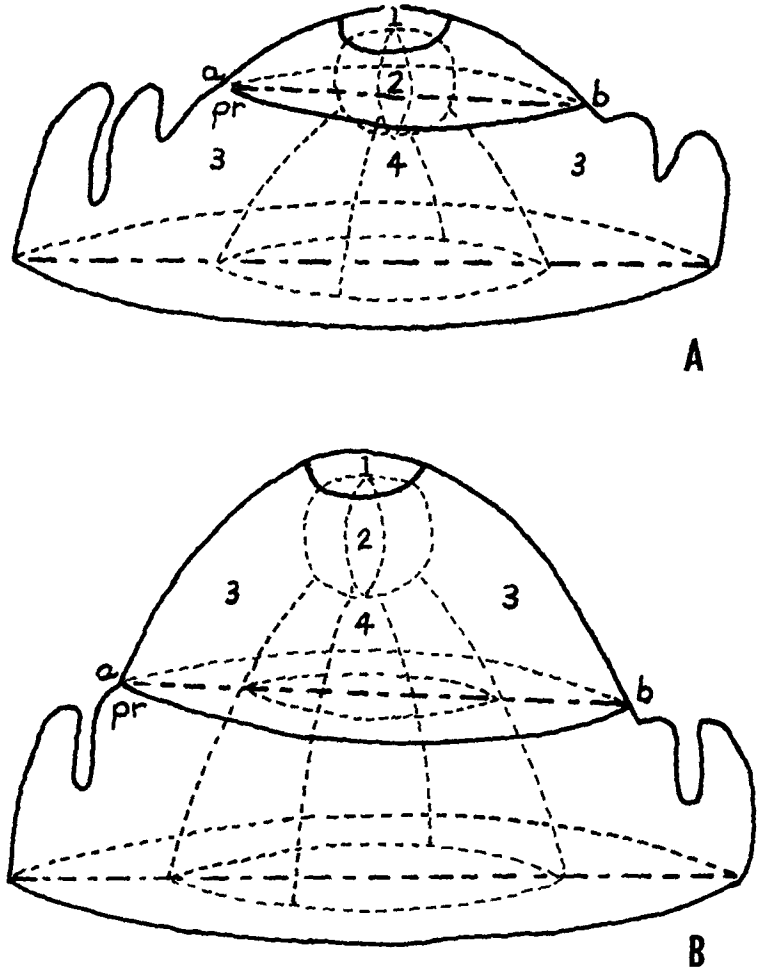


FIG. 1. Zonation in the shoot tip of *Abies concolor* during rest (*A*) and growth (*B*) phases. Zones are: *1.* apical initials; *2.* subterminal mother cells; *3.* peripheral zone; *4.* zone of central tissue. The plane *ab* represents the theoretical basal limits of the shoot apex. *pr* is the youngest observable leaf primordium (after Parke).

(3) new bud formation. The phase of new bud formation overlapped the bud-expansion phase. The ratio of height to diameter of the apex was higher during the formation of the new bud than during dormancy, but the ratio of depth of the apical meristem to diameter was, however, approximately constant all year. Hanawa (1967) noted differences in apical structure between dwarf shoots and lateral shoots. The lateral shoot is reported to pass through a juvenile stage similar to that of the dwarf shoot apex before acquiring an organization similar to that of the terminal (leading) apex.

For *Cephalotaxus drupacea*, Singh (1961) recognized four growth periods; shoot elongation and bud scale formation were considered distinct enough to be considered as separate phases. Dimensions (width and height) of the shoot apex fluctuate during the year. The diameter exhibited two minima, but the height only one, with maximum width and height occurring during the period of foliage leaf formation. Very little fluctuation, if any, could be determined for the dwarf (short) shoots of *Pseudolarix* (Kupila & Gifford, 1963).

Tepper (1963) compared dimensions and zonation of apices of the dormant leader and terminal branches in eight ponderosa pine trees. Apices of branches in upper positions in the crown tended to have larger diameters than those in lower positions. While no consistent trend in the variation of the height of the shoot apex could be demonstrated, the height/diameter (H/D) ratio of the apex increased in progressively lower positions in the crown. The H/D ratio of apices of small diameter was larger than that in those of large diameter. Cytohistological details were the same except for the increase in size of zones in apices of large diameter.

Cytohistological zonation in all the above species is basically similar, but authors have applied different terms to the various zones. Despite differences in size of apices and of zones, anticlinal and periclinal divisions occur in cells of many species at the summit in an axial position. These cells and their immediate subjacent derivatives are surrounded by a peripheral zone, the cells of which are often smaller, with greater stainable contents, and exhibit high mitotic activity preceding and during periods of appendage formation. A basal zone, the cells of which are concerned with the development of the pith, may function mitotically for variable periods of time in forming the pith. In some cases only one or two divisions occur before cell maturation. Variation in terminology is greatest for the zone that includes the summital cells. The present authors recommend use of the term "apical zone" to include those cells at the summit as well as the immediate basal derivatives. Usually these cells collectively share more cytological features than they share with cells of other zones. If desired, subdivisions (zone of apical initials and central-mother-cell zone) of the apical zone could be described. The "peripheral zone" is usually very clear and the cells of the surface layer may divide only anticlinally or both anticlinally and periclinally. The cells that give rise to the pith are the most difficult to categorize because of the variable role they play in development. Nevertheless, the terms "medullary zone" or "pith rib meristem" would seem to be appropriate.

The shoot apices of other conifers have been described. For three species of *Cupressus*, Pillai (1963b) described the common type of apical organization for conifers. There was no period of dormancy in *C. sempervirens* L., for which a seasonal study was made. During the less active months, periclinal divisions did not occur in the most apically placed cells, hence the outer layer had the appearance of a tunica.

Essentially the same type of zonation was reported to occur in *Picea smithiana* (Wall.) Boiss. (Shah & Thulasy, 1967). Jackman (1960) described apical structure for *Agathis, Podocarpus, Libocedrus,* and *Dacrydium.* For the latter three, periclinal divisions did occur in the surface summital cells, but only anticlinal divisions occurred in the surface layer (protoderm) proximal to the apical zone. The shoot apex of *Agathis australis* Steud. is similar to those described for *Araucaria* (Griffith, 1952) in having discrete surface layers in the apical zone. In fact, there are two discrete surface layers, and in this respect the apex of *Agathis* resembles those of *Araucaria excelsa* (Lamb.) R. Br. and *A. cunninghamii* Sweet. *Agathis* can be added to a group of other gymnosperms showing this type of organization, viz., *Thujopsis* (Seeliger, 1955), *Sciadopitys, Gnetum gnemon* L. (cited in Jackman, 1960), and *Ephedra* (Gifford, 1943; Seeliger, 1954). The latter genus is variable but a tunica is present with some exceptions.

Camefort (1950, 1956) applied the terminology used by Plantefol (1947) and Buvat (1952a, 1955) in his descriptions of vegetative apices of several gymnosperms. These concepts have been described in detail elsewhere (Gifford, 1954; Nougarède, 1965b; Cutter, 1965), but the essentials of the concept will be reviewed here. Phyllotaxis in most plants is described as consisting of two or three helices. Each helix ends in a lateral organogenic subapical region, the "anneau initial" or initiating ring (Fig. 6). The ring is considered to be self-perpetuating, and the last-formed primordium in a helix induces the formation of the next leaf in the same helix. The initiating ring surrounds a mitotically inactive centrally located region, the "méristème d'attente" or waiting meristem. Camefort (1956), however, applied the term "zone apicale" (apical zone) to the relatively inactive zone. The pith is said to be derived from the "méristème médullaire," situated below the "méristème d'attente" (Fig. 2A).

For gymnosperms this model was modified somewhat to accommodate cases where the shoot tip was greatly elongate (Fig. 2B). In these instances the anneau initial was visualized as consisting of a basal organogenic portion and a long region above it, the "zone d'entretien," which presumably is a zone of constant cell division or maintenance and which merges with the zone of organogenesis (see Nougarède, 1965b).

The concept of "anneau initial" was applied to the seedling apex of *Cryptomeria japonica* Don. by Tribot (1961). The leaves were said to be produced by the anneau initial. The meristematic nature of the anneau initial was correlated with the presence of large nucleoli and few vacuoles. The chondriome (mitochondria, proplastids), however, was the same in all regions of the apex. RNA was said to be more concentrated in the anneau initial, although this was illustrated only by drawings. Tribot (1961) also concluded that mitoses that occurred in the upper part of the flanks can be considered as augmenting the apical zone. The conclusions of this study illustrate, once again, the

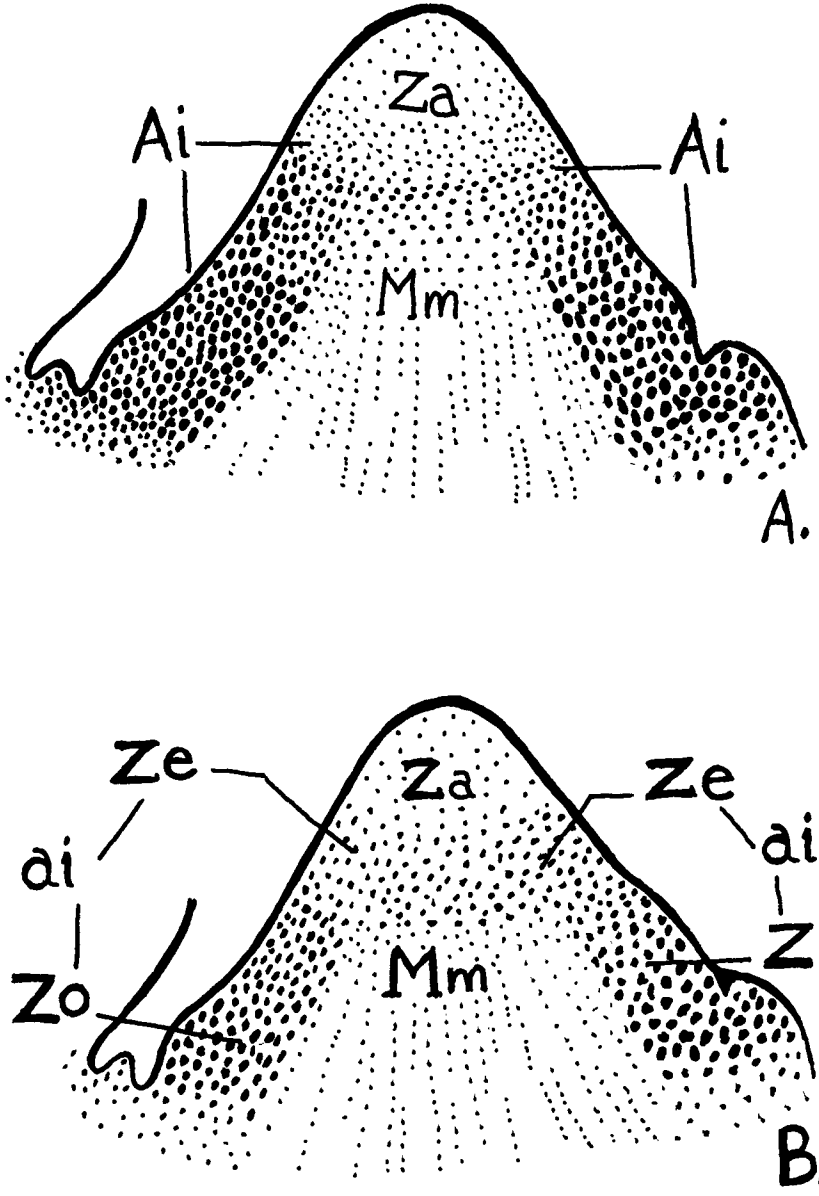


FIG. 2. Zonation in the shoot tip of *Picea excelsa* in an active growth stage. Density of stippling reflects degree of meristematic activity. *Za*, zone apicale (apical zone); *ai*, anneau initial (initiating ring); *Ze*; zone d'entretien (see text); *Zo*, zone d'organogenèse (zone of organogenesis); *Mm*, méristème médullaire (pith meristem). (*A*, after Camefort; *B*, after Nougarède).

marked divergence in opinion between various groups of investigators regarding overall development at the shoot apex.

The large percentage of nuclei labelled with H^3-thymidine in the lateral zone of the vegetative apex of *Pinus pinea* L. was said to be related to the presence of foliar helices and the functioning of an anneau initial (Taillandier, 1965). Undoubtedly the concentration of RNA was higher in the lateral zone as reported, but photographs used to demonstrate this point were taken of shoot tips exhibiting poor fixation.

The structure and cytohistologic aspects of the shoot apex of *Ephedra* have been extended through studies of several additional species. Adopting much the same concept of organization applied to the shoot apex of *Ephedra altissima* Desf. (Gifford, 1943), Seeliger (1954) observed some periclinal divisions in the tunica of *E. fragilis* var. *campylopoda* (C. A. Mey.) Stapf., five periclinal divisions being observed in 220 longitudinal sections. From a study of *E. monostachya* Dayes-Dujeu (1957) supported the concept of Plantefol and Buvat in that the author concluded that an anneau initial and "méristème médullaire" (pith rib meristem) construct each node. The internodes are constructed by intercalary meristems at the base of each node. Three phases of the plastochron were described in terms of size of the apex, number of divisions observed in each zone, structure of the nucleus, degree of vacuolation, and condition of the chondriome. At the beginning of the plastochron (stage I) there were few divisions in the apical zone and the nucleoli were small. There were abundant divisions in the flank regions (anneau initial) and the nucleoli were large. At stage II (growth of the apex) the apex was larger and the same regions were present, but much more evident. At stage III (maximal area phase, leaf buttress evident) the shoot apex showed maximum zonation. Despite differences in vacuolation and size of nucleoli the chondriome was similar in the various zones of the apex. More divisions in the apical zone were noted at stage III whereas they were few in number, if any, at other stages. Dayes-Dujeu (1957) concluded that cells of the apical zone were more active than in any investigated conifer. Paolillo & Gifford (1961) also were able to correlate mitotic activity in cells of the apical zone with a particular phase of the plastochron in *Ephedra altissima*. Development of the pith could be correlated with divisions in the apical region (see p. 209 this review).

A histochemical study of the seedling shoot apical meristem of *Pinus lambertiana* Dougl. by Fosket & Miksche (1966) provides evidence that cytological zonation can be correlated with biochemical differences between zones. Tests were performed for the identification of acid phosphatase (AP), succinic dehydrogenase (SD), and the SH groups of proteins. Zonation became apparent by eight days after planting the seed to include an initial zone (IZ), central mother cell zone (CMZ), peripheral zone (PZ), and rib meristem (RM). AP activity was high at five days after planting in the potential IZ and CMZ zones. After

these two zones assumed their usual characteristics, they no longer exhibited high AP activity. Following this reduction of AP activity, the two zones then showed a high SD activity. A study was not made of mitotic activity, but the two zones in question were at least metabolically active. At five days the concentration of cytoplasmic protein-bound sulfhydryl (SH) groups was high in the potential IZ, CMZ, and PZ and relatively lower in the RM. By the eighth day, SH was greatest in the PZ and young needle primordia. After the 13th day the apical initials contained relatively less protein-bound SH in both the nucleus and cytoplasm than in the PZ.

Seedlings also were subjected to X-radiation (500 R) for seven days. The pattern of AP was not greatly altered after one day of exposure (eight days after planting), but after 16 days AP was higher in amount in the IZ and CMZ of the irradiated apices than in the non-irradiated. Cytoplasmic SH was gradually lost during nine days of post radiation. The effect on SD was more immediate, showing an effect within one day after irradiation, particularly in the IZ and CMZ.

Irradiation completely inhibited not only the initiation of new needle primordia, but also the expansion of needle primordia formed prior to irradiation. X-ray treatment destroyed SD activity in the apical initial and CMZ zones but had little effect on SD activity in the subapical regions of the seedling. Fosket & Miksche (1966) concluded that the cells of the IZ and CMZ zones could possibly be producing substances that are responsible for initiation and early development of needle primordia. Hence, the zones of the apex are probably made up of cells that are biochemically as well as morphologically different.

Tepper (1964) reviewed earlier information on organization of the pine seedling and contributed information concerning the changes that occur at the shoot apex of *Pinus ponderosa* Dougl. during germination and growth of the seedling. Cell divisions in germinating embryos were first noted on the flanks of the apex near the cotyledons, a similar occurrence noted by Chouinard (1959) for *P. banksiana* Lamb. Cells were dividing actively in all regions of seedling shoot apices 12 days after planting, but no cytohistological zonation was evident. Leaf counts of 4- and 18-day-old seedlings indicated that, on the average, leaves were produced at a rate greater than one per day. Nucleolar differences in various zones were, however, apparent in 12-day-old seedlings as well as differences in plastid form and structure. Granular and filamentous mitochondria were present in all cells of the apex of 12-day-old plants. A zonate pattern similar to that of mature plants first appeared at 64 days. Changes in size of vacuoles and organelles accompanied the appearance of obvious zonation at the apex paralleling those described by Camefort (1956). Other changes in cell constituents were described, but not illustrated. Tepper (1964) reviewed earlier concepts of apical structure and discussed the relationships of his own results to the general problems of growth at the shoot apex.

Vanden Born (1963) made an intensive study of the distribution of several enzymes in the shoot tips of *Picea glauca* (Moench.) Voss to include peroxidase, cytochrome oxidase, phenoloxidase, succinic dehydrogenase, esterase, phosphatases, urease, and phosphorylase.

In general, peroxidase was more active in the peripheral region of the shoot tip except in the surface layer of cells. He concluded that the distribution of peroxidase may support the conclusion that this enzyme may be detected in advance of/or accompanying all cell divisions. For cytochrome oxidase Vanden Born (1963) suggested a correlation between presence of cytochrome oxidase and most rapid growth, whether by cell division or cell elongation. However, the lack of the enzyme in the peripheral zone at a time when leaf primordia are being initiated casts some doubt on the conclusions reached by the investigator. In the spring when shoot elongation was underway, succinic dehydrogenase was observed in the entire shoot tip. Staining was more intense in leaf primordia and in the outer layers of the apex during organization of a new terminal bud. It would appear that the central zone is not any less active than the outer layers of the apex. In elongating shoots high levels of esterase activity were noted only in young vascular tissues. When terminal buds began to develop, relatively strong activity was noted on the flanks of the apex and in young needle primordia. The enzyme also would appear to have been present in the surface layer of the shoot tip in the photograph used to demonstrate enzyme distribution. Acid phosphatase was observed to occur at the extreme apex and in the cortical tissue of elongating shoots, but the pattern changed later, the activity not being associated primarily with meristematic regions. Rather, it appeared to be localized between areas highly active in cell division and areas of more mature cells. When positive tests were obtained, alkaline phosphatase seemed to show the same distribution. For sulfhydryl groups, staining was greatest near the apex and in young primordia. Proteins were high in concentration in the apical cap, procambium, and secretory cells. For elongating shoots, RNA concentrations were high in the entire shoot tip and lower in underlying tissues with little or no changes occurring later. Vanden Born (1963) concluded that the histochemical evidence from his investigation supports the concept of the presence of an "anneau initial." He admitted that the visual estimate of DNA concentration (intensity of staining) supports the concept of greater activity on flanks of apex and hence, also, the existence of a quiescent central region, but he did not consider the dilution effect on the staining of large nuclei in the quiescent region. He called attention to the similarity in distribution of DNA and peroxidase which may support an association between the two, although he pointed out the inconclusive nature of the peroxidase test. Vanden Born stated that protein concentration was high in the apical cap, but in the discussion section he stated that proteins are high in the flanking regions. The results of Vanden Born (1963) and Fosket & Miksche (1966) indicate that succinic dehydrogenase is generally

correlated with active shoot growth, but the presence of acid phosphatase may not necessarily be associated with meristematically active regions.

B.) Transition to Reproductive Growth

Studies on transition to "flowering" generally have not been extended to gymnosperms except for a limited number of cases. It is probably important to distinguish between the initiation and development of microsporangiate and megasporangiate strobili. Irrespective of any implied homologies, the ovulate strobilus (megasporangiate strobilus) compares favorably with an angiosperm inflorescence and the pollen producing cone (microsporangiate) with a determinate flower. Just as for angiosperms, early transitional stages leading to reproductive structures are often difficult to obtain.

For *Chamaecyparis pisifera* Sieb. & Zucc., Hejnowicz (1957) concluded that the apex of the ovulate strobilus had essentially the same organization as that of the vegetative apex and that the intensity of cell division was not increased with "transition of apices from the vegetative to flowering state." Also, the outer layer at the tip behaved in a manner comparable to vegetative shoots with respect to planes of cell divisions. For *Larix decidua* Mill. the apices of long and short shoots have essentially the same structure, and are comparable to most gymnosperms (Gifford & Wetmore, 1957; Gifford, 1961). Because the expected positions of cones in *Larix* can be determined with some certainty, buds could be classified into the two types. For both micro- and megasporangiate shoots the organization of the apices was essentially the same as for vegetative shoots, except for dimensions of the apical or distal zone. In microsporangiate strobili, after sporophylls were initiated, cells of the apical zone underwent divisions that tended to obscure the zonate condition. This is perhaps to be expected since the microsporangiate strobilus is definitely determinate.

Gifford & Mirov (1960) summarized the information available up to 1960 with reference to the recorded dates of cone initiation in *Pinus* from widely separated geographical locations. In general, both types of strobili are initiated in late summer, fall, or even winter in North Temperate regions. For *P. ponderosa*, growing at Placerville, California, the ovulate strobilus was already 300 μ wide at the base and 125 μ high by mid-September. Growth of a main vegetative branch had ceased for the year, but the megasporangiate strobilus continued to grow through the winter. Organization of the apex was essentially similar to the vegetative apex. By March of the following year most of the bracts had been initiated, and by May 1 ovuliferous scales were present in the axils of bracts. Continued growth of the megasporangiate strobilus during the winter has also been reported for *Pinus resinosa* Ait. by Duff & Nolan (1958).

Taillandier (1966) studied seed-cone development in *Pinus maritima* Lam. using histochemical and autoradiographic techniques. She con-

cluded that cells of the apical axial zone gradually become active during the initiation of sterile bracts and fertile bracts, and zonation disappears. This quantitative approach is interesting, but some of the figures which were cited to demonstrate zonation or loss of zonation are confusing. Her *Fig. 2* (*Plate IV*) is said to represent an apex during formation of the first sterile bracts. The apex was described as being zonate, but it appears to be less zonate than one (*Fig. 1, Plate VII*) in which fertile bracts were being produced. Pyroninophilia (indication of RNA concentration) was not uniformly distributed over the apex and flanks of the apex in the latter figure. Pith rib meristem activity extended to within a few cells of the surface of the apex. This quantitative study is admirable but suffers from the perennial problem faced by histologists—that of judging the extent and limits of zones, which often becomes a subjective matter. Taillandier (1966) did not provide data on sizes of zones (particularly of the apical axial zone) during development.

In a study of the seed cone of *Pseudotsuga douglasii* [considered now to be *P. menziesii* (Mirbel) Franco], Owens & Smith (1964) concluded that there was no marked difference in the zonal pattern between lateral vegetative and reproductive buds. There was no reserved portion of the apical meristem concerned primarily with the formation of reproductive shoots. The vegetative apex showed as much variation during different times of the year as there was between vegetative and reproductive shoots during early stages of development.

A short shoot which terminates in an ovule was reported to arise in the axil of a scale on a short vegetative axis in *Taxus baccata* L. (Loze, 1965). Structure of the apex of the potential ovule-bearing shoot resembled that of a vegetative shoot apex until actual nucellus formation. At that time the "méristème d'attente" was said to become active. The integument was the last structure derived from the anneau initial, and the aril originated from the base of the integument.

Kemp (1959) concluded for *Torreya* that "both primary and secondary axis systems of the megasporangiate shoot resemble a vegetative dwarf shoot. They both originate as axillary mounds of uniformly meristematic cells, whose apices soon exhibit a zonal pattern comparable to that of the apex of the vegetative shoot of the same species." It is only at the time of actual ovule formation that vegetative zonal structure is modified by a type of coaxial growth, resulting eventually in determinate growth.

Development of the microsporangiate cone of *Cupressus arizonica* Greene following treatment with gibberellin was studied by Owens & Pharis (1967). Precocious branching was the first indication of transition from vegetative growth to reproductive. About 22 days after treatment potential strobilate apices were reported to assume a form distinguishable from vegetative apices, being shorter and wider. The mitotic frequency increased to 1.4, about three times that of the vegetative apex. Nucleoli were prominent and the cytoplasm became more dense. Transition of the reproductive apex involved the entire apex; the small subapical mother

cells and cells of the peripheral zone formed a continuous mantle of actively dividing cells.

The radioisotope H^3-thymidine was used successfully to study development of the pollen-producing cone in *Pinus maritima* (Taillandier, 1967). The reproductive buds were reported to be initiated in a manner similar to that of vegetative short shoots. The synthesis of DNA was localized initially in the lateral zone. From the time of formation of the last protective scales and during the initiation of microsporophylls, the incorporation of the isotope was homogeneous in the meristem. There is no reason to doubt the results except that the apex represented in *Figure 5* in the above cited paper does not appear to be median. This is a critical figure since it is said to represent the time of initiation of the first microsporophylls.

From the limited amount of information available, it would appear that the apices of developing megasporangiate strobili are much more similar to vegetative apices of the same species than are microsporangiate strobili. In general, this approaches the situation in angiosperms in which the organization of the inflorescent apex may become much less modified than the determinate apex of a flower.

III. ANGIOSPERMS

A.) Vegetative Shoot Apex

The following section is devoted to accounts of published descriptions of shoot apices in which the investigators employed mainly the usual techniques of sectioning and staining, and in some instances the use of isotopes for localization of cell constituents. In certain studies the behavior of naturally occurring chimerical plants could be related to apical organization, and these are described in detail. No systematic treatment has been attempted that would reflect a natural classification. In most instances the examples are identified as to their family affiliation according to Willis (1966).

Apical structure of seven species of *Bombax* (Bombacaceae) was described by Johnson & Tolbert (1960). In this article the authors introduced the term "metrameristem" to describe the central part of the shoot apex that maintains itself and contributes peripherally to growth (Fig. 3). The term metrameristem implies organic and topographic relationship to the remainder of the shoot apex. The zone would be equivalent to the surface initials and central mother cell zone in gymnosperms; to the central zone (to include tunica and corpus in an axial position) of angiosperms. If the mantle-core terminology is used, the metrameristem would be equivalent to the central part of the mantle and the central mother cell zone; or positionally to the "méristème d'attente" of the French cytohistologists. For *Bombax* there was variability in thickness of cell walls, vacuolation, and in the presence of plastids. A single layered tunica predominates in all seven species, with perhaps a second layer in

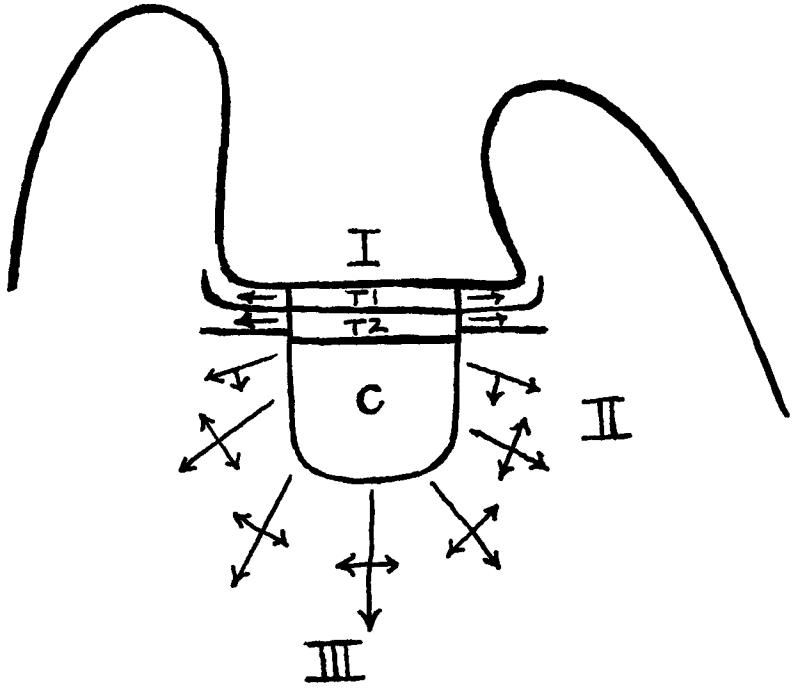


FIG. 3. Representative example of apical zonation in *Hibiscus syriacus*. Comparable zonation, except for form and size of apex, also described for *Bombax* and species of Malvaceae and Moraceae. *I*, metrameristem, to include tunica initials (T_1 and T_2) and corpus initials (C). *II*, flanking meristem; *III*, pith rib meristem (after Tolbert).

B. fendleri Benth. Johnson & Tolbert (1960) concluded that species in only seven genera of angiosperms surpass *Bombax* for size of the shoot apex. In two species (*B. ellipticum* H.B.K. and *sessile* Bkh.) there was little histological distinction between cells of the metrameristem, flanking, and pith rib meristems.

A very detailed cytohistochemical study was made of the shoot tip of *Brachychiton* (Sterculiaceae) by West & Gunckel (1968a,b). A zonate apex was described: central zone, flanking meristem, and pith rib meristem. Starch was reported to be present in the central zone and pith rib meristem, but absent in the flanking meristem. An "intercellular substance" in the central zone, often described for other plants, was identified as an acid polysaccharide. The types of cell-wall polysaccharides were determined for three transverse zones in the shoot tip: first 0.5 mm (initial growth phase), 0.5 mm to 3.5 mm (second growth phase), and 4 mm and beyond (third phase). The first growth phase was characterized by both radial expansion and cell elongation. Cell walls in the central zone were high in pectic substances, extremely low

in cellulose content, and about 20% each in hemicellulose and non-cellulosic polysaccharides. Cell walls in the second phase (characterized by radial growth) were approximately 46% non-cellulosic polysaccharides, 27% pectic substances, 20% hemicelluloses, and 7% cellulose.

The same authors (West & Gunckel, 1968b) analyzed growth phenomena and syntheses of RNA and protein that resulted in some unexpected results. Shoot tips of older plants of *Brachychiton acerifolium* Muell. and seedlings of *B. populneum* R. Br. were used in the study. The investigators considered the apices of both to be comparable since both showed zonation. For *B. acerifolium* the concentration of RNA in the flanking zone was nearly twice that in the central and pith rib meristem zones. On an RNA-per-cell basis, however, the pith rib meristem was highest, followed closely by the flanking meristem. Protein concentration was quite similar to RNA although the magnitude of difference between the flanking meristem and the other zones was not as great as with RNA. Protein content per cell in the flanking meristem was much closer to that of the central zone than the pith rib meristem, whereas the opposite was found for RNA content per cell.

RNA synthesis in *B. populneum* was measured by H^3-orotic acid treatment. After short exposure (two hours) to the orotic acid, about twice as much label was formed per cell in the flanking zone as in the central zone. With longer exposure to the isotope the ratio was about 40% in the central zone to 60% in the flanking zone. The investigators noted that the difference was not as great as might have been expected.

In the total transverse initial growth zone (0.5 segment), characterized by radial expansion and cell elongation, RNA and protein per cell increased sharply. In the 3.5 mm region (characterized by radial growth) the metabolites were relatively constant. Rates of movement of labelled RNA from nucleus to the cytoplasm was the same for both zones. Thus, the central zone deemed "quiescent" by some investigators is far from being metabolically inactive.

Tolbert & Johnson (1966) compared the structure of the vegetative apex in 40 species of the family Malvaceae. There was a wide range of size, shape, and zonation of apices within the family. Also, most of the species investigated had a more or less marked cytohistological zonation superimposed on the tunica corpus configuration. Possession of a single tunica layer was true of most species, but stratification of the upper corpus was common. The metrameristem (Johnson & Tolbert, 1960; Tolbert, 1961) was the focal point of the study (Fig. 3). There appeared to be a correlation between growth habit and distinctness with which the metrameristem was marked off from surrounding tissue. Most herbaceous species of the Malvaceae had an indistinctly marked metrameristem, whereas the shrubby trees and trees had distinctly marked ones. Lower shrubs and suffrutescent species may have either type. In regard to the metrameristem, Tolbert & Johnson (1966) stated that for the

Malvaceae all the cells of the shoot axis take their origin from this zone either directly or indirectly.

The concept of metrameristem was also adopted by Smith (1963) in a survey of apical structure in the Moraceae. The shoot apices of 42 species from 28 genera were studied. All, except two, had a tree habit with a wide range of size and shape of the shoot apices. Thirty-seven species exhibited some degree of cytohistological zonation superimposed on a tunica-corpus configuration. Ten species were considered highly zoned, eight moderately, 19 slightly, and only five with no evidence of zonation. Maximum or minimum zonation did not seem to be correlated with the tropical or temperate occurrence of a species or with its growth habit. It was concluded, however, that there is sufficient evidence to consider that a trend of increasing distinction of zonation does occur from the sub-family Moroideae to the Conocephaloideae, which may parallel secondary xylem specialization. A seasonal study was made of *Maclura pomifera* (Raf.) Schneid. Maximum cytohistological zonation occurred near the end of rapid shoot elongation. The metrameristem appeared to be subject to less size variation than the apex itself and Smith (1963) concluded that perhaps measurements of the metrameristem are more valuable for comparative purposes than those for the entire apex, particularly in plants with distinctly zoned apices.

A detailed study was made by Codaccioni (1962) of representatives of the Fagales to include descriptions of phyllotaxy and cytology of apical meristems in the winter condition, during bud expansion, and of the young seedling. Although the above author described the shoot apex of *Castanea* in terms used by French investigators, the apical zone was said to contribute some cells toward restoration of leaf sites during rapid growth of long shoots. Restoration of leaf sites is generally ascribed to the anneau initial by proponents of the "anneau initial-méristème d'attente" concept.

Kalbe (1962) described the shoot apices of several woody dicotyledons: *Euonymus europaeus* L., *E. japonicus* L. (Celastraceae), *Sambucus nigra* L. (Sambucaceae), and *Forsythia intermedia* Zabel (Oleaceae). Others studied in a more cursory way included *Syringa vulgaris* L. (Oleaceae), *Populus nigra* L. var. *italica* Du Roi (Salicaceae), and *Ilex aquifolium* L. (Aquifoliaceae). The terminology applied to these examples was that used by Guttenberg (1960, 1961): dermatogen, subdermatogen, and central meristem. The dermatogen initials give rise to the dermatogen (histogen), and the subdermatogen initials give rise to the subdermatogen (cortical histogen). The initials of the central meristem (termed the central mother cells) contribute ultimately to the flank meristem (histogen) and pith meristem (histogen). The procambium (histogen) arises from the flank meristem. This type (Type 1) of organization and development characterized *Euonymus europaeus*. For *Forsythia intermedia* (Type 2) the cortical histogen is said to be derived in part from the subdermatogen and in part from the flank

meristem. This concept was based upon a formalized histogenic description of development with very little consideration given to cytological characteristics of cells of the several regions.

Shoot apical structure of 63 species in 21 tribes of the three major groups of the Gramineae was described by Brown, Heimsch, and Emery (1957). These investigators reported a correlation between the number of tunica layers and certain other characters of systematic significance. The presence of two tunica layers was most common in the Festucoideae, whereas a single layer was most common in the Panicoideae. The authors suggested that the results of this investigation documented the first case in which the number of tunica layers seemed to be a character of systematic significance.

An intensive study of apical structure in monocotyledons and its relationship with color variegation was undertaken by Thielke (1954, 1955, 1959, 1962, 1963, 1964, 1965). In a study of the Commelinaceae she reported the presence of a two-layered tunica in *Commelina benghalensis* L. and *Tradescantia peruviensis*. The tunica was one-layered in four species of *Tradescantia*, in three species of *Zebrina*, in *Commelina forskalei* Vahl. and in *Aneilema papuanum* Warb. In *Tradescantia fluminensis* Vell. var. *albostriata* periclinal divisions occurred in the summital tunica cells that resulted in a mixture of tissues that was responsible for color variegation. If there was a reversion to the green form, a discrete tunica could be identified. Variegated forms in the family could be classified on a developmental basis according to whether they were sectorial chimeras or periclinal chimeras, and these, in turn, could be related to apical structure (Thielke, 1954). The variegated form of *Hemerocallis fulva* L. (Liliaceae) also was found to be a sectorial chimera, and this was made possible because leaves arise in very precise positions and hence the sectorial arrangement remained stable even though there was a stable tunica layer. The mesophyll was derived from underlying cells at the apex, some of which gave rise to chlorophyll deficient sectors (Thielke, 1955). That grasses with an unlayered vegetative apex can only form sectorial chimeras, not periclinal, was confirmed (Thielke, 1959). A developmental study was made of clones of species of *Saccharum* (Gramineae) and *Erianthes* (Thielke, 1960, 1962, 1963, 1964). In most clones there were periclinal divisions in summital tunica cells of young shoots, but this unstable condition was replaced by the presence of a stable tunica (divisions occurring only in the anticlinal plane). Again, sectorial patterning could be related to activity at the apex. Cytohistological localization of cytochrome oxidase could be correlated with changes at the apex. The enzyme was active in the terminal cells of young shoots, but activity was subterminal (present in the corpus) in older shoots, paralleling the structural changes that occurred (Thielke, 1965). Clones of *Saccharum spontaneum* L., however, did not show the anomalies of other species in that there was

always at least one definite tunica layer present in the apex throughout development (Thielke, 1960).

A study was made of 58 species in 33 genera of the Ericaceae and ten species in nine genera of the Empetraceae, Clethraceae, Pyrolaceae, and Diapensiaceae (Hara, 1958). The shoot apices were fairly uniform. They were generally low-domed and usually had one or two tunica layers. In each case there was a central zone, peripheral meristem, and pith rib meristem. The author suggested that tunica stratification has little phyletic significance in the group studied, whereas the type of marginal meristematic activity of leaves has considerable value.

Cladode development was studied in several species by Kaussmann (1955). In *Asparagus* and *Myrsiphyllum* (Liliaceae) and in members of the Ruscaceae (*Semele, Ruscus*) the organization of the main vegetative apex and that of laterals, early in development, are the same in that a two-layered tunica and underlying corpus could be identified. With development of the cladode a discrete second tunica layer tended to become obscure as a result of periclinal divisions, and the entire development became leaf-like in nature. Despite the pronounced flattened appearance of the cladodes of *Muehlenbeckia* (Polygonaceae), *Phyllanthus* (Euphorbiaceae), *Carmichaelia* (Leguminosae), and *Phyllocladus* (Phyllocladaceae), there was remarkable agreement with normal development of a shoot.

Hara (1962) studied the structure and seasonal activity of the vegetative apex of *Daphne pseudo-mezereum* A. Gray (Thymelaceae). There was considerable variation in the number of tunica layers with prominent seasonal fluctuations in tunica stratification. The number of tunica layers increased in September with maximum stratification occurring in March–April. Hara (1962) adopted the terminology of peripheral meristem and central zone (Fig. 4). Both were involved in the development of a "sector" and of a leaf primordium. "Depending on the state of development of a leaf primordium, the area between the apex and the primordium increases and becomes clearly distinguishable from the area of the leaf primordium." The area was referred to as the "evanescent apical cell connection" or simply the "cell connection." The area of cell connection is said to become expanded by divisions in this area as well as by divisions in the central zone. Hara (1962) admitted that the frequency of divisions was lower in the central zone, but he concluded that the zone must be a formative center. He suggested that the cell connection between the shoot apex and a leaf primordium in an apical sector plays a role in passing morphogenetic substances from the central zone to the primordium or vice versa. Hara (1961, 1962) concluded that "an apical sector differentiates into a leaf primordium and an 'evanescent apical cell connection' (or cell connection). The fully developed cell connection gives rise to a newly initiating sector and part of the stem." The region of the cell connection is somewhat comparable to the "anneau initial" in its original usage except for

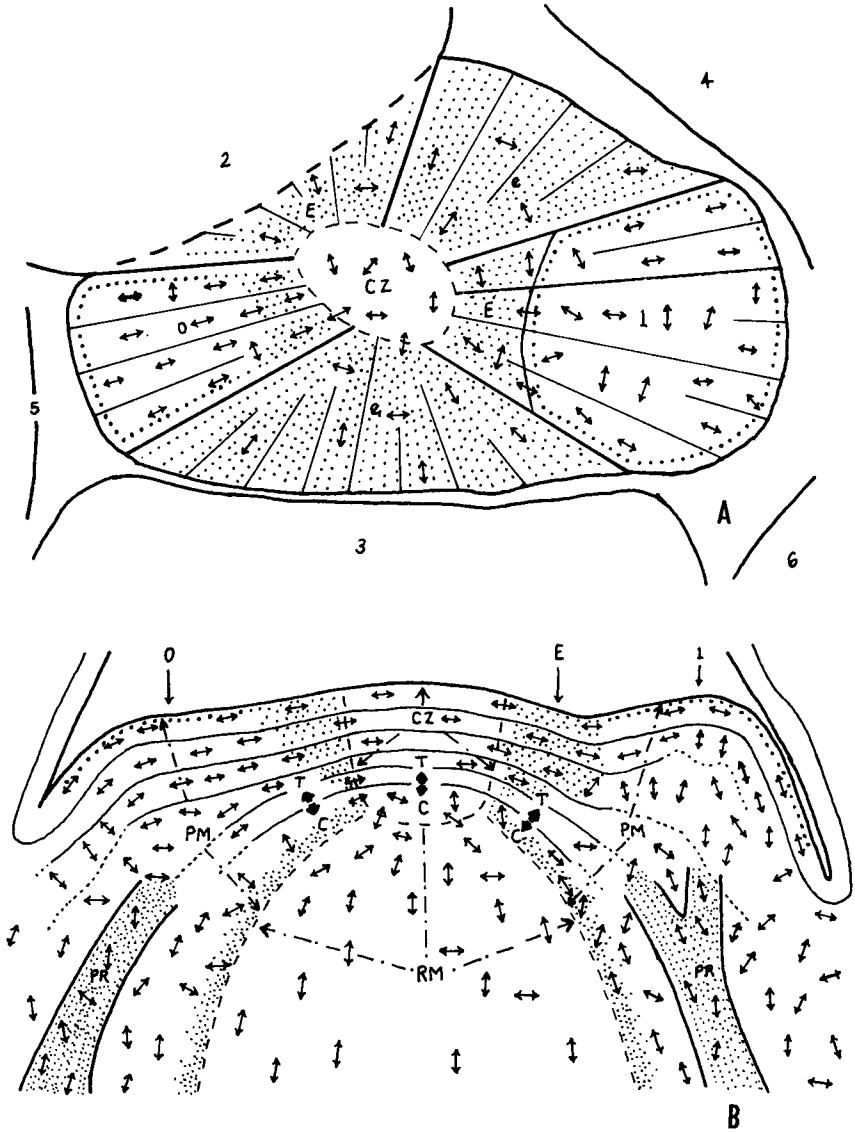


FIG. 4. Diagrams representing zonation and growth sectors of the shoot apex of *Daphne pseudo-mezereum*. *A*, transection at a level just below surface of apex; *B*, median longitudinal view. *CZ*, central zone; *PM*, peripheral meristem; *RM*, rib meristem; *T*, tunica; *C*, corpus; *PR*, procambium; *O*, initiating sector; *1–6*, successive sectors and their leaf primordia; *E*, evanescent apical cell connection (or cell connection) of developing sector; *e*, evanescent apical cell connection of developed or declining sector; arrows indicate planes of cell division (after Hara).

the author's (Hara) statement that the central zone is also a contributing zone.

Plastochronic changes of the shoot apex in *Euphorbia lathyris* L. (Euphorbiaceae) were studied by Soma (1958). The decussate leaf arrangement of this plant permitted the investigator to make a detailed and precise study of plastochronic changes. At maximal area phase the apex was essentially flat and measured 225 μ in one direction and 150 μ in the other. At minimal area the apex was a slightly rounded mound, being 125 μ wide in line with the newly initiated leaf primordia. The apex was described as having a 2-layered tunica, and a third tunica-like layer was present during maximal area phase. Following maximal area phase, the third layer was broken up by periclinal divisions, even in the central regions of the apex. Soma (1958) considered that there may be periodic shifting of the activities in the apical meristem from the central zone to the peripheral zone and from the latter to the former in relation to plastochronic changes of the shoot apex. He acknowledged the activity of a peripheral zone, but also called attention to the apparent increased activity of cells in the central part of the apical meristem during mid-stage of the plastochron. For *Euphorbia nerifolia* L. (Shah & Jani, 1964) one tunica layer was reported, although the second layer simulated a second one. The latter authors noted fluctuations in the width of the peripheral meristem, and a central meristem (includes tunica and corpus initials) was a permanent feature in all phases of a plastochron.

Our knowledge of apical structure for ranalean plants has been extended in recent years. In addition to those vesselless dicotyledons described by Gifford (1950, 1954), Ramji (1961a) has described the apex of *Sarcandra irvingbaileyi* Swamy (Chloranthaceae). The apex is larger than any other described vesselless dicotyledon, being 256–270 μ wide and 88 μ high during maximal area phase. During minimal phase the apex was essentially flat and measured 98 μ in width. The apex showed the common type of zonate pattern superimposed upon the tunica (2 layers) and corpus. During maximal area phase the apex exhibited a more stratified appearance because of a more orderly arrangement of cells of the corpus in the apical region. During minimal area phase a central vacuolate zone was reported to be absent (Ramji, 1961b). A comparison of maximal and minimal area phases suggested that cells of the more apical region must divide since the difference in cell numbers is so marked. Divisions were recorded in the central zone.

The shoot apex of *Polyalthia longifolia* Benth. and Hook. f. ex Hook. f. (Annonaceae) is small in comparison with other ranalean species, being only 68 μ wide and 44 μ high at maximal area phase (Ramji, 1960). There was a two-layered tunica with cells of the corpus often stratified into a seemingly third layer during growth to maximal phase. A zone of vacuolate cells was reported to comprise only a limited number of summital cells of the tunica.

For *Clematis* (Ranunculaceae) there is a two-layered tunica and a

shallow corpus, and the cells are cytologically uniform (Tepfer, 1960). No evidence was found for the presence of a "méristème d'attente" because mitoses appeared frequently in the central region of the tunica and corpus. In 28 apices 22 of them showed mitotic figures in the central region.

The apex of *Rauwolfia vomitoria* Afzl. (*Apocynaceae*) presents several interesting features during ontogeny (Mia, 1960). The apex of the embryo is flat and has two tunica layers. In the seedling the apex became slightly convex and flat to concave in the adult plant during minimal and maximal phases, respectively. In the adult plant the number of tunica layers increased to 3–5 layers, but this fluctuation was not related to the plastochron. During minimal area phase the cells of the tunica elongated in a direction parallel with the surface of the shoot apex. Corpus cells were larger than those of the tunica, but cells of both tunica and corpus were approximately uniform in staining qualities. Cells of the corpus were reported to be larger and more vacuolate at minimal area phase than in maximal phase. Groups of cells in both regions were surrounded by thick, common walls, a feature reported for other species (Ball, 1949; Gifford, 1950). A subjacent arc of cambium-like cells, considered to be a separate zone but derived from the corpus, gave rise to cells of the pith. An identifiable transitory flank meristem was present just prior to leaf initiation. The form of the apex in this species undoubtedly makes difficult the recognition of a flank meristem during all phases of a plastochron.

The origin and development of buds and a description of apical organization in *Boehmeria nivea* Gaud. (Urticaceae) have been provided by Kundu & Rao (1957). This species is an example of a seed plant with a "sunken" apex. The apex varied from 29–75μ in width, and the apex became only slightly concave at maximal area phase. The authors recognized two main regions: a central zone, comprising the two-layered tunica and corpus, and a peripheral zone. Each zone could be recognized by the stainability and size of cells. The pith was said to be derived from derivatives of the central zone and the inner derivatives of the peripheral zone. Since most of the peripheral zone was utilized in the formation of a leaf, the width of the central zone reflected the size (width) of the apex at minimal area phase. The authors concluded that cells of both the central zone and peripheral zone were involved in maintaining the shape and size of the apex. Buds were initiated from the second layer at the base of a leaf primordium, not from truly axial meristematic tissues as is true for many angiosperms.

The yearly cycle of activity of a tree such as *Acer pseudoplatanus* L. (Aceraceae) was shown to be correlated with cytochemical variations in various regions of the shoot apex (Catesson, 1960, 1964). The level of RNA was always high in the spring and summer, diminishing in autumn and winter at a time when lipids and starch were present in greater abundance. In May, for example, there was very little if any lipids or

starch in the apex at a time when the cells of the peripheral zone (anneau initial used by Catesson) exhibited a high concentration of RNA.

Interest has developed in the structure of the shoot apex of certain aquatic angiosperms. The organization of the plant body is somewhat different in these plants from other angiosperms. The shoot apex, likewise, exhibits different structural characteristics. For *Elodea* (Hydrocharitaceae) Savelkoul (1957) divided the apex into a distal zone and a proximal zone, the latter into a peripheral and a central zone. This manner of organization was based upon relative size of cells and the size, shape, and staining reactions of nuclei. Cell divisions were observed in the distal zone, which led Savelkoul (1957) to disagree with Buvat's earlier claims (e.g., 1952a) that cells located in a distal position (méristème d'attente) do not divide nor do they contribute to vegetative growth of the plants.

In an effort to explain this deviation from the concept of Buvat (1955), Lance-Nougarède & Loiseau (1960) concluded from a study of four aquatic or semi-aquatic plants that the "anneau initial" and "méristème d'attente" did not exist in these plants. The presence of an anneau initial and a méristème d'attente were said to be intimately related with medulated shoot axes. These aquatic plants have central vascular strands in the stem. The organogenesis of the stem, therefore, was considered to be inseparable from that of the leaves in medulated axes. In non-medulated plants the leaves are not associated with leaf gaps and have less influence on apical organization; the central procambium extends above the last-formed primordium, much as it does in many lower vascular plants (Freeberg & Wetmore, 1967) that have microphylls. Lance-Nougarède & Loiseau (1960) recognized an apical zone, the cells of which were uniformly rich in RNA. For two species (*Hippuris vulgaris* L., Hippuridaceae; *Callitriche stagnalis* Scop., Callitrichaceae) they described a subapical group of cells ("massif subapical") in which the concentration of RNA would appear to be greater than in the apical zone (although no special note was made of this feature). These cells surmount the central procambial strand. A comparable situation appeared to exist in *Ceratophyllum demersum* L. (Ceratophyllaceae), but the zone was not labelled (*Fig.* 3, Lance-Nougarède & Loiseau, 1960). If the subapical group is the initial source of procambial cells, it would seem that they should be included as a definite zone of the apical meristem since some of the cells, at least, lie above the last-formed leaf.

For the aerial shoots of *Hippuris vulgaris*, Jentsch (1960) reported no change in form during a plastochron because the leaves arise rather far from the shoot apex. There was variation in width of the apical cone, and this was correlated with the number of tunica layers, which varied from four to six. The number of layers varied with the individual plant, but the question remains whether a shoot changes in this respect during ontogeny. For the aerial shoots of *Myriophyllum brasiliense* Camb. (Haloragidaceae) and the submersed shoots of *M. pinnatum* (Walt.)

Britt., there were no plastochronic changes in form of the shoot apex. The tunica consisted of two layers in both species. No cytological details were given for *Hippuris* or *Myriophyllum.*

A seasonal study of the vegetative apex of *Myriophyllum heterophyllum* Michx. in the production of its three leaf types (submersed, transitional, and aerial) revealed great similarity in the histology of the apex, being constructed of two tunica layers and a corpus (England & Tolbert, 1964). Fluctuations were reported to occur in height and width of the apex during a plastochron. Periclinal divisions may occur in summital cells of T_2. The concept of "metrameristem" was applied to the shoot tip, and the metrameristem was considered to include tunica initials and corpus initials, distinguishable only by cell configuration and orientation. The outer cells of the corpus and the adjacent tunica layers formed a flanking meristem. No tests for RNA were made, but cells of the metrameristem appeared to be cytologically similar to their immediate derivatives.

In summary it would appear that for certain aquatic plants the apex does not have the general type of zonation common to many other angiosperms—an apical zone of large, vacuolate cells surrounded by a flank meristem or peripheral zone.

A survey of form and structure of embryonic shoot apices of 85 species in 26 families of dicotyledons revealed generally two types (Senghas, 1956b). The dormant embryonic shoot tip may be rather small with no primordia except the cotyledons, e.g., *Nicotiana, Senecio*; or, there are types in which plastochronic changes take place and leaves are formed of varying numbers, e.g., *Echinops, Astragalus, Pisum* (seven leaves formed). The number of tunica layers varied from one to two, but this was not correlated with the degree of development of the embryonic shoot.

For *Hydrangea* (Hydrangeaceae) and *Philadelphus* (Philadelphaceae) Jentsch (1956) concluded that the tunica was two-layered for both vegetative and floral shoots. Leaves arose in T_2. In *Deutzia* (Philadelphaceae) the apex possessed three tunica layers, and the initial divisions leading to leaf development occurred in the third tunica layer.

In the Commelinaceae the investigated species had a two-layered apex with leaves arising in T_2 (Rohweder, 1963). The latter worker adopted essentially the zonation concept of Rauh & Reznik (1953) with modifications. Rohweder (1963) concluded that the structure of the apex of a flower in the family agrees with that of the vegetative apex in layering and zonation, although the "Flankenmeristem" of the flower is less characteristic in its functioning than in the vegetative apex. No dyes were used, however, to localize specific cellular constituents.

Hageman (1963) concluded that the structure of apical meristems, whether in the vegetative or floral developmental phase, are fundamentally alike. He reported only quantitative differences between vegetative and inflorescent apices for *Oenothera biennis* L. (Onagraceae),

Digitalis purpurea L. (Scrophulariaceae), *Hesperis matronalis* L. (Cruciferae), and *Cheiranthus cheiri* L. (Cruciferae). He could not establish the important and essential role purported to be played by the "méristème d'attente" during flowering (Buvat, 1955).

B.) TRANSITION TO FLOWERING

1. **Dicotyledons.** Flowering in angiosperms is expressed in such diverse and varied ways that one wonders, on a developmental basis, whether it is possible to categorize them into any logical sequence. The flowering response may be expressed as the production of a single terminal flower, as an elongate, modified inflorescence with or without a terminal flower, as a compact capitulum, or as individual flowers in the axils of typical foliage leaves. Angiosperms respond in various ways to photoperiods, and there is good evidence that the apices of certain species exhibit varying modes of organization when subjected to photoperiods that do not favor flowering. Recently, investigators have favored species that clearly respond to a limited number of photoperiodic cycles (in some cases one inductive cycle being sufficient to induce flowering). These species, interesting as they are, may not be the best material for gaining a deeper insight into the general ontogeny of flowers and inflorescences. Woody perennials of temperate regions present other complications. Inflorescences or single flowers are often formed terminally or in the axils of leaves either in the spring of the current year or during the previous summer or fall. Hence, some caution should be observed in attempting to make generalizations about the histological details of the transition from vegetative growth to that of reproductive growth. Some of the conflicting reports concerning cytohistological transformation during flowering are probably the results of differences in sampling, choice of fixatives and dyes, and undoubtedly the adherence to preconceived ideas.

The concept of homology between vegetative and flowering shoots can be traced to the writings of Goethe, and there are those today who adhere to this basic concept. The organogenic studies of floral development by Strasburger supported the shoot-like nature of the flower, e.g., the order of development of floral parts in the majority of angiosperms is acropetal. Likewise, the results of early studies of vascularization of the flower supported the homologous concept. Studies of development at the histological level were slow in evolving. In 1938 Grégoire categorically opposed the theory of homology. For Grégoire the reproductive meristem possessed a structure fundamentally different from the vegetative meristem. A single flower or an inflorescence was not formed by the principal vegetative apex but, rather, originated from lateral buds. A meristematic mantle (manchon méristèmatique) was present in the apices of reproductive axes, but it was not formed from a transformation of the tunica-corpus layers present in the vegetative apex. The two

superficial layers of the manchon would give rise to the floral parts as well as contributing cells to a parenchymatous core (porte-méristème).

Grégoire's concept did not receive enthusiastic support and in the period that followed most investigators concluded that the floral apex and vegetative apices were quite similar, and in many instances the similarity in tunica-corpus organization of the two types of meristems was stressed.

In 1955 Vaughan described the morphology and growth of vegetative and reproductive apices of species of *Arabidopsis, Capsella* (Cruciferae), and *Anagallis* (Primulaceae). These examples represent two general types of inflorescences, but Vaughan concluded that the same type of tunica-corpus organization and cytohistological zonation occurred in vegetative and inflorescent apices of these species although their form may vary.

In contrast to Grégoire, Plantefol (1947) considered that the floral meristem originates by progressive transformation of the vegetative apex. According to Plantefol the "anneau initial" (see page 203 this review) would function during vegetative growth in the production of foliage leaves along a limited number of helices. Also, sepals are derived from the anneau initial, or it would finally be utilized in the formation of petals. Stamens and carpels would be derived from the residuum of the apex (Buvat, 1952a). This residuum was called the méristème d'attente (Bersillon, 1951) or waiting meristem (see page 203 this review), and Buvat applied the term to species with single flowers or inflorescences (Buvat, 1952a,b). This point of view was supported by Lance (1957) for several species and by Bersillon (1955a) for *Papaver* (Papaveraceae).

In the time period 1957–58 students and associates of Plantefol studied inflorescence development in *Beta* (Chenopodiaceae) and *Cleome* (Cleomaceae), the results of which appeared to be at variance with each other with reference to the concepts of anneau initial and méristème d'attente. For *Beta vulgaris* L. the méristème d'attente was described as undergoing dedifferentiation at the time of flowering in the formation of the inflorescence, including the terminal flower (Lance & Rondet, 1957, 1958). New lateral organogenic and axial zones were formed from the activated méristème d'attente. A homogeneous "meristematic mantle" (in the sense of Grégoire) did not exist; however, they could not reconcile the functioning of the new organogenic lateral zone with the original anneau initial of the vegetative shoot.

For *Cleome*, Hadj-Moustapha (1957, 1958) concluded that the inflorescence was constructed by the same anneau initial that was functional during vegetative growth. The initiating ring of the inflorescence was given the name "anneau initial inflorescentiel." Also, in individual axillary flower primordia there was an "anneau initial floral" and a méristème d'attente. No terminal flower is produced in the inflorescence of *Cleome*.

Plantefol (1957) attempted to reconcile these differences in ontogeny

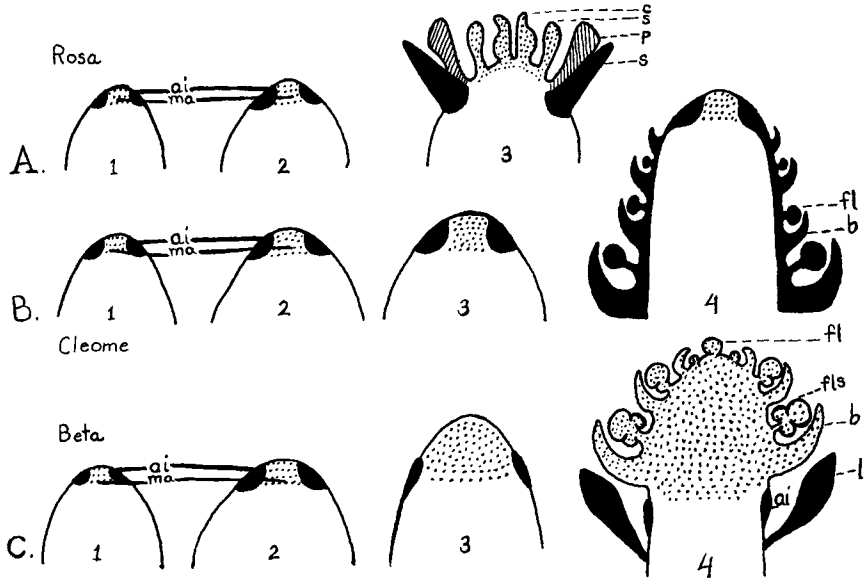


FIG. 5. Diagrammatic representation showing the utilization of the "anneau initial" (*ai*) and "méristème d'attente" (*ma*) in the formation of a single terminal flower (*A*, *Rosa*), an inflorescence without a terminal flower (*B*, *Cleome*), and an inflorescence with a terminal flower (*C*, *Beta*). Numbers 1–4 represent developmental stages for each example. *s*, sepals; *p*, petals; *st*, stamens; *c*, carpels; *b*, bracts; *fl(s)*, flower(s); *l*, leaves (after Plantefol).

of inflorescences. According to Plantefol the anneau initial and the méristème d'attente may play different roles at the time of flowering (Fig. 5). For *Cleome* the anneau initial was said to continue its function in the inflorescence. Also, the original méristème d'attente remained since no terminal flower was formed. In *Beta*, which ultimately forms a terminal flower, the méristème d'attente formed all of the inflorescence. Thus, the critical factor of what role is played by the vegetative anneau initial in flowering depends on whether a terminal flower is produced, according to Plantefol (1957).

Have the above generalizations of Plantefol been supported by the results of other investigations? To answer this question we would like to call attention to studies of floral development in certain selected examples. Philipson (1947) described the role of the flanking zone and its encroachment upon the central zone (roughly equivalent to the méristème d'attente in extent) during initiation of the capitulum in *Bellis perennis* L. (Compositae); recently, however, Philipson (1966) credits the American school with this concept. Bersillon (1955a,b) studied vegetative and floral apices of several genera and species in the Papaveraceae, with special emphasis on *Papaver somniferum* L. The

vegetative apices of all investigated species in the latter study were described in terms of anneau initial-méristème d'attente. During development of the flower the sepals were said to be derived from the anneau initial, whereas the other floral appendages were initiated in a meristematic mantle which in turn was described as having been derived, for the most part, from the méristème d'attente of the vegetative apex (comparable to *Rosa*, Fig. 5). He did not rule out the possibility of partial origin from the residue of the anneau initial. The pith of the flower was said to be the continuation of that of the vegetative shoot, a conclusion in variance with the original French concept. Development of procambium, vascularization, and anatomy of floral parts were included in the study. Floral development in *Papaver somniferum* was also studied by Sárkány & Percs (1957). For *Oenothera biennis*, the vegetative and inflorescent apices were described as being essentially similar. Also, the inflorescent apex exhibited plastochronic functioning (Bersillon, 1957). The same general type of development was described for *Reseda lutea* L. (Resedaceae) by Bersillon (1958). The latter considered zonation and functioning of the inflorescence to be analogous to that of the vegetative apex. There was no abrupt discontinuity between functioning of inflorescent and vegetative apices.

In the special case of *Jussieua grandiflora* Michx. (Onagraceae), zonation was evident only at maximal area phase during vegetative growth. During minimal area phase the apex was active throughout the apex. All regions of the apex participated in leaf-site preparation. A méristème médullaire in the original sense did not exist, and the reproductive apex was comparable to the vegetative apex. Flowers were produced in axils of vegetative leaves, and the passage to the reproductive phase was marked only by the appearance of flowers (Michaux, 1964).

In contrast to *Beta* (which ultimately forms a terminal flower and in which the anneau is considered to be absent after induction), *Agrimonia eupatoria* L. (Rosaceae) was reported to retain the organogenic functioning of the anneau initial and the inactivity of the méristème d'attente in the inflorescence (Phelouzat, 1957, 1964a). The méristème d'attente was said to participate directly in growth only when the terminal flower was formed, after the formation of sepals. Thirty to 90 lateral flowers were formed before the méristème d'attente assumed an active role. The latter author concluded that both modes of development (early activation of méristème d'attente vs. late) can exist for the same type of inflorescence, but in different species. The general conclusions arrived at by Plantefol (1957) would not seem to be borne out by the results of Phelouzat (1957, 1964a).

The remainder of this section will be devoted to discussions of flowering in representative species of genera in certain families of angiosperms.

Pharbitis nil Chois. (Japanese morning glory) has been used extensively in experiments on flowering, and much useful information of

general interest has been obtained from studies of this species (Hillman, 1962; Salisbury, 1963; Takimoto, 1969).

Although *Pharbitis nil* (Convolulaceae) forms flowers in the axils of vegetative leaves, the first flower to be formed, if the plant is induced to flower at the cotyledon stage, is terminal. Plants subjected to one long night (16 hours or more) at the time the cotyledons are fully expanded will flower. The developmental processes are very rapid; stamen primordia are evident within five days after the inductive night.

An histogenic study was made by Marushige (1965a,b,c) although cytological details were not included. Marushige (1965a) described the vegetative shoot apex of *Pharbitis nil* as having one tunica layer. Two days after seed hydration zonation of the apex became apparent, viz., central zone, peripheral zone, and rib meristem. A transitional zone, the cells of which were derived from the central zone, gave rise to cells of the peripheral zone and rib meristem. The vegetative apex of an 11-day-old seedling, and later the adult apex, exhibited even more pronounced zonation.

Under non-inductive long-day conditions the axillary buds of the first foliage leaf developed a zonation similar to the apex of the main shoot in the adult condition, but remained dormant (Marushige, 1965c). If the main axis was decapitated the axillary buds developed into floral primordia under inductive conditions. Under short-day conditions (following an initial five days under continuous light) the axillary bud apex developed an organization comparable to the adult shoot apex without passing through the juvenile stages characteristic of the main axis, in forming the flower.

When seedlings of *Pharbitis* with fully expanded cotyledons (five days after hydration) were subjected to an inductive dark period, they initiated a terminal flower bud after having produced four or five leaves, with single flower buds in their axils. Marushige (1965b) stated that the five-day-old seedlings have the juvenile type of apex (in organization). During the first three days of short-day cycles, the histology of the shoot apices differed very little from those plants of the same age grown under long-day conditions. After four or five inductive cycles the first morphological changes appeared in the apex. Cells of the peripheral zone exhibited large, light-staining nuclei, and their nucleoli became large and dark staining. After six inductive cycles sepals were initiated and the floral apex became organized into outer and inner zones. As the central and peripheral zones became transformed into the outer zone of the floral apex, the nucleoli became large in cells of these regions. However, when the apex increased in diameter prior to initiation of stamens, petals, and carpels, the nucleoli became indistinct despite the occurrence of active cell division. He stated that he could not relate nucleolar changes with plastochronic stages in the vegetative apices.

The main shoot apex of seedlings in *Pharbitis nil* is seemingly much

more sensitive to inductive conditions than the same apex of "adult plants," because the latter often do not produce terminal flowers even under optimum inductive conditions. Since the differences between apical organization in juvenile and adult plants is one of degree, the difference in sensitivity to induction is probably due to some other factor.

Cytohistological studies of development were conducted by Gifford (1963) and Healy (1964). The vegetative apex of *Pharbitis nil* was described by Gifford (1963) as having the common type of organization —central or apical zone, peripheral zone, and pith rib meristem. An increase in cytoplasmic RNA occurred within one day after the end of an inductive long night. This increase occurred throughout the apex, but more so in the central zone. A significant increase in total protein occurred two days after the end of the inductive night, which might be expected to result from increased RNA production. Sulfhydryls of the -SS- type increased with induction; they have been shown to be important in the timing process in flowering (Salisbury, 1959). The essentiality of nucleic acid metabolism in flowering has been studied using *Pharbitis* as the test plant (Zeevaart, 1962; Galun et al., 1964; also see Salisbury, 1963). Healy (1964) added significant details to the list of changes that occur during floral induction in *Pharbitis*. He reported a great increase in the number of ribosomes in the central zone and a lesser increase in the peripheral zone during the first day after the end of the inductive dark period. This increase occurred concomitantly with an increase in RNA. Dictyosome number and activity increased greatly from the second to the fourth day after the end of the inductive dark period. The endoplasmic reticulum (ER), which usually occurred as a single layer along the cell wall in vegetative apices, increased throughout the cytoplasm. There was apparently no increase in the concentration or change in the form of the mitochondria of the floral apex. Cells of the developing meristematic mantle had dense vacuoles, somewhat smaller and less numerous than those in the central zone and upper peripheral zone of the vegetative shoot apex. Healy (1964) concluded that post-inductive changes in *Pharbitis* fall into two groups: (1) those occurring during the first day after induction; and (2) those occurring after morphological change is observed in the apex. The first group includes an increase in RNA, ribosomes, and the activity of dictyosomes in the central zone, an increase in protein in the peripheral and central zones, and the beginning of starch digestion. The second group includes an increase in the number of dictyosomes and extent of ER in the developing meristematic mantle of the floral apex. Gifford (1963) and Healy (1964) both used controls (plants which received a light break in the middle of the dark period) and these resembled the vegetative plants in every respect. Therefore, the observed cytological changes were undoubtedly associated with floral induction.

Wada (1967, 1968) reported an increase in the mitotic index for *Pharbitis nil* at the end of a 24-hour dark period with an increased rate

of leaf production occurring at 48 hours (before the appearance of floral parts).

An early marked increase in rate of leaf production was true also for *Chenopodium amaranticolor* Coste and Reyn. (Thomas, 1961) and for *Chrysanthemum* (Schwabe, 1959).

Seedlings of *Datura stramonium* L. (Solanaceae), although not photoperiodically sensitive, form a terminal flower after the seventh or eight leaf is produced (Corson & Gifford, 1969). Subsequently, vegetative shoots are produced from buds in the axils of foliage leaves, and each of these shoots then ultimately terminates in a flower. These processes of branching and flowering are then repeated. Seedlings grown in plant-growth chambers (controlled conditions) exhibited a constant rate of leaf development which enabled the investigator to obtain and study specific apical stages (Corson, 1969; see page 195, this review). The mitotic index of an upper axial region was significantly lower than in other zones where mitotic indices were not significantly different. During floral transition the mitotic index of the axial zone increased significantly. Little, if any, cytohistological zonation could be detected in vegetative apices using common staining methods, but staining with pyronin did reveal a slight zonate pattern for RNA distribution. The concentration of RNA was less in the axial apical zone. With transition to flowering there was a general increase in the mitotic index of all zones, and a significant increase in the mitotic index of the axial apical zone paralleled the increase in the concentrations of RNA and total proteins. The concentration of RNA in the upper axial region, although higher than in the vegetative apex, remained lower than in the peripheral regions at the time of late transition to flowering. The authors (Corson & Gifford, 1969) were unable to establish an abrupt or marked dedifferentiation of a definite méristème d'attente-like zone during transition to flowering.

Morphology and ontogeny of the inflorescences of several species of *Nicotiana* (Solanaceae) were studied intensively by Diomaiuto-Bonnand (1962, 1966; also see Bonnand, 1959a,b, 1960). In the species examined (*N. glutinosa* L., *tabacum* L., *rustica* L., and *nudicaulis* Watson) the inflorescence is basically a modified cyme. The development of the apex from the vegetative condition to flowering was described and the terminology of previous French investigators was utilized. In certain instances reversions of the flowering axis to the vegetative occurred. One of the buds of the cyme could become a leaf-producing bud. Several reversions could occur on the same inflorescent axis. Diomaiuto-Bonnand (1966) concluded that this anomalous situation was the consequence of an unstable state of equilibrium between the floral and vegetative states, particularly in the greenhouse when light intensity may have been insufficient for normal growth.

Waterkeyen et al. (1965) and Nitsch & Nitsch (1965) were able to correlate gross morphological changes of the apex in the course of

induction with changes in endogenous growth substances for the cultivar "Maryland Mammoth" (short-day plant) of *N. tabacum* and the long-day species *N. sylvestris* Spegazzini and Comes. Instead of one or two auxins, which previous workers had found in *Nicotiana*, there appeared to be a whole constellation of active endogenous growth substances, both auxins and gibberellins. Chromatographic investigations revealed the existence of at least five different endogenous growth substances which varied in concentrations in the apex, immature leaves, and mature leaves and which also varied in time during photoperiodic induction to flowering.

Members of the Chenopodiaceae, particularly *Chenopodium*, have become favorite species for cytohistological and physiological investigations of flowering. Mention has already been made (page 166) of the descriptions of flowering in *Beta vulgaris* by Lance & Rondet (1957, 1958) and the importance given to the relatively inactive central zone (méristème d'attente) during the first year of growth. From a dedifferentiation of the méristème d'attente two zones are said to arise: (1) a lateral organogenic zone and (2) an axial apical zone; but the authors could not reconcile the organogenetic lateral zone with the anneau initial of the vegetative apex on a functional basis.

Cytohistological and autoradiographic studies were made of shoot development and floral induction in *Chenopodium album* L. by Gifford & Tepper (1961, 1962a,b). On a short-day regime the plastochronic interval decreased and bud primordia were formed precociously. The entire primary inflorescence was considered to be a compound dichasium, and after seven or eight days a terminal flower was formed on the main inflorescent axis which was, in fact, the continuation of the vegetative axis.

The vegetative shoot apex had a biseriate tunica, the cells of which near the summit were quite vacuolate, and the nucleoli of cells of T_2 were larger than those in any other region of the apex. The apex had the structure of what has been described as the intermediate stage (see page 185, this review).

Upon photoinduction the apex gradually became stratified with four to five layers being present. After four inductive cycles the nucleoli of all cells of the apex tended to be of uniform size. Mitotic activity increased with photoinduction, as might be expected, followed by a definite increase in RNA as well as in total protein by the fourth day after the beginning of the inductive regime. Also, cytoplasmic histone increased in concentration reaching a maximum by the fourth day (Gifford, 1963).

Alterations in cytoplasmic structure of *Chenopodium album* as early as three hours after the end of the photoinductive period which promotes flowering was noted by Gifford & Stewart (1965). The endoplasmic reticulum showed an altered distribution, and there was evidence of an increase in acid phosphatase production. Dictyosomes increased in number per cell by the end of the second inductive cycle.

Chenopodium rubrum L. has proved to be an interesting species in

that photoinduction can be achieved in the cotyledon stage. Cumming (1969) has described this response in considerable detail. Seidlová and Štichová (1968) concluded for *C. rubrum* that two inductive short days from the time of germination were necessary to induce flowering.

In a study of nucleic acid inhibitors Watson & Matthews (1966) showed that actinomycin-D and 2-thiouracil inhibited floral initiation in *Chenopodium amaranticolor* when applied to plants before the end of the inductive dark period. Actinomycin did not suppress floral differentiation if applied to plants more than 48 hours after the inductive dark cycle, whereas 2-thiouracil retained its ability to interfere with floral differentiation when applied to plants several days after the inductive dark period.

A new ^{32}P-labelled, ribonuclease-resistant RNA component was detected in apical buds following floral induction and was associated with DNA, probably as a DNA-RNA hybrid. This component was not detected in non-induced apical buds. They concluded that DNA-RNA hybrids may be needed for the synthesis of messenger RNA that is needed for floral bud differentiation.

Considerable attention has been devoted to the ontogeny and cytohistology of the inflorescence and flowers in the Cruciferae. As mentioned elsewhere in this review (page 166) Vaughn (1955) concluded for *Capsella bursa-pastoris* (L.) Medic. and *Arabidopsis thaliana* (L.) Heynh. that vegetative and inflorescent apices were structurally similar. A central initiation zone was reported to be present in all stages of inflorescence development. Earlier Chakravarti (1953) reported an increase in apical layering during growth of the seedling and in the inflorescence. Three or four tunica layers were described for the inflorescent apex. Senghas (1956a) emphasized the changes in size and form of the apex that took place in *Capsella* during ontogeny. The size increased, and finally only a small portion of its volume was utilized in primordium formation. He described a centrally positioned zone ("Zentral-mutterzellen" meristem) which he concluded was a self-perpetuating zone. This zone would be roughly equivalent in size to the méristème d'attente of the French workers. He attempted to reconcile the French view with his own, and he noted that a meristematic-like mantle was present in the inflorescent apex.

A very intensive and thorough study of flowering in *Sinapis alba* L. (= *Brassica alba* Rabenh.) has been accomplished by Bernier and his associates using cytological and autoradiographic techniques (Bernier, 1961, 1962, 1964; Bernier & Dath, 1962; Bernier & Bronchart, 1963, 1964; Dath, 1963; Remacle—Dath, 1965; Bernier, Kinet, & Bronchart, 1967). The shoot apex was described for plants grown under floral inductive conditions (long days) and under non-inductive conditions (short days) and during the transfer from short days to long days or from long days to short days. Vegetative apices of plants grown under inductive or non-inductive conditions were similar initially, but the vegetative phase was reported not

to exceed 9–12 days in length after germination under long-day conditions. The vegetative apex has a biseriate tunica and exhibits a cytohistological zonate appearance as described for other Cruciferae (Senghas, 1956a; Chakravarti, 1953). Bernier (1962, 1964) used the terms "central zone," "peripheral zone," and "pith-rib meristem" for the three prominent zones of the apex in *Sinapis alba*. The zonate organization was clearly demonstrated by the unequal distribution of RNA as revealed by Unna's methyl green-pyronin mixture and by autoradiographic studies of synthesis of DNA (Bernier & Bronchart, 1963). Bernier (1964) concluded that centrally located apical cells did divide and even in rare cases furnished cells to the peripheral zone and pith-rib meristem. However, the inverse passage of cells of the flank to the central zone was more frequent, according to that author. He concluded that *Sinapis* has a typical anneau initial as defined by French investigators. An average of 1.1 foliage leaf primordia were formed during the first nine days after germination, at which time the apex underwent transition to flowering under long days. Transition to the "prefloral stage," which requires about one or two days, involved primarily an activation of the central zone. A mantle-like zone of superficial layers of cells was formed, but the apex remained somewhat zonate with RNA concentration being lower in the axial region. The "floral stage was reached but the apex resembled that of a vegetative apex." The inflorescence apex was thus constituted and plastochronic functioning continued, but the time interval was shortened (Bernier, 1964). In this instance the anneau initial did not disappear during transition to flowering (Bernier, 1962).

Plants grown under short-day conditions remained vegetative, but their apices increased in size and took on special characteristics by the 20th day after germination. The upper part of the corpus became more stratified, RNA concentration increased and nucleoli, which were previously small in this zone, became uniform in size throughout the apex. Foliage leaves continued to be produced although at a slower rate than for plants growing vegetatively under long days; their origin was reported to be from the third or fourth layer of the peripheral zone, rather than from the second.

Short-day plants transferred to long days when they were 60 days old showed changes as soon as one day after transfer. RNA concentration increased, particularly in cells of the four or five layers in the central zone; cells of the pith-rib meristem were less active mitotically and underwent precocious vacuolation. The inflorescent apex then continued plastochronic functioning similar to that of the inflorescent apex observed for plants growing under long days from the time of germination. Bernier (1962, 1964) considered the apex of *Sinapis alba* grown continuously under short days to be sufficiently different from the apex of a vegetative plant (up to about 12 days old) grown under long days to qualify it as a "phase intermédiaire" apex, intermediate in the sense that subtle modifications occurred which permitted a rapid transition

to the reproductive condition when plants were subjected to even one long day. Similar conclusions were reached for other examples (Lance, 1957; also see page 186, this review). Certain abnormalities were observed for *Sinapis alba* when plants were transferred from long days (inductive conditions) to short days at the time of flowering. In some instances flowers were initiated by an apex having vegetative characteristics, or a foliar primordium could be initiated by a meristem which had partially or totally lost its vegetative characteristics. In one instance a young leaf and flower were produced next to each other. It was concluded that these abnormalities can best be explained by assuming that modifications can occur in the functioning of floral or leaf-generating centers under special environmental conditions. Similar results have been observed for *Alyssum maritimum* Lam. [= *Lobularia maritima* (L.) Desv.] by Nougarède & Rondet (1962).

Recently Bernier, Kinet, & Bronchart (1967) analyzed in considerable detail the cellular events that occurred during induction in *Sinapis alba* transferred from short days to long days (inductive condition). The following events occurred in both the peripheral and central zones of the apex: 1) a rise in the mitotic index culminating about 30 hours after the beginning of the long day—interpreted to be the release of many otherwise arrested nuclei at the G_2 phase; 2) a stimulation of DNA synthesis reaching a maximum at the 38th hour; 3) an increase in nucleolar diameter culminating about the 54th hour; 4) an increase in cell volume culminating at the 62nd hour; 5) a second rise in the mitotic index reaching a maximum at 62 hours. Flower buds were initiated coordinate with the second rise in mitotic activity. Floral induction was considered to occur during the first rise of the mitotic index which led to subsequent steps. It is interesting to note that there was a daily rhythm in DNA synthesis and in mitotic activity in both the central and peripheral zones for the controls, the peripheral zone being higher. This type of periodicity was lost under inductive conditions and both zones showed paralleling increases, with that of the peripheral zone always being higher. Thus, both zones were responsive to the flowering stimulus, and the peripheral zone of the inflorescence could be considered as a region with modified potentials. There was no abrupt change in regard to the origin of the inflorescent axis—e.g., from the central zone alone. This may be a reflection of the "intermediate" stage which was reached by the apex of plants grown for 65 days on short days before induction. Similar experiments, as described above, on 9- to 12-day-old plants grown on long days (inductive) would be of interest in determining the roles of the two zones at the time of transition under continuous inductive conditions.

The action of gibberellic acid on the mitotic activity of each zone of a shoot apex was studied for two species which revealed quite different reactions to the hormone (Bernier, Bronchart, & Jacqmard, 1964; Jacqmard, 1964, 1967, 1968). Flowering in *Rudbeckia bicolor* Nutt.

(Compositae) occurs naturally in long days, but flowering can be achieved by application of gibberellic acid similar to other reports (e.g., Lang, 1956). For *Rudbeckia bicolor* the apex acquired the "prefloral" state under long days by 42 days after treatment with GA (Jacqmard, 1964). On short days the plant remained vegetative indefinitely and ultimately reached the "intermediate" stage, but was still vegetative in its functioning (see page 187, this review). Applications of GA hastened the acquisition of the intermediate stage and ultimately led to flowering. To determine the mechanism of control by GA, 60-day-old plants of *Rudbeckia* were treated with one application of GA. The mitotic index increased and DNA synthesis was stimulated after a lag period. The lag period for DNA synthesis was shorter than the lag period for mitotic stimulation. This fact suggested that the primary role of the hormone was to control the progression of cells from the pre-synthetic phase, G_1, of the mitotic cycle to the phase of DNA synthesis, S. Also, the investigator (Jacqmard, 1968) suggested that the role of GA was to control the progression of cells from the post-synthetic phase, G_2, of the mitotic cycle to cell division.

For *Rudbeckia*, stimulation of cell division occurred mainly in the central zone, which resulted in enlargement of the zone pior to floret initiation. For *Perilla nankinensis* (Lour.) Decne. (Labiatae), activation was more general, affecting the central and peripheral zones and resulting in an acceleration of plastochronic functioning, but did not lead to flowering (Bernier, Bronchart, & Jacqmard, 1964).

Geum urbanum L. (Rosaceae) is a perennial plant that normally requires vernalization for flowering. After winter, under natural conditions, flowers are formed from axillary buds. Under experimental conditions buds in the axils of young leaves developed into flowering shoots if the plants were kept at 3°C for eight weeks, which was considered a minimal time for flowering (Trân Thanh Vân, 1965a,b). The axillary buds which were potentially responsive began meristematic activity and had a high level of RNA as indicated by a greater affinity for pyronin. If the plant was subjected to a cold period at this time, the axillary bud meristem passed from the transition stage to floral phases. If, on the contrary, the bud was not subjected to a cold period, the bud failed to develop as a flowering axis and assumed a vegetative role. Trân Thanh Vân (1965a,b) concluded that cold treatment simply accentuates an initial meristematic condition and a minimum of elongation.

Factors other than cold treatment were effective in promoting flowering of the axillary buds (termed "sensitive buds"), viz., enhanced mineral nutrition, excision of the terminal bud, and treatment of axillary buds with kinetin. It was concluded that the effect of a cold treatment is primarily to release the axillary buds from apical dominance and to increase their meristematic capabilities. The terminal bud also could become floral if a plant were subjected to 30 weeks or more of cold treatment. A combined treatment of cold and gibberellic acid provoked

flowering of the terminal bud after 14 weeks. With the added effect of high mineral nutrition, activation occurred within 10 weeks and flowering occurred shortly thereafter.

It was concluded that vernalization in *Geum* not only promotes an aptitude for flowering where none existed, but is a process that prevents the inhibition of an already existing potential for flowering (Chouard & Trân Thanh Vân, 1962, 1965).

Phelouzat (1964b) considered the floriferous shoot of *Geum urbanum* to be a ramified vegetative structure producing a terminal flower. It was stated that the apex functions as in a vegetative shoot by activity of an anneau initial.

For *Coleus* (Labiatae) Jacobs & Morrow (1961) made a quantitative study of mitotic figures in relation to development in the apical meristem of the vegetative shoot. There was a diurnal rhythm in the initiation of leaf primordia, and the number of mitotic figures in the apical meristem was closely and significantly correlated with the height of the apical meristem; but the distal 10–20 μ of the meristem consistently had fewer mitotic figures than the more proximal regions. Contrary to expectations there was no evidence for a marked diurnal rhythm in percentage of cells showing mitotic figures. There was a large increase at 11:00 p.m. in the absolute number of mitotic figures, and this was considered only to be a reflection of the much higher apical meristem at that time, associated with a phase of the plastochron.

Earlier Clowes (1959) found that for *Coleus* shoots injected with labelled adenine, there was no zone of the apex without labelled nuclei. Nearly all nuclei were labelled if the plant was fed for a longer period. Clowes was attempting to show that the time a nucleus spends in mitosis is a small and variable fraction of the time spent in interphase. Use of isotopes is a better guide to meristematic activity than determining mitotic frequencies from counts of figures (e.g., Lance, 1957), because the time taken to produce a visible label can be varied to suit the frequency of mitosis.

Saint-Côme (1965) emphasized the importance of lateral cells of the apex in restoring sites for leaf initiation, although occasional divisions did occur in summital cells. Under 16-hour photoperiods plants entered the reproductive phase after the production of seven to nine pairs of leaves. In short days of eight hours light, the reproductive phase began after 15 to 17 pairs of leaves were formed. Comparisons of percentages of mitoses and of labelled nuclei (treated with H^3-thymidine for six hours) were made for the vegetative apex in various phases of a plastochron, during the prefloral phase (early transition) and the later, clearly reproductive phase. Saint-Côme concluded that the differences in mitotic activity and synthesis of DNA were very significant between the "zone axiale préflorale" and "zone axiale" of a vegetative shoot, but there were no significant differences between the lateral zones. Although the term "zone laterale" is used throughout the text, Saint-Côme con-

cluded in the summary that an "anneau initial" does exist in *Coleus*, presumably in the sense it was used originally (Plantefol, 1947). Also, an intermediate phase was recognized (see page 186, this review), but very little information was presented to support the conclusion. More conclusive evidence was presented in a later publication (Saint-Côme, 1966).

The responses of *Perilla nankinensis* (Labiatae) grown under inductive conditions (eight-hour photoperiod) and under non-inductive conditions (16-hour photoperiod) have been described in some detail (Nougarède, Bronchart, Bernier, & Rondet, 1964; Lance-Nougarède & Bronchart, 1965; Nougarède & Bronchart, 1965; Bernier, 1966). The following account pertains mainly to the structural changes occurring at the shoot apex. A general discussion of flowering in *Perilla* can be found in a recent review paper by Zeevaart (1969). A zonate apical structure was described for the vegetative plant under an eight-hour photoperiod (Nougarède et al., 1964). Future leaf sites were described as being organized by cells lateral to a "zone apicale." The term "méristème de flanc" was used for what is presumably the "anneau initial" of previous publications by French investigators. A detailed cytological study using techniques of light microscopy revealed that the cells of the two outer surface layers of the apical zone were large and vacuolate, but at minimal area phase these cells differed very little from lateral cells. This situation brought the above authors to believe that the outer two layers of the apical zone participate in regeneration of leaf sites, but they concluded to the contrary because the lateral cells were the most highly pyroninophilic of the apex. However, no actual data were provided on distribution of mitoses, etc. From an inspection of available line drawings it would appear that mitochondria of the two outer layers of cells of both the apical and lateral zones were about the same size. In later publications, however, it was shown that a greater percentage of labelled nuclei (H^3-thymidine) could be demonstrated in the total lateral zone than in the apical zone (Lance-Nougarède & Bronchart, 1965; Nougarède & Bronchart, 1965; Bernier, 1966), although the apical zone was not inactive; this fact was not reflected in the autoradiographs selected to show isotope incorporation. There was also greater concentration of RNA in the anneau initial (Lance-Nougarède & Bronchart, 1965), peripheral zone (Bernier, 1966), or lateral zone (Nougarède & Bronchart, 1965) than in the apical zone. In transition to flowering (from 22 to 28 days after germination) nucleolar dimensions were said to increase. Also, an increase took place in pyroninophilia for the axial and lateral zones, but particularly so for the apical zone (central zone as cited by Zeevaart, 1969). Nucleolar diameter does not seem to be greater for the apical zone when compared with illustrations of the vegetative apex (Nougarède et al., 1964). Zeevaart (1969) described the transition stage as being prefloral and cited the paper by Nougarède et al. (1964), but the latter authors used the term "transitoire" because

this stage was presumably different from the "prefloral" stage of other examples. However, the term prefloral was used later by Lance-Nougarède & Bronchart (1965). Following the transitory stage, the apex entered the reproductive phase. Four to six pairs of bracts, each with a floral meristem, were produced. At this stage the number of ribosomes per $5\mu^2$ was reduced in both the apical and lateral zones with a considerable reduction of DNA synthesis occurring in the central zone. The terminal apex degenerated after about 45 days.

For plants grown under 16-hour photoperiods (non-inductive) the apex functioned as for a plant on short days, up to about 20 days. At this time zonation was still visible, although the nucleoli of the apical zone became larger than those of the peripheral zone. Ribosomal counts in the apical and lateral zones approached each other, but that of the apical zone was larger than for the vegetative phase of plants on short days. The apex then entered the so-called "intermediate phase," continuing in this condition as long as the plant remained in a non-inductive situation (see page 187, this review).

An added structural feature for *Perilla nankinensis* kept in non-inductive conditions for a long period of time (93 days) was noted by Nougarède et al. (1964). The apex became strongly rounded in form and increased in height. Intercalated between the lateral cells and the axial cells was a "transition zone" where the cells were reported to acquire little by little the meristematic characteristics of the cells of the flank. The authors provided no labelled photomicrographs to illustrate this condition, only line drawings. Therefore, it is not clear what the transition zone represents or how the activity of this zone functions in relation to the flank meristem which is equated to the anneau initial.

For other investigations on the physiology and cytology of flowering in *Perilla* the reader is referred to Zeevaart (1969).

In *Teucrium scorodonia* L. (Labiatae) Lance-Nougarède (1961b) reported that an inflorescence is formed in which at its inception an activation of the apical zone (presumably a méristème d'attente) occurred as shown by an increase in RNA; the apical zone remained active during initiation of bracts. No terminal flower was formed, and the apical cells underwent differentiation at the end of the summer.

In *Alyssum maritimum*, vegetative organization was maintained in the reproductive meristem. Floral meristems were formed by a type of anneau initial (Lance-Nougarède, 1961b). As the latter author noted, reproductive meristems do not behave uniformly in a fashion that is generally accepted for vegetative apices.

The development of a functional vegetative apex from a flower was reported by Brulfert (1965a,b). In the case of "proliferous" flowers of *Anagallis* (Primulaceae), the apical part of the floral meristem became organized into a vegetative apex under certain photoperiodic regimes. Gibberellic acid also was effective in accelerating the production of "proliferous" flowers.

For *Kalanchoë* cv. "Brilliant star" (Crassulaceae), Stein & Stein (1960) reported that the general architecture of the shoot apex remained the same during vegetative and reproductive growth. There were two tunica layers in both the vegetative and floral meristems. Transition to the reproductive condition under inductive conditions was reported to take place in about ten days, whereas for another clone, transition occurred after eight or nine short days (Fredericq, 1960), and the lateral parts of the apex, particularly young leaves, stained deeply with pyronin which revealed high concentrations of RNA.

2. **Monocotyledons.** Barnard (1955, 1957a) surveyed floral histogenesis in representative examples of the Gramineae. Apices were described as having a two-layered tunica, although Barnard preferred the terms "dermatogen" (T_1) and "hypodermis" (T_2). For *Triticum aestivum* L., the transition from the vegetative to the reproductive stage commenced when there were from five to seven expanded or externally visible leaves. The first morphological indication of spike formation was rapid elongation of the apical dome. However, he did not determine when changes from vegetative growth to production of reproductive structures became irreversible. For one species each of *Lolium, Bromus, Danthonia, Ehrhartia, Bambusa, Stipa,* and *Triticum,* the foliage leaf, glume, lemma, palea, lodicule, and carpel originated by periclinal divisions in the hypodermis followed by similar divisions in the dermatogen. Barnard (1957a) disagreed with Holt (1954), who arrived at different conclusions for *Phalaris* and *Dactylis* for the origin of appendages and branches. Barnard (1957a) concluded that floral structures, on the basis of their modes of origin, may be classified as cauline or foliar, depending on their site of origin. Those taking their origin from the hypodermis and dermatogen would be foliar, while those taking their origin from underlying layers would be cauline. He suggested that the flower in the Gramineae may be regarded as a branch system. In all of the above studies no histochemical techniques were employed.

Increased stratification with time at the apex was stressed by Opatrná et al. (1964) for *Triticum aestivum.* Zonation was reported to become more evident with age. With the elongation of the apex and the appearance of the first bract, zonation was less clear and gradually disappeared when bracts and spikelets of the upper half of the ear were initiated. The term tunica was not used, but the apex appeared to have a biseriate tunica during later stages.

Cytohistochemical studies were made by Poux (1957, 1958, 1960a,b,c) on spring and winter varieties of *Triticum vulgare* Vill. and *T. sativum* Lam. In the vegetative condition the apex was described in terms of méristème d'attente and anneau initial. For spring wheat, sown in spring, the plant remained vegetative up to the seven-leaf stage, at which time a profound dedifferentiation of the méristème d'attente was purported to occur as revealed by increased staining of RNA. Poux concluded that in the vegetative apex growth is nearly exclusively subapical and essen-

tially apical in the reproductive condition. The inflorescence of a winter variety (*T. sativum*) sowed in spring formed slowly after having produced about 20 leaves instead of seven. The extra foliage leaves were produced by an apical meristem that underwent the beginning of transformation to the reproductive state but remained cytologically and functionally in an intermediate state (see page 186, this review) based on the form of the apex, disappearance of plastochronic functioning, and intensity of apical activity.

The number and total volume of cells produced in the apical region of the shoot of rye were measured at different stages of development (Sunderland, 1961). For non-vernalized seedlings of winter rye, grown in July, cells of the apical region divided about once every 1.8 days during the first week of growth, and there was about a 2.2 fold increase in volume of each cell generation. Four weeks later the apices were still vegetative, but the rate had fallen off to once every 5.8 days, and there was slightly less than a 2.2 fold increase in volume of each cell generation. The average cell volume decreased as the apices developed.

In vernalized seedlings more cells were produced during the first four days of growth than in non-vernalized seedlings, but as in the latter, the rate of production fell during vegetative development. At the time of transition to reproductive growth the cells divided about once every 2.0 days, and then there was about a 2.2-fold increase in volume of each succeeding cell generation. From four to eight days after transition, the cells divided once every 1.4 days and there was a 2.4 fold increase in the volume of each succeeding generation of cells.

A very intensive cytohistological study was made of floral induction in *Lolium temulentum* L. (Knox & Evans, 1966), which responds to one inductive cycle. In vegetative shoots RNA concentration was reported to be uniformly high at the summit. Day-II apices presented the same situation, but by day III the first sign of a change was a build up of RNA at future sites of spikelet primordia, in the axils of bracts. The same results were obtained with acridine orange staining of RNA. There was no obvious reduction in nucleohistone staining as revealed by staining with alkaline fast green or ammoniacal silver. After acetylation or deamination the nuclei of the apex and those down the flanks (three outermost layers) were almost free of bromphenol blue staining for histones, while nuclei in the central core of the shoot tip all showed some staining. The nuclei that stained were presumably arginine rich, while those that did not were lysine rich. There was no evidence of an increase in cytoplasmic histone staining, although there was some cytoplasmic staining at all stages of induction, particularly in central cells below the summit of the main axis. Localization of the staining was not determined precisely although possibilities were suggested.

Localization of RNA and protein through use of isotopes and autoradiography confirmed earlier work in that the greatest increase in incorporation by evoked apices was in cells of axillary bud sites (Knox

& Evans, 1968). Although relatively inactive in vegetative controls, incorporation of precursors by cells in axillary bud sites increased two- to three-fold. They concluded that there was no evidence for a méristème d'attente at the main vegetative apex, and they compared the inactive, but potentially responsive axillary bud sites to the central zone of dicotyledonous apices. Attractive as this analogy might be, it should be pointed out that most if not all of the bud sites were not organogenetically functional at the time of floral evocation. The authors preferred the term evocation for events occurring at responsive sites and reserved the term induction for events occurring in the leaves during the flowering process (Knox & Evans, 1968).

The views of Poux (cited above) and Knox & Evans (1966) are rather divergent on the first events of flowering in the Gramineae, but this may be a reflection of generic differences or methods employed. Knox & Evans used thin cryostat sections after first fixing material in formalin, while Poux's study was based on an examination of chemically fixed, paraffin-embedded material.

Evans & Wardlaw (1966) have contributed to our knowledge of the velocities of movement of assimilates and the floral stimulus. Simultaneous determinations of velocities of translocation out of the seventh leaf of *Lolium temulentum* yielded estimates of 1–2.4 cm/hr for the floral stimulus and 77–105 cm/hr for C^{14}-labelled assimilates.

They concluded that assimilates and florigen may both move in the phloem but the floral stimulus is mediated by cytoplasmic streaming in parietal cytoplasm of sieve tubes or in phloem parenchyma and fibers. Auxin and gibberellin followed the pattern of the floral stimulus in regard to movement.

Histogenesis of the shoot apex in *Hordeum distichon* L. from early stages of embryogeny through development of the spike was investigated by Klaus (1966). The shoot apex was reported to have a one-layered tunica (author used term "protoderm") throughout ontogeny. Spikelets of single flowers were produced in an acropetal sequence, being initiated in the subprotodermal layer. Stamens took their origin from cells beneath the subprotoderm. The carpels developed near the tip of the floret axis, and there was no evidence of a tricarpellary condition.

Barley plants treated with gibberellin (GA) resulted in several cytological and developmental changes (Im, 1961, 1962, 1963). Treatment just prior to spikelet differentiation enhanced "heading" by four or five days. However, there was no variation of spikelet number between GA treatment and controls. Irregular divisions were reported to occur in the apex, which resulted in decreasing the number of tunica layers. Nuclear-cytoplasmic ratios were reduced for the corpus. GA treatment promoted hydrolysis of polysaccharides and also affected RNA and protein metabolism. Mitochondrial number increased in apices of treated plants, and the total amounts of free amino acids were markedly increased with GA treatment under long-day conditions.

In a study of development from the dormant embryo to later stages in *Avena sativa* L., Ikenberry (1966) reported a 2-layered tunica to be present in late vegetative development. Nine leaves were formed before reproductive growth occurred. Size of the vegetative apex increased along with an increase in number of cells, while the length of the plastochron decreased.

The effects of mineral nutrition, especially nitrogen, phosphorus, and potassium on the structure and physiology of vegetative and inflorescent apices of rice were studied by Shimizu (1959, 1960a). The author described rather specific alterations in topography and histology of the rice-plant shoot apex related to mineral nutrition. With high nitrogen the apex was a low dome and the number of tunica layers was increased to three or sometimes four layers. Under conditions of high phosphorus the apex was somewhat cylindrical and the tunica consisted of one or two layers accompanied by an increase in irregularity of divisions in the corpus. High nitrogen increased plastochronic functioning, whereas high phosphorus had the opposite effect. Potassium appeared to modify the extreme response to high nitrogen or phosphorus. Under normal development Shimizu (1959) reported an increase in stratification of the tunica late in the vegetative phase followed by a decrease in stratification following transition to flowering. The time of panicle differentiation was affected by mineral nutrition—earliest in plants treated with high phosphorus, latest in high nitrogen. The effects of mineral nutrition on time of panicle differentiation appeared to be quantitative rather than qualitative. For wheat Shimizu (1960b) concluded that phosphorus has an important role to play in the transition from the vegetative to the reproductive state, and the results of mineral nutrition studies for wheat appeared to be the same as for rice.

Cytohistological localization and analyses of polysaccharides, RNA, amino acids, and soluble sugars were made by Murakami (1960, 1962) on the shoot apex of the rice plant. The main result of this investigation was that polysaccharides appeared to be deposited initially, even momentarily, in tissue having meristematic potentialities. At the moment of an organogenic event, for example, the concentration of polysaccharides decreases rapidly followed by increases in sugars, amino acids, RNA, protein, etc. Whether the conclusions regarding these sequential steps can be applied generally awaits further investigation.

A rather thorough study of apical growth, leaf development, and histogenesis of the stem in the rice plant was made by Kaufman (1959a,b,c). Based on relative height of the shoot tip (term used here to include shoot apex *per se* and very young leaf primordia), the rice plant apex is of the "short type" (Sharman, 1942), but in older vegetative and transitional (to flowering) apices the shoot tip is relatively long. The vegetative shoot apex *per se* became larger during successive plastochrons (also see Ledin, 1954), and the tunica increased from one layer to two with the corpus tending toward stratification. During transition

to flowering, enlargement of the apex was particularly evident. Contrary to earlier views Kaufman (1959a) reported fluctuations in size of the apex of the rice plant during a plastochron. A biseriate tunica was less stable in primary and secondary branches with spikelet apices having only one tunica layer. Besides making important contributions to leaf histogenesis of the rice plant, Kaufman (1959b) provided quantitative data on growth of various parts of the leaf in relation to successive plastochrons.

For rice, variety Elon-elon, Zamora (1959) confirmed the occurrence of a two-layered tunica for the vegetative plant but emphasized the occurrence of a zonate corpus. The size of the apex was reported to be much greater than that for the variety Caloro (Kaufman, 1959a).

Day length was reported to affect size, shape, and number of primordia present at the completion of the vegetative phase for two varieties of *Avena sativa.* The shoot apex of plants grown under continuous illumination was smaller, both in height and width, than for plants kept under a daily regime of 12 hours of light. Also, under the latter environmental condition more primordia were present at the completion of the vegetative phase. Continuous illumination hastened the transition to flowering (Tayal et al., 1961).

Barnard (1957b, 1958) concluded from histological studies that, just as for the Gramineae, one can characterize cauline and foliar-type structures for the reproductive parts of the Cyperaceae and Juncaceae. Periclinal divisions in cells of the outer corpus and hypodermis (T_2) characterized the formation of flowers and stamen primordia for three species of three genera of the Cyperaceae and three species in two genera of the Juncaceae.

Floral histogenesis was studied by Barnard (1960) for *Bulbine bulbosa* R. Br. and *Stypandra glauca* R. Br. (both in Liliaceae). In both cases the apex of the floral axis had a two-layered tunica and bracts arose through periclinal divisions of cells in the inner layer of the tunica. In both species the perianth members originated in the same manner as bracts, through periclinal divisions of cells in T_2. Periclinal divisions may occur later in cells of the outer tunica layer. Stamens, as reported for certain other monocotyledons, were initiated by periclinal divisions in both hypodermal (T_2) and subhypodermal cells. Barnard (1960) concluded that a two-layered tunica seems to be a very common feature of both vegetative and floral apices in monocotyledons.

Growth of the banana plant (Musaceae) is extremely interesting, and its development throughout phases of the life cycle has been described recently by two groups of investigators (Barker & Steward, 1962a,b; Fahn et al., 1963). The shoot apex increases greatly in size during growth from a vegetative lateral bud to the flowering condition. Barker & Steward (1962a) reported very little demarcation of zones in the vegetative apex. They recognized a sharply defined surface layer, and a second one was usually detectable. Fahn et al. (1963) were more

insistent on the occurrence of a zonate pattern in the apex, particularly in the presence of an active central mother cell zone below the tunica. A subjacent cambium-like zone was described, but to the present reviewers it was not too well delineated in the figures cited. Fahn et al. (1963) stated that the apex of Dwarf Cavendish banana can be classified with the "Opuntia type" of Popham (1951). Lateral buds were not formed by the apical meristem but were separated from it in time and by a space of ten interposed leaves, and they arose adventitiously (Barker & Steward, 1962a).

During vegetative growth the leaves were dominant structures of the main axis, which remained as a short "pseudostem." A leaf could elongate ten feet in eight days and could achieve a growth rate of one inch per hour. The vegetative shoot tip, which was in a central depression and was a broad flattened dome, increased in height and became a pointed cone during transition to flowering.

There is agreement that an outer zone of actively dividing cells becomes at least three layers deep (up to eight layers, Fahn et al., 1963), and this is correlated with rib-meristem activity of subjacent cells. Flowers of a "hand" of bananas were said to develop simultaneously (Ram et al., 1962), not successively as reported by Fahn (1953).

Pineapple plants (*Ananas comosus* Merr., var. Smooth Cayenne) can be made to flower by treatment with acetylene (Gifford, 1969). Cytological changes occurred as early as three days after treatment which involved nuclear changes and an increase in ribonucleic acid (RNA) in the cytoplasm of cells of the apical zone. A marked increase in height of the apex occurred by the ninth day, which was preceded by rib-meristem activity in the central core. All component parts of the inflorescence were present by the 21st day after treatment.

Van Overbeek & Cruzado (1948) were able to induce flowering in pineapple by placing the plant in a horizontal position for a minimum of three days.

Ethylene and applied auxin are also effective in bringing about flowering in the pineapple. Burg & Burg (1966) have provided evidence that applied auxin mimics the effect of ethylene by stimulating ethylene formation, and that it is the ethylene, not auxin, that causes the pineapple to flower. In their experiment detectable ethylene production occurred one day after treatment with auxin and by three days the level was quite high.

Under all experimental conditions it would appear that three days after treatment may be the critical time in which induction occurs.

3. **"Intermediate" condition.** As described earlier many studies have been made on the behavior of the vegetative meristem during change to the reproductive condition in response to one or several photoinductive cycles. Fewer comparative studies have been made on cytohistological changes that occur in the meristem of a plant subjected to continuous photoperiods which are either favorable or unfavorable to flowering.

For absolute or quantitative long-day or short-day plants transferred from non-inductive to inductive conditions, the transition to the reproductive phase is often very rapid. A partial list of the changes that may occur include: (1) shortening of the plastochron, (2) precocious formation and development of axillary buds, (3) changes in shape of the apex, (4) general increase in mitotic activity, particularly in the central zone, apical zone, méristème d'attente, or metrameristem, whichever term is preferred, (5) changes in nucleolar size and volume, (6) increase in RNA concentration, and (7) increase in stratification and loss of zonation characteristic of earlier phases. The above list includes only some of the more obvious changes. Additional details can be found in the earlier part of this section and in recent reviews (Cutter, 1965; Nougarède, 1965a,b, 1967; Nougarède & Bernier, 1966).

The organization of the seedling apex may differ from that of the older plant. Lance (1957) reported that the shoot apex differs from that of an earlier juvenile stage if the plant is grown continuously under conditions that do not favor flowering, or are entirely inhibitory for a species. For example, a quantitative short-day plant can be prevented from flowering for varying periods of time by increasing the light period during a 24-hour period beyond a critical length. An absolute short-day plant can be prevented from flowering indefinitely under extended light periods. In these examples the plant by-passes floral induction, but the shoot apex gradually assumes a structure somewhat different from the previous juvenile phase, although foliage leaves continue to be produced. Lance (1957) and Poux (1957) described this condition as being "intermediate" in that apical organization is intermediate between the vegetative (juvenile) phase and that of the flowering condition.

One example will suffice to illustrate this phenomenon. Nougarède, Gifford & Rondet (1965) compared apical structure of *Amaranthus retroflexus* L. (quantitative short-day plant) grown continuously on a photoperiod of either eight or sixteen hours. The former regime favors flowering and the latter delays flowering for some time (up to the 14–20-leaf stage). When the plant reaches the 4–5-leaf stage under an eight-hour photoperiod, the shoot apex undergoes rapid transition to flowering—displayed by an increase in stratification and in RNA concentration, resulting in a mantle of 3 to 4 layers of cells which stain uniformly. If plants are grown continuously under a 16-hour photoperiod, transition to flowering does not occur at the 4–5-leaf stage, but the apex acquires a modified type of organization marked by differential responses of various zones of the apex. Cells of the tunica acquire a large nucleolar volume, but RNA concentration remains low. RNA concentration increases in cells of the corpus in an axial position, but nucleolar volume becomes less than that of the tunica. The frequently stated positive correlation that exists between nucleolar size and RNA production does not occur in this instance. The characteristics of the entire axial zone (to include tunica and corpus) of the juvenile seedling stage are

not apparent. Foliage leaves continue to be formed, however. With transition to flowering (at 14–20-leaf stage), marked by the production of bracts, there is an increase in concentration of RNA in cells of the central part of the corpus and in cells of the peripheral zone, resulting in the formation of a subtunica mantle. It is clear that shoot apices of this species in transition under different photoperiods have quite different cytological characteristics.

Some other examples of species showing generally the so-called intermediate condition under conditions unfavorable for flowering are as follows: Absolute short-day plant—*Chenopodium album* (Gifford & Tepper, 1962a,b), *Perilla nankinensis* (Nougarède et al., 1964); absolute long-day—*Sinapis alba* (Bernier, 1962), *Rudbeckia bicolor* (Jacqmard, 1964); quantitative long-day—*Coleus blumei* Benth. (Saint-Côme, 1965), *Chrysanthemum segetum* L. and *Aster sinensis* L. (Lance, 1957); requiring vernalization—*Geum urbanum* (Trân Thanh Vân, 1965a,b).

Acquisition of the "intermediate" phase or condition is clear and unmistakable. Interpretation is somewhat more difficult. Does the altered structural organization truly represent a preparation for flowering, or is it a corollary of an altered physiological state not necessarily related to flowering? Indeed, some species are more readily induced to flower after they exhibit the intermediate condition. Others are reported not to flower as rapidly after being transferred from non-inductive conditions to inductive conditions (see Nougarède & Bernier, 1966, p. 28).

To restrict the term "vegetative" shoot apex to the seedling stage for the plants in question would seem to be too narrow a use of the term even though one of us (EMG) previously gave endorsement to this concept (Nougarède et al., 1965). Since some additional changes do occur at the apex of the so-called intermediate apex before actual irreversible transformation takes place, perhaps it would be better to refer to the stage as the "adult" vegetative shoot apex phase. Clearly the "intermediate" apex is producing vegetative leaves and undergoes, in many instances, the usual plastochronic functioning.

C.) Chimeras and Changes Induced by Chemicals and Ionizing Radiation

The occurrence of natural or induced chimeras has been utilized by investigators in various ways in attempts to prove or disprove the importance of apically located cells in shoot development. In instances where rather stable layers occur in the shoot apex as revealed, for example, by the use of colchicine, the independence of the tunica and corpus can be fairly well established. The researches of Satina et al. (1940) and Satina & Blakeslee (1941, 1943) are well known in this respect. Clowes (1957, 1961a) and Gifford (1954) considered this information to be important in understanding the behavior of shoot apical meristems, especially in reference to the functioning of summital cells. The results obtained by Derman & Bain (1944) and by Bain & Derman (1944) on

induced chimeras in shoots of *Vaccinium macrocarpon* Ait. (Ericaceae) come under the same category.

A study was made by Thompson & Olmo (1963) to determine the apical structure of 62 different clones of the grapevine (of natural and colchicine origin) suspected to be tetraploid. In most buds there was a biseriate tunica, but it varied from one to three layers. Occasional periclinal divisions occurred in T_2 which accounted for the homogeneity of all tissues beneath the superficial layer. The following types of periclinal chimeras were observed (first number refers to the outer layer and the second to the remainder of the cells of the apex): 2–4, 4–2, 4–4, 2–2. On the basis of these cytological studies Thompson & Olmo (1963) concluded that the generally accepted concept that the grape shoot has two discrete tunica layers needs to be reevaluated because of the instability of L-II. It would appear that the grapevine is not particularly suitable materal for answering questions of cell lineages and subsequent patterns of tissue ploidy unless one were able to analyze growth immediately after a change in ploidy at the apex. However, the stabilization of a 2–4 from a 2–4–2 would tend to favor the concept that divisions do occur in the more medianly positioned cells rather than being limited to peripheral cells.

Nougarède (1965b, 1967) minimized the importance of induced polyploidy in answering problems pertinent to the functioning of apical meristems, because the manner in which the induced chimeras are formed is not known (that is, which cells in the apex are responsible for the initial production of the chimera). The results of these studies do not answer the questions of whether there are or are not active cells in the most apical region of the shoot apex. In the reviewers' opinion, this position is somewhat justified because once a periclinal chimera is established then the criteria used for mitotic activity are the same as those for unchanged or "normal" apices. Chimeras resulting from grafting experiments present some of the same problems, although the conclusion (Nougarède, 1967) that the information provided by this type of experiment is not useful in interpreting normal or nonchimeric apices seems unjustified to the present reviewers. If "normal" and expected organogenesis results from a graft, the usual processes of growth are probably occurring at the apex. In summary, the production of periclinal chimeras, if they involve the summital cells, tend to support the generalized tunica-corpus or mantle-core concepts for angiosperms, within the framework of occasional adjustments between zones of the apex. These adjustments naturally would result in the production of variable patterns in the primary plant body as well as in the secondary body.

The results of investigations on chimeras described above do not assist specifically in resolving questions of activity of various zones of the apex, whether one adheres to the anneau initial concept or to the concept that the most apically positioned cells are important in histogenesis. Recently Thielke (1959, 1962, 1963, 1964) has been able to

relate the occurrence of divisions in specific zones of the shoot apex with the external appearance and internal structure of the shoot (also see page 158, this review). For many clones of certain species of *Saccharum* she was able to demonstrate that periclinal divisions are common in summital cells of the apex during early growth of a shoot. Only later did a stable tunica layer become organized. A sectorial pattern was associated with a non-layered apex (periclinal divisions occurring in surface cells at the summit), and a periclinal pattern was associated with at least a one-layered tunica. In *Tradescantia fluminensis* var. *albostriata*, periclinal divisions in summital tunica cells resulted in a mixture of tissues and hence the leaves were variegated. With increased greening of subsequent leaves, a stable tunica reappeared. These results demonstrate a correlation between the evident results of morphogenesis and activities of specific cells of the shoot apex—namely, the summital cells.

Analyses of cell lineages, contributions of various zones, etc., of the shoot apex are difficult to perform on large zonate apices. The smaller the apex the easier it is to delineate cell lineages. A most significant study in this respect has been provided by Bartels (1956; 1960a,b; 1961), who described apical ontogeny of the small shoot apex of *Epilobium hirsutum* L. (Onagraceae). Descriptions were based upon forms that had genetically controlled chlorophyll deficiencies, and hence markers were available for analysis. Shoots clearly exhibited sectorial type chimeras. Phyllotaxis is decussate, and the mutant sectors could be correlated precisely with functioning of the apical meristem. During development of the seedling a three-layered apical meristem was formed in which Bartels distinguished central cells (Zentralzellen) for the surface layer (L-I central cells), for a second layer (L-II), and for a third layer (L-III). Four L-II cells occupy, in a regular fashion, the central portion of the second layer. These four cells divided only anticlinally, and they were considered to be the ultimate source of cells for portions of the leaf and axis. The apparent preciseness in the manner in which these sectorial chimeras were formed argues strongly for the presence of a limited number of source cells at the tip of the vegetative apex. This conclusion would seem more reasonable than an attempt to relate these patterns to the occurrence of a mutation in one cell of a large population of cells within subjacent tissues—e.g., in the "anneau initial."

Additional support for the idea that specific apical initials are present in an apex was provided recently by analyses of mericlinal sectors in periclinal chimeras for several species (Stewart & Dermen, 1970). The dimensions of mericlinal sectors in periclinal chimeras, resulting from adjustments in apical layers, were used to determine the number of apical initials present at the summit of the shoot axis. In some cases changes in variegation, involving wide sectors (up to ⅓ or ½ the circumference of the stem) extended for many nodes, up to 100 in some

instances. Stewart & Dermen (1970) contended that if apical initials were in a ring or collar (e.g., anneau initial) around a distal quiescent zone (e.g., méristème d'attente), a change in one cell would result in a long, narrow sector. Long, wide sectors are better explained by assuming adjustments have occurred in a limited number of distal (summital) cells. The above authors concluded that the ultimate origin of cells during primary growth is from 1–3 apical cells in each of the apical layers. By indirect methods it was concluded that the apical initials of *Ligustrum ovalifolium* Hassk. divided only once in 12 days during the formation of 3 nodes and that this was sufficient for the initials to be the *ultimate* source of all cells of the shoot.

In recent years some interest has been generated in the possibility of determining the number of initial cells of shoot apices by analyzing the mutants formed after application of ionizing radiations. Evidence obtained from morphological mutants of the coffee plant led Moh (1961) to ask the question whether the coffee plant develops from one initial cell in the shoot apex of an embryo. Seeds of *Coffea arabica* L. (Rubiaceae) irradiated with neutrons or gamma rays gave rise to a high frequency of morphological mutants. Sectorial changes were not produced, but rather whole plant "mutants" were formed. A mutant showed changes in leaf morphology and internode length. The causal reason being the result of temporary physiological disturbances was ruled out because the changes were permanent. Coffee embryos at the time of irradiation had a uniseriate tunica and a corpus without any evidence of a special, solitary apical cell. With radiation one might expect to obtain a sectorial chimera, but regardless of dosage, the "mutant" character appeared as a whole-plant response. With the exception of the epidermis, Moh (1961) speculated that cells forming other parts of the shoot appear to be derivatives of only one of the initials in the corpus.

From an analysis of chromosome aberrations induced by X rays on barley embryos Caldecott & Smith (1952) concluded that all microsporocytes in a barley spike usually can be traced back to a single cell in the dormant embryo.

The segregation ratio of chlorophyll mutants (induced by X-ray treatment of dormant embryos) was used by Gaul (1959, 1961) to estimate the size of mutated spike-sectors in barley. It was found that the mean sector size increased with both the dose and later emergence of tillers. The data suggested that the generative tissue of each of the first spikes may be represented in the dormant embryo by perhaps four initial cells per potential spike primordium. With increasing radiation damage an increased number of these initial cells per spike primordium were killed without presumably being replaced, and the mean size of a mutated sector became greater. With 30,000 R the spikes usually appear to be non-chimerical, which would mean that each of them was derived from one surviving initial per spike primordium. Later formed tillers were often developed from secondary axillary buds. If the latter were derived

from a mutated corpus initial cell of the primary axillary bud, then the chimera would include even more than one spike.

A hypothetical model-system was described by Weiling (1962) in which the number of surviving initials and their effect on development supported the conclusions of Gaul cited above for the barley plant but which would not necessarily be applicable to certain other plants (e.g., the pea plant).

The analyses of mutations induced by ionizing radiations on seeds have provided an interesting approach to problems of apical growth. It is unfortunate, however, that supporting histological evidence has not been supplied to complement these analyses. For example, one would like to know more precisely the actual location of the original mutated cells, particularly for chimerical plants of the sectorial type. If one adheres to the anneau initial concept, the results could perhaps be reconciled with the theory. Completely mutated spikes of barley would favor the concept that the more apical cells of an apex have a dominant influence on development. With extensive radiation damage of the apical meristem, the one effective surviving cell could be located apically or laterally in the apex. Upon reorganization of the apex the derivatives of this cell would populate the entire spike, but this information would not necessarily be pertinent in explaining the functioning of an organized apical meristem. In this regard, responses of adult shoots to radiations would be more informative than the responses of irradiated dormant embryos.

More recently somatic mutations have been induced by gamma rays in the embryos of maize in order to follow albino-tissue patterns in mature plants (Steffensen, 1968). Mature plants were analyzed for their patterns of albino tissue. The location and frequency of these patterns were correlated with cell numbers at various sites of the shoot apex in order to deduce the number of cells contributing to each frequency class. Various sectorial patterns were diagrammed and the position of their albino tissue was explained in relation to the location of a specific cell in the apex. Apparently the cells at the crest of the apex contribute daughter tissue to nearly one half of the upper part of the maize plant.

The effects of acute and chronic doses of irradiation on growing shoots could conceviably contribute to an understanding of apical structure and growth of normal shoots. The results of such studies are inconclusive and somewhat ambiguous. No attempt will be made here to review the entire subject of the effects of ionizing radiation on shoot apical meristems; the reader is referred to pertinent literature where references to additional articles and discussions occur (e.g., Pratt, 1959, 1960, 1963; Stein & Sparrow, 1963). For the concord grape the entire shoot apical region was more sensitive than mature stem tissues and partially differentiated leaves. After doses of 2000 R of X rays or two hours of thermal neutrons there was loss of differential staining, plasmolysis occurred, and there was final collapse of the 2-layered tunica

and of the corpus; there was no group of distinctive central cells in the normal apex (Pratt, 1959). For diploid and cytochimerical varieties of apples, the superficial layer was maintained, but relative amounts of 2n and 4n underlying layers of tissues were changed. After irradiation the central cells below the first layer of apical and axillary meristems were conspicuous by their failure to stain normally. Such radiation-damaged cells did not divide. The cells lateral and basal to them hypertrophied and divided. Normal development did not occur until a newly organized shoot apex was formed in several ways. No new primordia were produced on the original apex, which was damaged by irradiation. The cells lateral and basal to the damaged areas could hypertrophy, divide, and either crush the damaged cells and heal the apical meristem, or contribute to the formation of a substitute meristem lateral to the damage (Pratt, 1963). One could conclude, perhaps, that the more centrally placed cells were more sensitive to radiation damage, although the author did not emphasize this possibility. In any event the lateral cells of the damaged apex itself did not continue producing leaves, which one might expect if the results of surgical experiments, used to support the general concepts of méristème d'attente and multiple helices, were applied to this case (see Nougarède, 1965b).

Also for *Kalanchoë* (Crassulaceae), the apex could be damaged by 330 R/20-hour day by Co^{60} (Stein & Sparrow, 1963). In contrast with apple, meristematic activity resumed in the same apex after 26 days and leaves were produced. Recovery of the apex was discussed in terms of several possibilities. Cell selection could operate through cell death or the multiplication of certain cell types. No evidence was found for this, nor for a méristème d'attente which could act as a reservoir of radiation-resistant cells as described by Clowes (1963, and other references cited therein) for certain root tips. Stein & Sparrow (1963) suggested that initial radiation damage may lead to a temporary deficiency of some physiological prerequisite to cell division.

While the results of investigations on chimeras, use of colchicine, and ionizing radiation do not provide indisputable evidence regarding the histogenic and/or organogenic role of each zone of an apex, the conclusion is inescapable that apically positioned cells do have a histogenic function. The degree to which they function probably depends upon the species and is related to the size of the apex, rapidity of leaf initiation, and phyllotactic pattern.

IV. COMPARATIVE CELL-DIVISION RATES IN VEGETATIVE AND REPRODUCTIVE APICES

Various aspects of the process of cell division have been of interest to biologists for a long time. Edgar (1961) noted that most of the investigations on cell division before 1930 were concerned primarily with periodicity in root and shoot meristems. The principal aim of these investigators was to demonstrate the presence of rhythms from fluctua-

tions in numbers of cell divisions in sections cut from shoot and root apices which had been fixed at definite intervals throughout a 24-hour period. Counts were made on standard areas of apices so that comparisons could be made. If rhythms were established, experiments were sometimes made to test the effects of different environmental factors on this rhythm.

The work of Karsten (cf. Chamberlain, 1916) was apparently the first concerned with periodicity of mitoses in shoot apices of higher plants. Rhythms were reported to be present in shoot apices of *Pisum* and *Zea* with a maximum number of mitoses occurring during the dark periods. Edgar (1961) pointed out that the investigations of Karsten were the only ones involving cell division in shoot apices during the pre-1930 period.

In recent years two lines of research have been in progress concerning cell division in shoot apices. Pertinent to this review are the investigations primarily concerned with spatial distribution of mitoses and mitotic rates within the shoot apex. This line of research was prompted by Buvat's (1952a) hypothesis that the central apical portion of vegetative shoot apices is a méristème d'attente (after Bersillon, 1951), or waiting meristem, where cell divisions are rare or lacking.

The hypothesis generated some controversy and has led to a considerable amount of fascinating research concerning relative cell division rates in various regions of the shoot apex. Whether the original hypothesis of Buvat (1952a) is accepted or not is not the prime concern of this portion of the review. Rather, the methods used and the results obtained by investigators of both schools in an effort to test the hypothesis are considered. In order to obtain reliable data on relative mitotic activity within certain regions of the shoot apex, certain other factors which have been shown to influence cell division rates also will be considered.

A.) Mitotic Fluctuations with Respect to the 24-Hour Cycle

Lance (1952) was concerned more with spatial distribution of dividing nuclei of *Vicia faba* L., but she also studied diurnal rhythms in the shoot apex. She found a maximum number of mitoses in the longitudinal section of a 250 × 300 μ region of the apex at 9 a.m., a second lower peak at 9 p.m., and a minimum number of mitoses at midnight. Savelkoul (1957), who collected *Elodea* shoot apices every three hours for 24 hours grown at a constant temperature (21–23 C), did not detect fluctuations in mitoses with respect to time. A similar lack of evidence for diurnal rhythms in cell division was reported in *Chrysanthemum morifolium* shoot apices collected every hour by Popham (1958), who also was concerned with distribution and orientation of mitotic figures. In 1961 Jacobs & Morrow studied mitotic figures in relation to the development of the apical meristem of *Coleus blumei* and considered their data similar to that of Abbe and coworkers (Abbe & Phinney, 1951; Abbe, Phinney, & Baer, 1951), who found an increase in the size of the apex of *Zea* during a plastochron as well as an increase in apical volume during ontogeny.

The round-the-clock collection of apices by Jacobs & Morrow (1961) revealed no diurnal rhythm in the percentage of cells showing mitotic figures. They attributed the large increase in the absolute number of mitotic figures in the middle of the dark period to a diurnal rhythm in the initiation of new leaf primordia which was correlated with the attainment of the maximum height of the apex. They, along with Edgar (1961), were the first to take into account the possible effects of the plastochron stage on mitotic rhythms. A portion of Edgar's (1961) study of shoot apices (taken from a hedge of *Lonicera nitida* Wils.) was concerned with mitotic fluctuations with respect to time of day. She found significant maxima of mitotic activity based on percent of cells in mitosis in specific regions at 11 p.m., 2 a.m., 7 a.m., and 11 a.m. These results were based on apices of the same stage of the plastochron.

Denne (1966b,c) ran a sophisticated set of experiments on *Trifolium repens* L. and *Tradescantia fluminensis* Vell. and demonstrated a very complex interaction of temperature and light. In plants grown in 12-hour light and fluctuating temperatures, there was a marked peak in the mitotic index (cells in mitosis expressed as a percent of the total number of cells per zone) in all regions except the summit (S) towards the end of the light period and a minimum close to the end of the dark period. Plants grown in 12-hour light and constant temperatures showed fluctuations in the mitotic index, but these were not significant at the 5% level. On the other hand, plants grown in 8-hour light and constant temperatures did display a significantly higher mitotic index during the light hours in all regions. Denne (1966b,c) was careful to show that the plastochron stage did not depend on time of day and, therefore, the diurnal rhythm in cell divisions was independent of the plastochron stage in *Trifolium* and *Tradescantia*, contrary to the results obtained with *Coleus* by Jacobs & Morrow (1961). Interested in floral transition studies in *Sinapis alba*, Bernier, Kinet, & Bronchart (1967) found a daily rhythm in DNA synthesis and in mitotic index in both the central and peripheral zones of vegetative apices with a single maximum around midnight. However, during floral transition, the rhythmical activity of the meristematic cells disappeared at least during the first three days after the beginning of induction. Jacqmard (1967, 1968) studied the effects of GA on the mitotic activity and DNA synthesis of the apical bud of *Rudbeckia bicolor* and noted daily changes in the mitotic index and in the index of labelled nuclei in various regions of control plants. However, these daily fluctuations were found not to be significant statistically at the 5% level. A real daily rhythm was found only in the index of nuclei synthesizing DNA in both the pith-rib meristem and the subapical pith where the highest percentages of labelled nuclei were observed during the night. That incorporation of P^{32} into the leaves of *Lolium temulentum* was highest during midday also suggests a diurnal fluctuation in nucleic acid synthesis (Rijven & Evans, 1967b). Studying transition to flowering in the shoot apex of *Datura stramonium* L., Corson (1969) was not able to

detect significant fluctuations in the mitotic index or mitotic rates with respect to time of day in various regions of the vegetative or floral transition apex.

B.) Mitotic Fluctuations with Respect to the Plastochron Stage

Before 1961 mitotic fluctuations as a result of plastochronal changes had not been considered (cf. p. 23 Edgar, 1961). Jacobs & Morrow (1961) attributed the rise in numbers of mitoses in the *Coleus* shoot apex not to the time of day but to the time of leaf initiation, or the beginning of the plastochron, which occurred between 11 p.m. and 1 a.m. Edgar (1961) found no change in the mitotic index in various plastochronal stages of the apex of *Lonicera* fixed at the same hour of the day. Denne (1966a) noted cyclic fluctuations in the mitotic index of the flank regions during the plastochron in *Trifolium*. The mitotic index increased to a peak at the beginning of the plastochron in the flank area which was to produce the next primordium. The flank destined to produce the following primordium had a high mitotic index at the end of the plastochron. However, the fluctuations were sometimes only suggestive trends since they were not always found to be significant at the 5% level. The mitotic index of the summit region remained constant during the plastochron. Similar results were obtained in *Tradescantia* (Denne, 1966c) and *Datura stramonium* (Corson, 1969).

Berg (1970) studied the relation between plastochron stage, apical organization, and mitotic activity based on incorporation of thymidine-C^{14} in the vegetative shoot apex of *Chrysanthemum morifolium* 'Albatross.' He found no differences in number or distribution of labelled nuclei during three stages of the plastochron. Uniform distribution of labelled nuclei throughout the apex was apparent.

C.) Mitotic Changes during Floral Transition

A considerable amount of information has been published concerning changes in mitotic activity in various regions of shoot apices as flowering approaches (Bernier, 1964, 1965; Bernier, Kinet, & Bronchart, 1967; Bonnand, 1959a; Buvat, 1952a, 1955; Corson, 1969; Gifford, 1954, 1963; Gifford & Tepper, 1961, 1962a; Jacobs & Raghaven, 1962; Jacqmard, 1964; Nougarède, Bronchart, Bernier, & Rondet, 1964; Nougarède 1965a,b; Nougarède & Bronchart, 1965; Nougarède, Gifford, & Rondet, 1965; Philipson, 1947; Rijven & Evans, 1967a,b; Saint-Côme, 1965; Sunderland, 1961; Thomas, 1963; Wetmore, Gifford, & Green, 1959; Wardlaw, 1957a; and others). In general, it can be concluded that mitotic activity of the axial apical cells of the vegetative shoot apex increases during floral transition, thus becoming equal to or almost equal to the mitotic activity of the peripheral cells. However, there appears to be an overall increase in mitotic activity of all regions upon floral transition in the apices studied.

Certainly not all of the factors which affect mitotic activity have been considered here. As pointed out by Edgar (1961), "since the experi-

ments had been carried out under such a variety of conditions, there was no definite information as to the effects of light and temperature on mitosis in shoot and root apices." Also, it is difficult to compare results involving comparative mitotic activity within a shoot apex because of the variety of conditions used. It would seem essential to the present authors that if mitotic activity is to be compared between certain regions of the shoot apex, the effects of factors such as light, temperature, time of day, and apical stage must be carefully controlled so that the results can be compared with others.

D.) Measuring Relative Mitotic Activity in Various Zones of the Shoot Apex

Comparative mitotic rates within various zones of the shoot apex were first based on the number of cells in mitosis (Bersillon, 1951; Bonnand, 1959a; Buvat, 1952a, 1953, 1955; Camefort, 1956; Catesson, 1953; Gifford & Tepper, 1961, 1962a,b; Hsü, 1944; Jacobs & Raghaven, 1962; Lance, 1952; Millington & Fisk, 1956; Popham, 1958; Popham & Chan, 1950; and others).

Buvat (1952a) used a method (Buvat & Genèves, 1951) of superimposing camera lucida drawings of several sections of *Myosurus, Lupinus,* or *Cheiranthus,* and he noted that there were no mitotic figures in the most axial apical cells while the surrounding peripheral cells had them. He concluded that the axial region was a méristème d'attente (after Bersillon, 1951) which had no vegetative histogenetic function. Lance (1952), using the same technique, noted few mitotic figures in the axial region of the vegetative apex of *Vicia faba.* That cell divisions were present in the axial region was verified in *Luzula* (Juncaceae) (Catesson, 1953), *Triticum* (Buvat, 1953), and *Chrysanthemum* (Popham & Chan, 1950). Buvat (1955) modified his original hypothesis and admitted that divisions do occur in cells of méristème d'attente. Even though mitotic activity was present in the axial region, it was relatively low. The axial region is not considered a region of initials but is passive (Buvat, 1955). As pointed out by Romberger (1963) and Clowes (1961a), Buvat does not ask us to believe that summit cells never divide. He admits that they may be mother cells but only by virtue of their position, not because they have special inherent qualities. On the other hand, the "anneau initial" (initiating ring) is said to have special qualities (see page 147, this review). Others have also found fewer but some divisions in the apical axial regions of *Nicotiana tabacum* (Bonnand, 1959a), *Vicia faba* (Ball, 1960), *Chenopodium album* (Gifford & Tepper, 1961, 1962a,b), *Chrysanthemum morifolium* (Popham, 1958), *Xanthium pennsylvanicum* (Millington & Fisk, 1956), *Perilla* (Jacobs & Raghaven, 1962), and some gymnosperms (Camefort, 1956).

Labelled precursors of DNA synthesis have also been used by investigators to determine relative mitotic activity expressed in terms of numbers of labelled nuclei. Results from these studies support

the evidence that divisions do occur in axial apical cells. Partanen & Gifford (1958) treated several species of plants with various concentrations of phosphorus-32 by immersing their roots or cut stems in the solution for periods of one to seven days. Using autoradiographic techniques they concluded that DNA synthesis does occur in the central axial zones of the species studied. Injecting labelled adenine into the stem of *Coleus* or immersing plants of *Vallisneria* (Hydrocharitaceae), *Elodea*, or *Cabomba* (Cabombaceae) into a radioactive solution of adenine, Clowes (1959) observed the distribution of DNA synthesis in the shoot apices and concluded that there was no méristème d'attente. Evidence of mitotic activity in the region of the méristème d'attente was obtained with labelled adenine by Lance-Nougarède (1961a) for the meristems of *Lupinus albus* L. and *Teucrium scorodonia* (Labiatae). Radioactive nucleosides seem best incorporated into the apices of shoots when applied directly to the tip (Gifford, Kupila, & Yamaguchi, 1963; Gifford, 1960) or injected just below the apex (Clowes, 1959; Lance-Nougarède 1961a). Phosphorus-32 readily moves to the shoot when roots are immersed in the solution (Gifford & Tepper, 1962a; Partanen & Gifford, 1958) or when it is applied to leaves (Rijven & Evans, 1967b).

The use of numbers of mitotic figures as representative of actual cell division rates was criticized by many investigators as insufficient evidence because it was recognized that total numbers of cells as well as size of cells varied with different regions of the apex (Cutter, 1959; Clowes, 1959; Gifford, 1954; Nougarède, 1965a; Wardlaw, 1957b; and others). It was believed that the number of cells in division expressed as a percent of the total number of cells counted (mitotic index) would better represent true mitotic rates. Investigators of the shoot apex did present their data in terms of the mitotic index (Berg, 1966; Bernier, 1964, 1965; Bernier, Kinet, & Bronchart, 1967; Corson, 1969; Denne, 1966a,b,c; Edgar, 1961; Jacobs & Morrow, 1961; Jacqmard, 1964, 1967, 1968; Nougarède 1965a; Nougarède & Bronchart, 1965; Nougarède, Gifford, & Rondet, 1965; Saint-Côme, 1965; Savelkoul, 1957; Thomas, 1963; and others). Mitotic indices were calculated by (1) direct observation of dividing nuclei expressed as a percentage of the total number of cells observed in a particular region of the shoot apex, or (2) expressed as a percentage of the total number of cells on a slide when the squash technique was used. Mitotic indices were also calculated from shoots treated with radioactive percursors of DNA synthesis and processed with autoradiographic techniques. Most reports indicated results similar to those obtained when mitotic activity was expressed in terms of numbers of mitoses only. The mitotic index was lower in the axial region of the vegetative apex as compared to that of the peripheral region. In floral transition studies, the mitotic index of the central region increased to equal or almost equal that of the peripheral region.

Several authors have shown that the mitotic index does not necessarily represent the true rate of cell division (Brown, 1951; Clowes, 1961b;

Edgar, 1961; Evans, Neary, & Tonkinson, 1957; Hoffman, 1949). "The time taken for the initiation of a new cell must be considered as including both the time taken in mitosis and the time taken in interphase; in other words, rapidity of growth depends on the length of the whole of the mitotic cycle and not on the length of mitosis. Therefore a high mitotic index does not necessarily imply a rapid growth rate" (Edgar, 1961, p. 41). Jacobs & Morrow (1961), who found a lower percentage of dividing cells in the distal end of the shoot apex of *Coleus*, did not know if the lower percentage of distal cells showing mitotic figures was a reflection of a slower rate at which the cells were undergoing mitosis, or if the duration of mitosis was less there, or both. As pointed out by Evans, Neary, & Tonkinson (1957), an increase in the mitotic index can mean either a relatively greater slowing down of the mitotic phase as compared with the time in interphase or a speeding up of the rate of progress through interphase while the mitotic phase stays constant or slows down. So an increase in the mitotic index could be associated with either an increase or a decrease in the rate of passage of the cells through the mitotic cycle. Similarly a decrease in the mitotic index could be interpreted in two ways. This was illustrated factually by Clowes (1961b), who calculated the rate of the entire mitotic cycle (T) of cap initials of *Zea* root tips to be once every 12 hours, whereas the central stelar cells divided every 29 hours, and the cells of the endodermis (22 μ behind the quiescent center) divided once every 35 hours. However, the observed mitotic indices for these regions were 15.7, 16.7, and 17.7 respectively. Certainly, here, the mitotic index does not represent the rate of cell division.

Mitotic rates have been determined by the use of several methods such has histoautoradiography, colchicine application, time-lapse photography, and volume-time relations. With the use of time-lapse photography, rates of cell division were determined for the surface cells of shoot apices (Ball, 1960; Soma & Ball, 1963; Ball & Soma, 1965). A rate of cell division of 22.5 to 24.5 hours was found to remain fairly constant in surface cells of shoot apices of *Vicia faba* cultured in various sugar concentrations (Ball & Soma, 1965). Because of the relative ease of applying radioactive materials or colchicine to roots, more information has been reported for mitotic rates in roots than in shoots. Evans, Neary, & Tonkinson (1957) presented a method for the calculation of mitotic rates with the application of colchicine using *Vicia faba* root tips. They demonstrated that colchicine applied in certain concentrations over a period of time will arrest chromosomes in metaphase sufficiently to allow for the determination of mitotic rates. Three concentrations of colchicine (0.1%, 0.05%, 0.025%) were found to have similar capacities for metaphase accumulation during treatments of one to six hours duration. With increased durations, the weaker concentrations were more efficient, while the stronger concentrations inhibited the rate of entry of cells into mitosis thus increasing the time spent in interphase. They

found a mitotic time (τ) of 3.9 hours and a complete cycle time (T) of 24.6 hours in whole mount squashes of the root apex.

Clowes (1961b) was apparently the first to use histoautoradiography and the application of colchicine on sectioned material whereby rates could be compared between various regions. He found both T (duration of mitotic cycle) and τ (duration of mitosis) to vary in different regions of the *Zea* root tip. Since cells of one region took less time in mitosis than did cells of another region (from τ), he concluded that the mitotic index did not accurately describe mitotic activity in the *Zea* root tip. In her study of the shoot apex of *Lonicera* grown under natural conditions, Edgar (1961), relating the number of new cells produced over a known period of time, calculated the duration of mitosis to be 5.1 hours and the duration of the complete mitotic cycle to be 44.8 hours in the upper layer of the apex during a plastochron. Although these rates were calculated for only one region, she noted the possibility that, since the mitotic index varied with the position in the apex, the duration probably also varied. She compared her results with those of Sunderland & Brown (1956), who measured the volume and total number of cells before and after a specific time period for fragments of the apex of *Lupinus albus* containing several internodes squashed on slides. An average value of one cell division every three days (72 hours) was reported which, according to Edgar (1961), was the first calculation of cell division time in shoot apices. Edgar (1961) pointed out that cell-division rates in roots seem to be faster than in shoots based on investigations of root tips prior to her study (Laughlin, 1919; Brown, 1951; Gray & Scholes, 1951; Evans, Neary, & Tonkinson, 1957; and others).

Sunderland (1961) studied cell division and expansion in the growth of the shoot apex of rye and lupine in the vegetative and floral states. Rates were calculated by measuring the volume and total cell number in apices before and after a specified time period. In the vegetative state of non-vernalized rye, he found that cells divided every 1.8 days at first, and later every 5.8 days. Cells divided once every 2 days and later once every 1.4 days in reproductive apices. In the vegetative apex of lupine, cells divided once every 1.3 to 1.8 days and once every 1.4 days during floral transition.

Using the methods of Clowes (1961b) and Evans, Neary, & Tonkinson (1957), Berg (1966) calculated the rates of mitosis in specific zones of the vegetative shoot apex of *Chrysanthemum* grown in an environmental chamber with a temperature of 20 C and 20 hours of light (1800–2000 ft-c). By applying a 0.25% solution of colchicine to the apex by means of a cotton tuft after removing the tips of the young leaf primordia, Berg determined that the cells of the flank zones divided every 70–73 hours, the cells of the axial zone divided about half as often, and the cells of the rib meristem divided about 0.7 times as frequently as those of the flank meristem. The rates determined for each zone were not

out of line with the time required for the apex to double in volume, which was calculated to be about 50 hours (the cells in the apical dome probably divide only on the average of once every 50 hours). Berg (1966) attributed the high rates calculated by accumulation of metaphases to the fact that they were based on increase in percent metaphases between 3 and 10 hours after applying colchicine, while metaphases had not actually begun to accumulate in the apical dome by the third hour.

Denne (1966a), using a modified technique of Clowes (1961b), calculated the rates of mitoses of specific zones of the vegetative shoot apex of *Trifolium* grown under short-day conditions in the greenhouse and treated with a 0.05% solution of colchicine applied to a plastic tube lined with a damp filter paper covering the shoot after the young leaves had been bent back. The duration of mitosis (τ) was about the same for the cells of all regions, varying from 3.3 to 4.2 hours. The duration of the mitotic cycle (T) varied between zones: the cells of the summit zone divided on the average of once every 108 hours, the cells of the two flank zones divided once every 69.4 and 86.9 hours, and the cells of the rib meristem divided once every 136.5 hours. By comparing the above results with her mitotic index studies, Denne (1966a) concluded that the mitotic index did represent actual cell division activity in the shoot apex of *Trifolium.*

Although rates were not determined, a thorough investigation of mitotic indices was made on the shoot apex of *Sinapis alba* during floral transition (Bernier, Kinet, & Bronchart, 1967). A rise in the mitotic index of both the peripheral and central zones as early as 18 hours after induction was detected. A second peak in the mitotic index occurred about 62 hours after induction. The second peak corresponded to the initiation of the first flower buds. The mitotic index of the pith-rib meristem remained low but showed a sharp increase in the induced meristem between 22 and 30 hours after the beginning of the inductive cycle. With the use of labelled thymidine and autoradiography, DNA synthesis was found to occur after the first mitotic index peak. It was concluded that a great number of cells in G_2 (the post DNA synthesis period of interphase) was built up and that the floral stimulus activated mitosis. It was suggested that short days considerably increased the length of G_2 in the meristematic cells and that they remained in G_2 and entered mitosis in response to the floral stimulus (also see page 175, this review).

Changes in mitotic activity expressed in terms of the mitotic index as well as calculated rates of mitosis by accumulation of metaphases (after Evans, Neary, & Tonkinson, 1957; Clowes, 1961a; Denne, 1966a) were studied in certain zones of the vegetative and transition shoot apex of *Datura stramonium.* This species was found to flower after a certain number of leaves (7–8) were produced when grown at 20 C and in 8 hours of light (1800–2000 ft-c) and 16 hours of darkness (Corson, 1969).

A 0.5% solution of colchicine was applied to a piece of cotton positioned between young leaves close to the apex. Lower concentrations of colchicine, as used by other investigators, did not seem to be as effective on *Datura*. Mitotic rates were based on the accumulation of metaphases between 4 and 8 hours after the application of colchicine. It was found that the duration of mitosis (τ) could be considered constant for the cells of the various zones whose values varied between 1.86 and 2.15 hours in the vegetative apex and 1.49 and 2.06 hours in the floral transition apex. These variations were less than those reported by Denne (1966a), who also considered the duration of mitosis (τ) to be constant for cells in various regions of the vegetative shoot apex of *Trifolium*. The values in hours for the duration of the complete mitotic cycle (T) in *Datura* varied between zones and increased during floral transition as shown below:

	Summit	Flank 1	Flank 2	Central
Veg.	76.2	35.7	36.2	32.7
Trans.	46.2	29.0	26.2	25.8

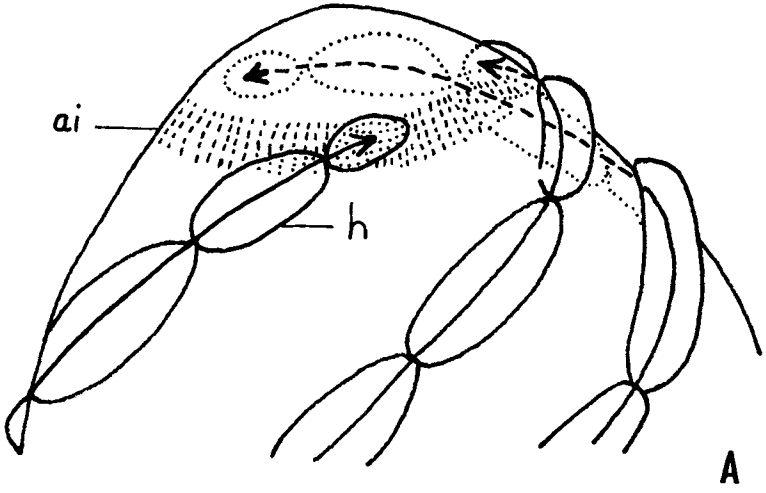
While Clowes (1961b) demonstrated that the time a cell spent in mitosis (τ) varied in zones of *Zea* root tips, Corson (1969) and Denne (1966a) considered (τ) to be constant. From the relation (from Evans et al., 1957)

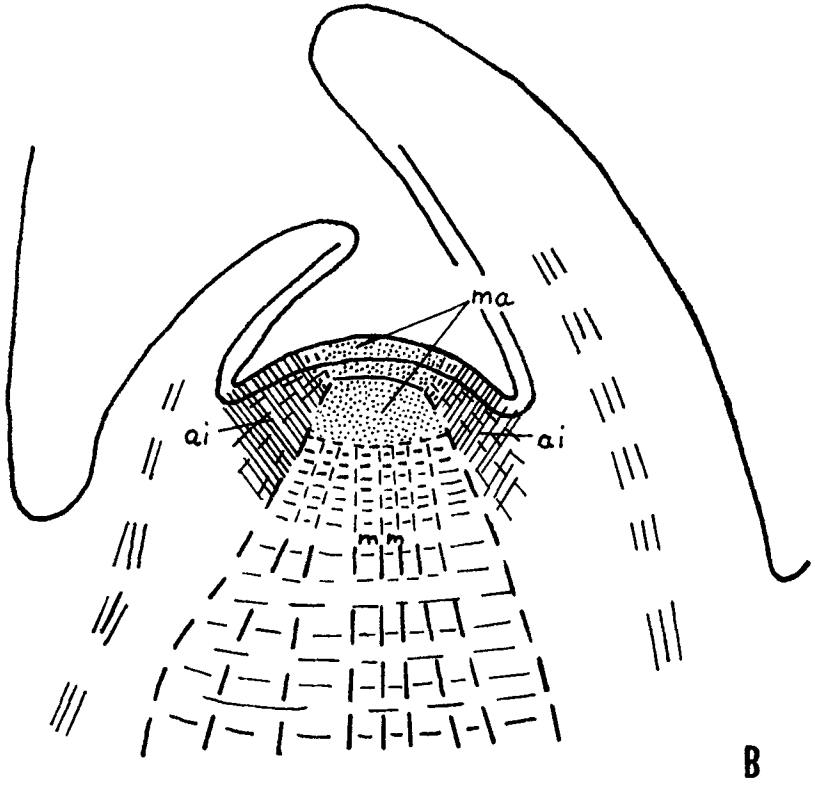
$$\mathrm{MI} \simeq \frac{\tau}{\mathrm{T}} \log_e \frac{2\text{-}l}{1\text{-}l}$$

where $l = 0$ (assume all cells to be dividing)

it can be seen if (τ) is constant and 1/T (number of divisions per cell per hour) varies in the same proportion with the mitotic index, the mitotic index can be considered representative of actual mitotic rates. Corson (1969), as did Denne (1966a), concluded that since relative changes in the mitotic index were proportional to the relative changes in 1/T, the mitotic index is a useful and accurate means of expressing mitotic activity in vegetative and floral transition shoot apices of *Datura*.

Because information with regards to the effects of various factors on mitotic activity such as plastochron stage, diurnal periodicity, temperature and light appears to vary with the investigator and his material, it would seem essential to the present authors that consideration of these factors must be made in order to obtain reliable data that can be compared with other work. Although rates of cell division in meristematic regions have been studied for decades, investigations of comparative mitotic rates of specific regions of vegetative and floral transition shoot apices are few in number. Future work involving many species is, indeed, necessary before we can be certain of the role that the mitotic index plays in representing mitotic activity.


ai
h
A


ma
ai
ai
B

V. COMMENTS ON THE "MÉRISTÈME D'ATTENTE-ANNEAU INITIAL" CONCEPT

In 1959 Cutter stated that some sort of authoritative statement was needed from morphologists who support the French-oriented concept of shoot apical structure. Such a statement was needed to clarify the present views on the theory of multiple helices and their generative centers that produce leaves. A very extensive review of the subject was published recently by A. Nougarède (1965b) in a volume dedicated to L. Plantefol—the originator of the concept of multiple helices and its attendant implications. The following section pertains primarily to an analysis of that publication.

The very heart of the morphological and histological implications of shoot development, as held by most French morphologists, depends upon the theory of multiple helices as envisioned by Plantefol (1947). This theory has been reviewed several times by various authors, but a brief explanation is deemed necessary in order for the following discussion to be understandable (also see page 166, this review). According to Plantefol's concept, leaves are disposed on the stem along "foliar helices" and the leaves are in continuity throughout the length of each helix (Fig. 6). The terminal portions of each helix end in foliar generative centers which function in a subapical zone—the "anneau initial." Nougarède (1965b) stated that in the spirit of the concept (Plantefol's) the anneau initial maintains its proliferative activity without it being necessary to call upon axial initials endowed with special organogenetic properties. We shall return to this subject later in the text.

Following the original announcement of the theory of multiple helices, a major effort was and has been devoted to cytohistological studies, the uses of which are directed toward support of the theory. Nougarède (1965b, 1967) recognizes that other workers at an earlier date, particularly A. S. Foster (1938), described the existence of zonate patterns in vegetative shoot apices, but that they did not abandon the idea that there are initials at the distal end of the shoot apex. In a series of articles Buvat (1950, 1951, 1952a,b, 1953, 1955) established the cytohistological concept and terminology used subsequently by proponents of the theory of multiple helices. Basically, the terminology consists of "méristème d'attente" for the most apical region (which may

---

←

FIG. 6. *A*, schematic representation of the vegetative shoot tip according to the concept of "multiple helices." The leaves appear in continuity the length of each foliar helix (*h*), ending in the initiating ring or "anneau initial" (*ai*) where a new leaf will be initiated (after Plantefol). *B*, diagram, shoot apex of *Cheiranthus cheiri* based on the concept of an inactive apical zone ("méristème d'attente," *ma*) an active lateral zone ("anneau initial," *ai*), and a rib-meristem (méristème médullaire) which gives rise to the pith. At the time of flowering the "méristème d'attente" would become active (after Buvat).

encompass tunica and corpus), "anneau initial" referred to earlier, and the "méristème médullaire," the meristem giving rise to the pith (Fig. 6). Terms for the same regions used by most anatomists at the time were "central zone," roughly comparable to the méristème d'attente in volume, "peripheral zone," a flanking zone in which leaves take their origin from the more superficial layers of the zone, and the "pith-rib meristem," the progenitor of the pith. Buvat (1952a, 1953) concluded that the méristème d'attente has no organogenetic or histogenetic role during vegetative development. Evidence was put forward to prove that the méristème d'attente is without divisions during vegetative growth (Buvat, 1962a; Lance, 1953). This original position has been modified somewhat (to be discussed later).

Throughout the literature pertaining to the concepts of multiple helices, méristème d'attente, anneau initial, etc., proponents of these theories have emphasized the importance of RNA as an indicator of cell activity. Zonate vegetative apices, stained with pyronin or azure B, for example, generally reveal a deeper staining in cells of the flank than in cells of other regions. Since these dyes stain RNA, the results were cited as evidence for the more active role of the anneau initial, although quantitative data were not supplied. Quantitative studies have shown recently that the average content of RNA, as well as DNA and protein, was the same in all regions of the shoot apex of *Pisum sativum* c.v. Lincoln, although differences in rates of cell division did occur (Lyndon, 1970a,b). Additional quantitative studies are needed to determine if the results for *Pisum* are generally true for other species.

Nougarède (1965b) notes in her extensive review that there were reactions by other morphologists, principally those writing in the English language, to the original pronouncement of Buvat. She points out the Anglo-Saxon reticence to accept the new idea. In another section of the review entitled "Les prétendus arguments de quelques chercheurs de langue anglaise," Nougarède discusses mainly the work and views of Newman and Ball in which these workers reported on the results of direct observations of the apex. Newman (1956) made direct observations on the apex of *Tropaeolum majus* L. and noted changes in organization of the superficial layers of the meristem. He concluded that divisions must occur in the axial cells. Using isolated segments of the shoot, 10 mm in length, Ball (1960) cultured the apices of several angiosperms (*Tropaeolum, Vicia, Asparagus, Lupinus*) and was able to photograph the surface of the shoot apex by time-lapse photography. Ball concluded that cells in the superficial layer of the axial zone divided as frequently as those on the flanks. Because the culture medium contained gibberellin and coconut milk, Nougarède (1965b) concluded that these observations only demonstrated that divisions can be induced by substances in the culture medium. However, it should be noted that the results of rather profound surgical operations are used to support the theory of "multiple helices" and the existence of a self-perpetuating "anneau initial." A

choice between these two experimental alternatives is perhaps difficult to make.

Loiseau (1962), a supporter of the concept of multiple helices, also reported an active mitotic role for surface cells of the apex of *Impatiens roylei* Walp. This was accomplished by observing the displacement of small drops of India ink from the apex to the flanks. Movement was more rapid when the ink spots (and the cells below them) reached the flanks of the apex.

A complaint was registered by Nougarède (1965b) to the effect that it is difficult to know what is meant precisely by an initial cell as used by authors writing in the English language. This complaint is perhaps justified and will be discussed later. Nougarède reviewed Newman's (1961) concept of apical organization. Newman described the type "duplex" as being characteristic of an apex which can be described in terms of tunica and corpus in which there are two superimposed regions of growth, each taking its origin from initial cells for each zone. The type "simplex," encountered in gymnosperms, exhibits a single superficial zone of initials. As a third type, Newman recognized the type of organization exhibited by ferns and lower vascular plants in which there is very clearly one apical cell at the shoot apex. This he designated as the "monoplex" type (Fig. 7).

In 1965 Newman extended and reiterated some of his ideas on apical organization published in 1961. His main theme is the concept of a continuing meristematic residue (initial cells) and the general meristem. Newman (1965) argued that to understand structure and functioning of a meristem of any kind, it is necessary to analyze it into two components: (1) "Firstly, a source of cellular structure, the continuing meristematic residue, which, for convenience, may be called the initial cell (or cells) and from which emergence, though very slow, is continuous and of long duration." (2) "Secondly, a region of elaboration, the 'general meristem,' which contains a phase of great rapidity, is continuous, but of only short duration." The analogy he used is a filament with a terminal cell which divides and, hence, could be referred to as an apical cell (initial). When the apical cell divides, the new terminal cell remains (distal cell of two daughter cells); the proximal cell may divide several times before it and its derivatives mature. The function (source of structure) of the original apical cell is "passed" on to the new terminal cell which results from the transverse division (Fig. 7). Newman, then, has stressed the long-term continuing function of cells at the distal end of the shoot apex. In this regard Nougarède (1965b) has not understood the intent of Newman's arguments.

In an earlier publication Newman (1956) made an analogy between organization of the vascular cambial region and the shoot apex. He suggested that there is a parallel between the two in the sense that there is an initiating region and an adjacent meristematic region. The initiating layer of the cambium is functionally consistent, but the width of the

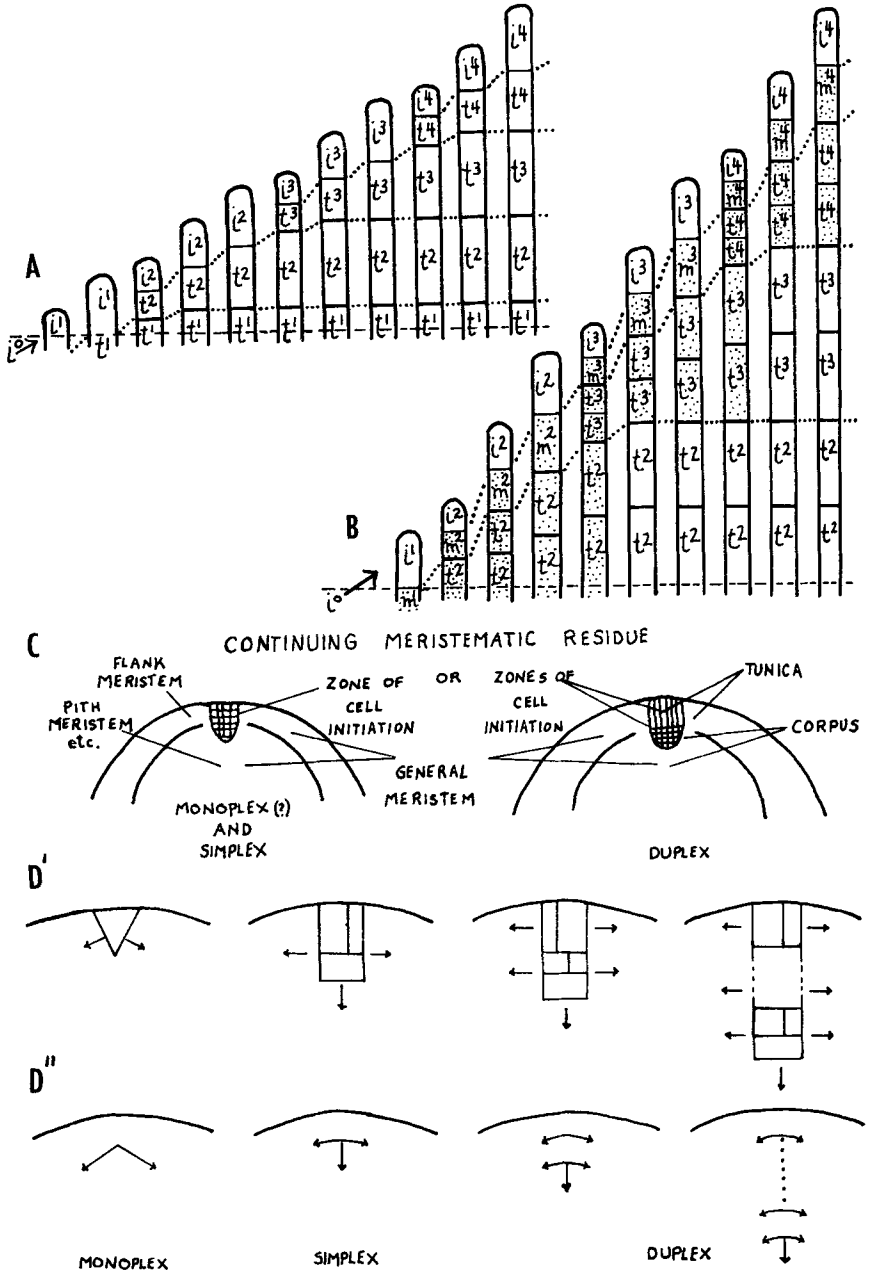


FIG. 7. Schematic diagrams explaining the concepts of the continuing meristematic residue and the general meristem (after Newman). *A*. The continuing meristematic residue as the terminal cell of a filament with division only in the terminal cell, i^0, i^1, i^2, etc. *B*. As in *A*, except that the lower daughter cell (m) of a division of the continuing meristematic residue (i^1, i^2, etc.)

zones of phloem and xylem mother cells varies. In a study of *Thuja occidentalis* L. Bannan (1955) reported a greater percentage of divisions in the xylem mother cells than in the initiating layer during the development of early wood in a vigorous tree. Bannan (1955) indicated that "The ontogenetic sequence in the cambial region has features of resemblance with that in the stem tip. In the latter a small number of initials produce central mother cells which in turn give rise to the tissue systems of the stem." Nougarède (1965b) discussed Newman's (1961) concept by stating that in a division of the cambial initial one cell is formed that retains the faculty to divide, while the other gradually becomes specialized. However, in the example cited above (*Thuja*) the derivatives may divide several times, and only then do these cells undergo differentiation and maturation.

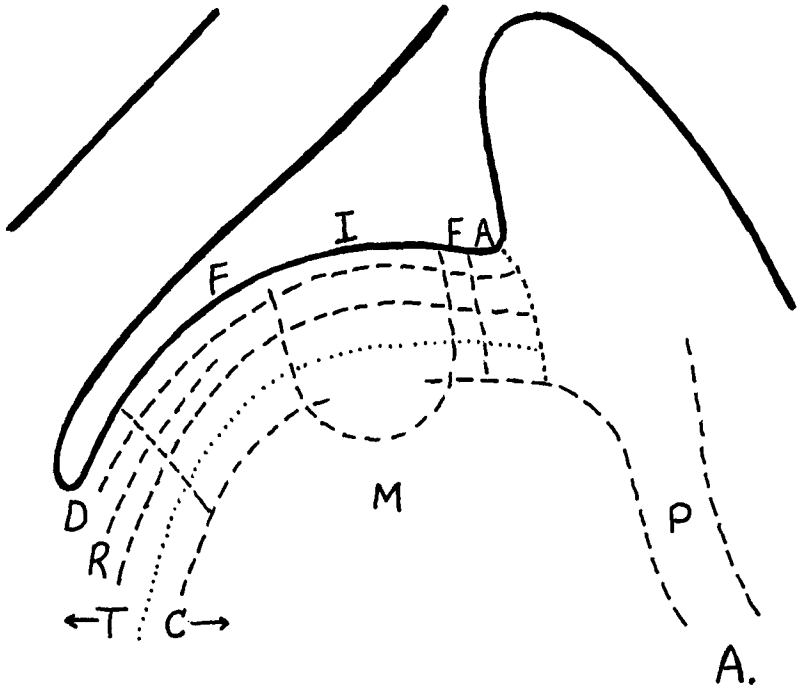
The same argument could be made for marginal meristems of leaves. Maksymowych & Erickson (1960) have shown that the mitotic index of marginally positioned cells in young leaves is lower than those removed from the margin, yet marginal initials do divide to maintain the system.

Hagemann (1960) described apical structure for *Cyclamen* (Primulaceae), *Magnolia* (Magnoliaceae), *Liriodendron* (Magnoliaceae), and *Peperomia* (Peperomiaceae). For angiosperms he recognized a central initial zone (central mother cell complex) and a flank meristem (zone of organogenesis). The two together constitute the shoot apex (Vegetationspunkt). The pith-rib meristem, procambium, cortical meristem, and dermatogen collectively would be the zone of histogenesis. Collectively all the zones would constitute the vegetative cone (Vegetationskegel) or zone of primary growth. Hagemann (1960) utilized the apex of *Liriodendron tulipifera* L. to illustrate his concept (Fig. 8). Near the edge of the central initial zone, where a leaf was expected, there was initially a center of mitotic activity (Mitosefeld). From this site the flank meristem was activated (Fig. 8). Hagemann raised the question of whether a part of the flank meristem is used up after each plastochron and is then replenished from the central initial zone or whether the flank meristem is only activated at the margin of the initial zone at the place where a leaf will arise. The result, in any event, was a ring-shaped zone that gives rise to the peripheral part of an internode and to a leaf. Nougarède (1965b) believes that in spite of the divergence in the use of terms Hagemann's concept agrees with the French concept. To the present reviewers the two concepts are fundamentally different

---

←

divides and constitutes the general meristem. *C*. Correlations between the concepts of the meristematic residue and general meristem and descriptions of shoot apical meristems. *D'*, *D''*. Diagrams of the basic types of continuing meristematic residue in minimum form (*D'*) and minimum activity (*D''*); in the right-hand diagrams of the duplex type, the broken lines indicate an unspecified number of layers with anticlinal division.


I
FA
F
M
D
R
T
C
P
A.

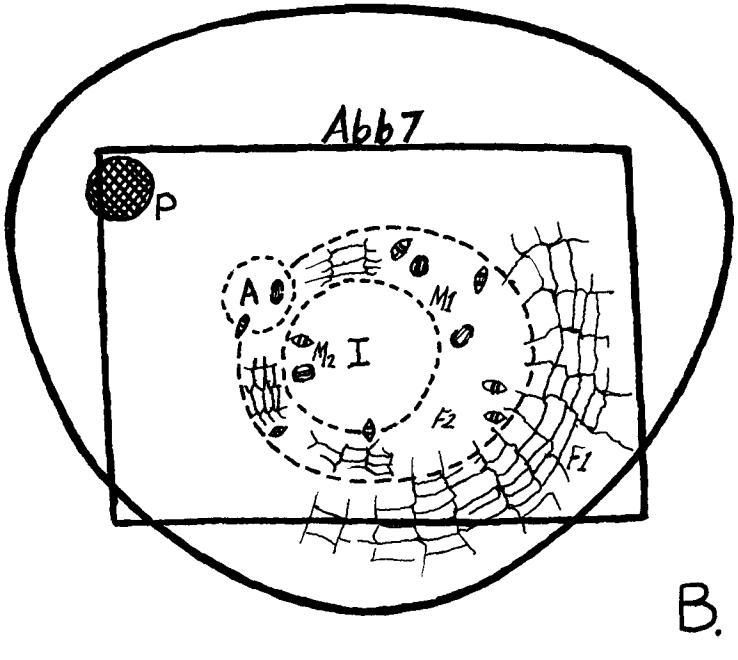


Abb7
P
A
M1
M2
I
F2
F1
B.

since Hagemann stressed the importance of the initial zone (apical) in initiating each repetitive event (plastochron). Furthermore, for *Peperomia*, a plant with a small apex, Hagemann (1960) stated that the flank meristem was renewed directly from cells of the apical initial zone at each plastochron.

In a section entitled "Les tendances nouvelles de l'école américaine," Nougarède (1965b) recounted the results of certain researchers in the United States. She asserted that French researchers have posed important questions to histologists. She claims that recent publications in the English language reflect a new indecision on the part of workers who publish in English (see page 202 of above cited review). In this connection she cited the publications of Paolillo & Gifford (1961) on *Ephedra*, Tucker (1962) on *Michelia* (Magnoliaceae), Tepfer (1960) for *Clematis*, and Gifford & Tepper (1961) for *Chenopodium*.

For *Ephedra altissima* Paolillo & Gifford (1961) were able to determine with little doubt the existence of divisions in a group of subapical cells which could be related to definite phases of the plastochron. It could also be established that transverse divisions in the subapical zone provided the elements for a difference in height apparent between minimal and maximal area phases of the plastochron. In addition, from a careful analysis of growth of the apex, it was concluded that the concept of "anneau initial" (original sense) cannot be satisfactorily applied to the apex of *Ephedra altissima*. It was concluded that, "regardless of which perspective one chooses to adopt, the distal cells are the source of all the other cells of the apex." Lance (1957) suggested that all apices may vary by degrees from the model. Paolillo & Gifford (1961) disagreed with Nougarède (1965b) over what the model should be and stated: "The model consisting of an independent anneau initial and méristème d'attente seems to represent the extreme case rather than the rule." This statement would hardly constitute indecision.

It is quite apparent that the main obstacle in any understanding and agreement upon organization of the vegetative apex revolves around the use and meaning of the term "initial." Two more of the apparent inconsistencies and/or contradictions of writers in the English language were cited by Nougarède (1965b). The organization of the shoot apex

---

←

FIG. 8. *A*. Schematic representation of median longitudinal section, shoot apex of *Liriodendron tulipifera* showing zonation and tunica corpus organization. *I*, initial zone; *F*, flank meristem; *A*, axillary meristem; *M*, pith meristem; *D*, dermatogen (protoderm); *R*, cortical meristem; *T*, *C*, boundary between derivatives of tunica and corpus; *P*, procambium. *B*. Diagram, surface view of shoot apex of *L. tulipifera* near the summit. *I*, initial zone; *F*1, portion of flank meristem associated with stipule growth; *F*2, portion of flank meristem associated with mitotic field, M_1; M_2, mitotic field or center where the youngest leaf is expected to arise; *A*, axillary meristem and procambium (*P*) of an older leaf primordium.

of *Michelia fuscata* Blume was described by Tucker (1962), who recognized a central initial zone in which divisions are rare and generally localized at the periphery of the zone. In spite of this observation Nougarède (1965b) maintained that Tucker attributed an active role for the central initial zone.

Johnson & Tolbert (1960) introduced the term "metrameristem" for the zone which comprises the surface initials and central mother cell zone of gymnosperms, the central zone in angiosperms, if tunica and corpus are recognized, or the central part of mantle and central mother cell zone if the mantle-core concept (Popham, 1951) is adopted. Johnson & Tolbert (1960) used the term "metrameristem" according to Nougarède, in spite of the weak mitotic activity of the zone. Nougarède suggested that the terms "axial cells" could be used in place of "initials" which would emphasize the interdependence of diverse zones rather than implying the acquisition of special organogenetic properties. Those who use the term "initials" for centrally located cells have never implied that they had organogenetic functions, *per se*. The above examples point up forcefully the misunderstanding of terms and their usage by both schools of thought. Tucker (1962), Johnson & Tolbert (1960), and Tolbert (1961) have used the terms "central initials" or "metrameristem" in the sense that the origin of cells is directly or indirectly from the zone in question. This zone is the ultimate site of origin of cells. However, derivative cells may be more meristematic, persisting in the meristematic state for varying lengths of time. Restated, the central or axial initials are part of a growth system in which the zone under consideration moves on in time, occupying space and leaving behind it tissues in various stages of meristematic activity, differentiation, and maturity.

New trends of the French school. In the earlier publications of Buvat (e.g., 1952a, 1953) and those of Lance (1952, 1953) attempts were made to demonstrate the lack of mitotic activity in the méristème d'attente of certain shoot apices. The contention was made that the apical zone (méristème d'attente) was inactive mitotically until flowering occurred and was carried passively along by the anneau initial. In 1955 Buvat recognized that a few mitoses could occur in the méristème d'attente, but claimed that their role was minimal in relation to the role played by the anneau initial. Nougarède (1965b, p. 318) admitted that the occurrence of divisions in the méristème d'attente is detrimental to their original contention, but the presence of these divisions is compatible with the functioning of a "méristème de flanc." It is interesting to note that the latter term was used instead of anneau initial—a departure from the original terminology.

Several examples are discussed by Nougarède (1965b) that pertain to problems posed by the organization of the "adult" vegetative shoot apex. The mode of establishment of the anneau initial during germination was described for *Chrysanthemum segetum*. The anneau initial, once established, is said to be responsible for the restoration of sites of leaf

initiation. The method of regeneration of the anneau initial was described in detail for *Leucanthemum parthenium* L. The term "méristème de flanc" (flank meristem) was used interchangeably with anneau initial; the former term was used earlier by writers in the English language but devoid of any self-perpetuating connotation. The use of the term "méristème de flanc" is a new tendency on the part of cytologists in France. Other examples were described (*Alyssum maritimum, Beta vulgaris, Veronica teucrium* L., and *Teucrium scorodonia*) in which selected photomicrographs were used to support the concept of anneau initial. By identical processes Nougarède (1965b) related the results of others to the importance of the anneau initial in the restoration of leaf sites.

She acknowledged the existence of mitoses in an axial position (presumably in the méristème d'attente) in certain examples investigated by researchers writing in the French language. In one such example, *Scabiosa ukranica* L. (Dipsacaceae), she reported the occurrence of periclinal divisions in axial positioned cells, but considers them to be insufficient in number to augment the lateral regions. Nougarède concluded that axial divisions or even the participation of axial cells in lateral organogenesis cannot invalidate the existence of the flank meristem nor diminish its role. Again one notes the use of the term flank meristem instead of anneau initial. One must conclude that flank meristem is now equated with anneau initial.

The second example is somewhat curious. The model was established, but Nougarède stated that not all apices function according to the model. For example, in *Impatiens biflora* Walter and *I. balfouri* Hook. (Balsaminaceae), at each plastochron, cellular material is "borrowed" from the axial zone since the leaves cut into or encroach upon most of the axial meristem. According to Loiseau (1959), as cited by Nougarède (1965b), this divergent type is derived from the classical type (i.e., clear separation of méristème d'attente and anneau initial). It would appear that the term "borrow" is a most unfortunate choice of words. She circumvents the direct statement that it is the anneau initial that "borrows" the cellular material.

In a section entitled "Détermination de la véritable valeur des divisions axiales," Nougarède again expressed the view that the results obtained by Newman (1956) and Ball (1960) on direct observations of divisions in superficial layers of isolated apices cannot provide useful information for normal morphogenesis.

Nougarède (1965b) reported that during early phases of vegetative growth of *Tropaeolum majus* the apex functions according to their scheme—restoration of leaf sites, presumably by the anneau initial. Starting with the initiation of the 7th to the 13th leaf, the apical axial zone is said to become enriched in RNA, to include the cytoplasm and nucleoli. It is difficult, however, when one compares the apices of young plants (*Plate V, Fig. 4, Plate VI, Fig. 1*) with those of older plants

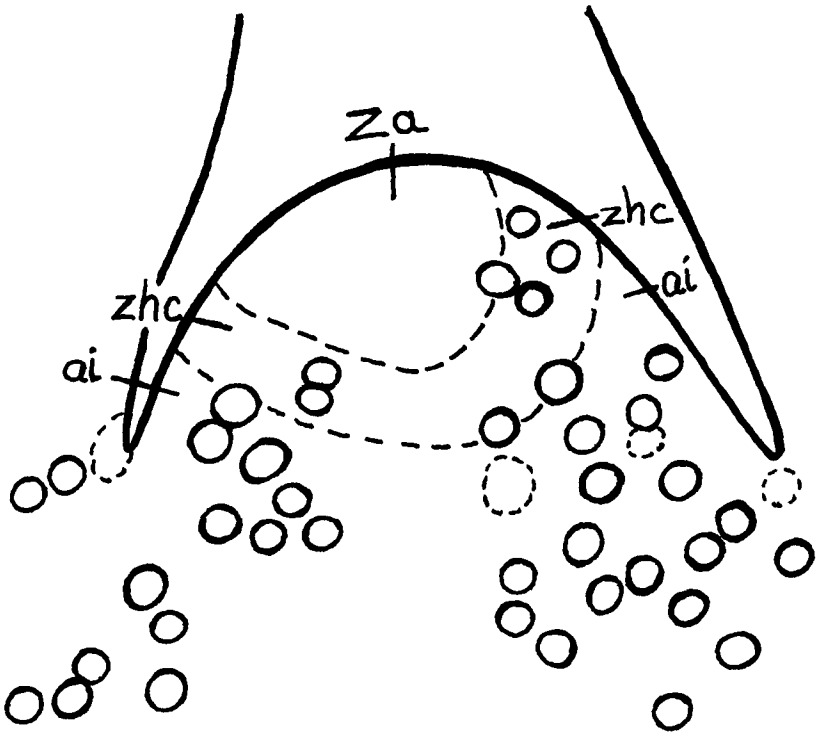


FIG. 9. Diagram, autoradiograph of shoot apex of *Chenopodium album* treated with H^3-thymidine. Nuclei labelled by the isotope are shown as circles. *Az*, "zone axiale"; *zhc*, "zone d'harmonisation de croissance"; *ai*, "anneau initial" (after Nougarède, based on original preparation by Gifford; see text for explanation).

(*Plate VI, Fig. 2*) to note any marked difference in size or intensity of staining of nucleoli in the apical zone. After 30 days, regeneration of the apex after each leaf initiation is said to be accomplished uniformly by divisions in the apical dome. *Tropaeolum* does present an interesting morphogenetic problem in that foliage leaves are continually produced while axillary flowers may be formed after the 7th foliage leaf is initiated. Nougarède concluded that the presence of frequent mitoses in axial apical cells in *Tropaeolum majus* does not have any particular significance. However, the shoot apex continues to undergo plastochronic functioning, but there is no evidence of a méristème d'attente or anneau initial. Irrespective of which theory of apical functioning one adopts the situation in *Tropaeolum* is of interest and has been discussed by Philipson (1966).

In another section entitled "Controles apportés par l'examen de l'incorporation de substances marquées," Nougarède (1965b) made an admirable attempt to bridge the gap between divergent views on apical

organization and growth. A transverse region between an apical zone and the anneau initial, termed the "zone d'harmonisation de croissance," was recognized for the apex of *Chenopodium album* (Fig. 9). Earlier, Gifford (1960) described the results of an experiment using H^3-thymidine in which labelled nuclei were noted to be above what would be considered the limits of the anneau initial if such a designation were used for *C. album*. The zone in question perhaps could be looked upon as a transition region or be comparable to the "zone d'entretien" (see page 148, this review) described for certain growth phases of gymnosperm apices. Acceptance of this interpretation would constitute a partial rapprochement between investigators holding divergent viewpoints on apical growth.

VI. CONCLUSIONS

Nearly all, if not all, plants which have been investigated show cytohistological zonation in their shoot apices regardless of taxonomic affinities. Modern studies have centered around this phenomenon as well as being concerned with mitotic indices and rates of cell division in the various zones. The concept of multiple helices, although difficult to prove to the satisfaction of all investigators, was the basis of renewed interest in shoot apices. The original position of French investigators regarding the degree of inactivity of centrally located cells of the vegetative shoot apices of seed plants has been shown to be an extreme one and, by their own admission, modified to include examples where divisions do occur in the "méristème d'attente." The central problem revolves around the meaning and use of the term "initial." Proponents of the "méristème d'attente-anneau initial" concept rejected what they termed the "classical" apical initials as being important in histogenesis and organogenesis of the shoot. Instead, for them, the lateral anneau initial (initiating ring) constitutes the formative region of primary importance. Those who look upon the more apically placed cells as being important in vegetative growth have probably not defined precisely their use of such terms as "apical initial" or "initial zone." Although not always so stated, the "neoclassicists" have regarded the apically positioned cells as being the ultimate source of cells of the shoot. Obviously the apical cells cannot and do not give rise *directly* to all cells of the primary plant body. For example, stated simply, two cells which result from an anticlinal division in the peripheral region of an apical meristem, prior to actual leaf initiation, could not have had their origin directly from the apical zone. The adherents of the "anneau initial" concept state that it is unnecessary to call upon apical initial cells with special endowments to provide cells for leaf initiation; this is accomplished by the self-perpetuating anneau initial. If the latter view is adopted, one can only conclude that the anneau initial of the French school has special endowments and has a special, privileged role to play.

Would it not be better to view apical structure from an holistic view-

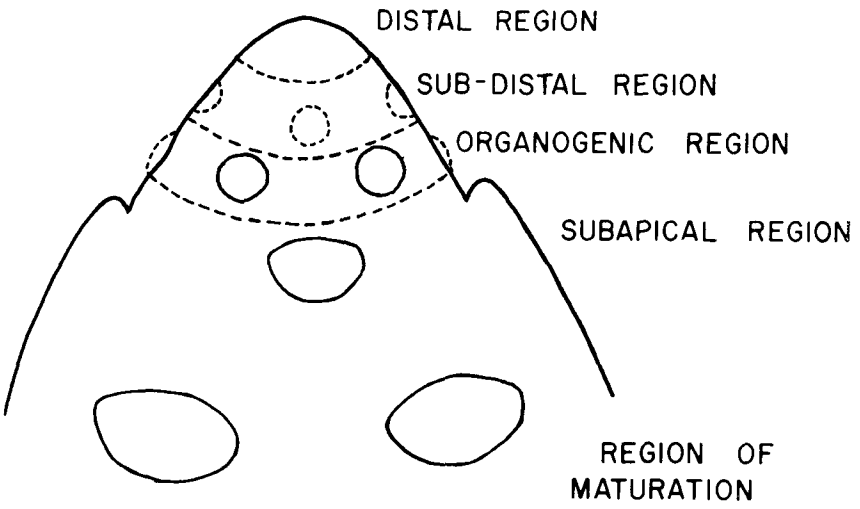


FIG. 10. Diagrammatic representation of a shoot apex, with whorled phyllotaxis, as a system of inter-related zones (after Wardlaw). See text for explanation.

point? Wardlaw (1957a,b,c; 1965a,b; 1968) especially, and many others in passing, have called attention to this concept. According to this idea, the activity of a cell (or cells) depends upon its position in the integrated whole—performing its role in response to gradients of physiologically active substances. Restated, the shoot tip is a system of integrated and inter-related zones. These are (Fig. 10) (1) the distal region, comprising a group of initial cells arranged in one or more tiers and constituting the focal point of the meristem upon which the integrity of the shoot depends; (2) the sub-distal region consisting of superficial layers of meristematic cells. In this region there is maximum interplay between active substances diffusing acropetally and basipetally, resulting in the inception of growth centers; (3) the organogenic region in which outgrowth of leaf primordia takes place and tissue differentiation has its inception or becomes more conspicuous; (4) the subapical region characterized by widening and elongation of the shoot (by continued cell division and cell elongation) and by further differentiation of internal cells; and (5) the region of maturation (varying in distance from the tip of the shoot).

The transverse tiers or zones described above can be more easily visualized in plants with whorled leaf arrangement, as shown in Fig. 10, but the concept can be applied to other phyllotactic patterns if adequate consideration is given to longitudinal adjustments and the overlapping of certain zones or regions.

In the authors' opinion the description provided by Wardlaw has considerable merit, but investigators of shoot development usually wish to describe the organization of shoot apices in more precise histogenic

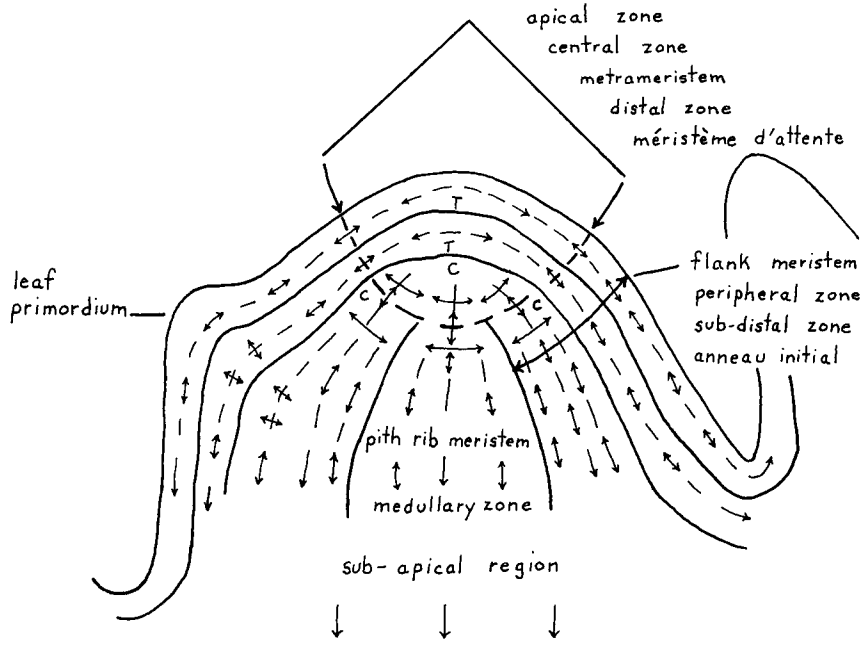


FIG. 11. Diagrammatic representation of zonation and growth in the vegetative shoot apex of a hypothetical dicotyledon. Most of the descriptive terms commonly in use for various zones are indicated. *T*, tunica; *C*, corpus.

terms. The present authors, with some degree of trepidation, would like to describe a hypothetical example of a dicotyledonous shoot apex using some of the descriptive terms that have been applied to an apex (Fig. 11). For this purpose terms are used without regard to preference in their usage. Tunica and corpus are recognized because they do have topographical value. Zonation is a feature of most described examples, and it is reflected in the diagram by the setting off of an apical zone from the subjacent peripheral zone and pith-rib meristem. Leaf initiation in the example is shown to occur in the second tunica layer with contributions being made from the peripheral part of the corpus. No group of cells is considered to occupy a permanent position, although there is evidence for permanency of varying degrees for apical (summital) initials; the displacement of cells is principally in a basipetal direction. The distance between any two arrows indicates the degree of mitotic activity. The shorter the distance, the greater the mitotic activity. The plant is considered to have a helical or distichous phyllotactic pattern and is siphonostelic.

There is considerable variation between species with regard to the degree of mitotic activity of the centrally located apical cells (irrespective of the zonal designation). Size of the apex, its form, and phyllotactic pattern of the plant undoubtedly determine or are correlative phenomena

of mitotic activity. In smaller apices the axial apical cells appear to have higher mitotic indices than larger ones since often the proportion of the apex involved in leaf initiation is greater than in the second type. The rapidity with which leaves are "carried" away from the apex may also affect and/or be correlated with apical mitotic activity. Mitotic indices of the apical axial zone may be significantly lower than for other zones for some species, but the degree of quiescence generally is not of the order reported for the "quiescent center" in roots. Also, the production of lateral appendages of exogenous origin in the shoot probably accounts for the difference to some degree.

Rather profound histochemical and morphogenetic changes may occur with transition to flowering in angiosperms. While all zones in the vegetative shoot apex usually show changes during flowering, the apical axial zone (distal zone, central zone, metrameristem, méristème d'attente, etc.) often is most affected, particularly when a terminal flower is formed rather soon after floral induction. Changes in mitotic rates, zonation, distribution and concentration of RNA, and form of the apex may occur before the meristem becomes determinate. Changes are often more subtle if an inflorescence is formed, especially if a terminal flower is never formed or is produced rather late in inflorescence ontogeny.

ACKNOWLEDGMENT

The authors would like to express their appreciation to Miss Jeanette Gifford for her valuable assistance in the preparation of the illustrations.

LITERATURE CITED

ABBE, E. C. & B. O. PHINNEY. 1951. The growth of the shoot apex in maize: External features. Amer. Jour. Bot. **38**: 737–744.

————, ————, & D. F. BAER. 1951. The growth of the shoot apex in maize: Internal features. Amer. Jour. Bot. **38**: 744–751.

BAIN, H. F. & H. DERMAN. 1944. Sectorial polyploidy and phyllotaxy in the cranberry (*Vaccinium macrocarpon* Ait.). Amer. Jour. Bot. **31**: 581–587.

BALL, E. 1949. The shoot apex and normal plant of *Lupinus albus* L., bases for experimental morphology. Amer. Jour. Bot. **36**: 440–454.

————. 1960. Cell divisions in living shoot apices. Phytomorph. **10**: 377–396.

———— & K. SOMA. 1965. Effect of sugar concentration on growth of the shoot apex of *Vicia faba*. *In*: Proc. International Congress on Tissue Culture. P. R. White (Ed). McCutchan Publ. Co., Berkeley. pp. 269–285.

BANNAN, M. W. 1955. The vascular cambium and radial growth in *Thuja occidentalis* L. Can. Jour. Bot. **33**: 113–138.

BARKER, W. G. & F. C. STEWARD. 1962a. Growth and development of the banana plant. I. The growing regions of the vegetative shoot. Ann. Bot. N.S. **26**: 389–411.

———— & ————. 1962b. Growth and development of the banana plant. II. The transition from the vegetative to the floral shoot in *Musa acuminata* cv. Gros Michel. Ann. Bot. N.S. **26**: 413–423.

BARNARD, C. 1955. Histogenesis of the inflorescence and flower of *Triticum aestivum* L. Austral. Jour. Bot. **3**: 1–20.

———. 1957a. Floral histogenesis in the monocotyledons. I. The Gramineae. Austral. Jour. Bot. **5**: 1–20.

———. 1957b. Floral histogenesis in the monocotyledons. II. The Cyperaceae. Austral. Jour. Bot. **5**: 115–128.

———. 1958. Floral histogenesis in the monocotyledons. III. The Juncaceae. Austral. Jour. Bot. **6**: 285–298.

———. 1960. Floral histogenesis in the monocotyledons. IV. The Liliaceae. Austral. Jour. Bot. **8**: 213–225.

BARTELS, F. 1956. Zur Entwicklung der Keimpflanze von *Epilobium hirsutum*. I. Die im Proembryo ablaufenden Zellteilungen bis zum beginnenden "herzförmigen Embryo." Flora **144**: 105–120.

———. 1960a. Zur Entwicklung der Keimpflanze von *Epilobium hirsutum*. II. Die im Vegetationspunkt während eines Plastochrons ablaufenden Zellteilungen. Flora **149**: 206–224.

———. 1960b. Zur Entwicklung der Keimpflanze von *Epilobium hirsutum*. III. Wachstumstendenzen, die zur Entwicklung eines Laubblattprimordiums und Keimblattes führen. Flora **149**: 225–242.

———. 1961. Zur Entwicklung der Keimpflanze von *Epilobium hirsutum*. IV. Der Nachweis eines Scheitelzellenwachstums. Flora **150**: 552–571.

BERG, A. R. 1966. Growth and cell division rates in the shoot apex of *Chrysanthemum morifolium*. Ph.D. dissertation, Univ. of Calif., Davis, Calif.

———. 1970. Relation of plastochron to anatomy and growth in the shoot apex of *Chrysanthemum*. Amer. Jour. Bot. **57**: 24–32.

BERNIER, G. 1961. Ontogénie du méristème apical de *Sinapis alba* L. (Crucifères) depuis la graine jusqu'a l'edification de l'inflorescence. Comp. Rend. Acad. des Sci. (Paris) **252**: 2750–2752.

———. 1962. Evolution of the apical meristem of *Sinapis alba* L. (long-day plant) in long days, in short days and during the transfer from short days to long days. Caryologia **15**: 303–325.

———. 1964. Étude histophysiologique et histochimique de l'évolution du méristème apical de *Sinapis alba* L., cultivé en milieu conditionné et en diverses durées de jour favorables ou défavorables à la mise à fleurs. Mémoires, Acad. Royale Belgique, Classe des Sciences **16**: 1–150.

———. 1965. The morphogenetic role of the apical meristem in higher plants. Les Phytohormones et L'organogènese. Les Congrès et Colloq., Univ. de Liège **38**: 151–211.

———. 1966. Evolution of nucleic acid metabolism during the ontogenetic development of apical meristems. *In*: Differentiation of apical meristems and some problems of ecological regulation of development of plants. Proceedings of a symposium, Praha-Nitra, 1964. pp. 115–119.

——— & R. BRONCHART. 1963. Application de la technique d'histoautoradiographie à l'étude de l'incorporation de thymidine tritiée dans les méristèmes caulinaires. Bull. Soc. Royale Sci., Liège **32**: 269–283.

——— & ———. 1964. The steps of floral induction in *Sinapis alba* L. Die Naturwissenschaften **19**: 469–470.

———, ———, & A. JACQMARD. 1964. Action of gibberellic acid on the mitotic activity of the different zones of the shoot apex of *Rudbeckia bicolor* and *Perilla nankinensis*. Planta **61**: 236–244.

——— & M. T. DATH. 1962. A propos de quelques cas d'apparition de fleurs le long d'entre-noeuds végétatifs ou de feuilles le long de l'inflorescence chez *Sinapis alba* L. Rev. Cytol. et Biol Vég. **25**: 241–252.

———, J.-M. KINET, & R. BRONCHART. 1967. Cellular events at the meristem during floral induction in *Sinapis alba* L. Physiol. Vég. **5**: 311–324.

BERSILLON, G. 1951. Sur le point végétatif de *Papaver somniferum* L.: structure et fonctionnement. Comp. Rend. Acad. des Sci. (Paris) **232**: 2470–2472.

———. 1955a. Sur la formation du bouton chez le *Papaver somniferum* L. Comp. Rend. Acad. des Sci. (Paris) **240:** 903–905.

———. 1955b. Recherches sur les Papavéracées contribution à l'étude du développement des dicotylédones herbacées. Ann. des Sci. Nat., Bot. **11**, **16:** 225–443.

———. 1957. Ontogénie comparée de la tige feuillée et de l'inflorescence chez *Oenothera biennis* L. Comp. Rend. Acad. des Sci. (Paris) **245:** 1455–1458.

———. 1958. L'inflorescence terminale de *Reseda lutea* L.; ses rapports avec la pousse végétative. Rev. de Cytol. et Biol. Vég. **19:** 185–197.

BONNAND, J. 1959a. Transformation de l'apex végétatif de *Nicotiana tabacum* L. (variété white burley) en apex inflorescentiel. Compt. Rend. Acad. des Sci. (Paris) **248:** 1209–1211.

———. 1959b. *Nicotiana tabacum* L. Point végétatif et morphologie. Rev. Cytol. et Biol. Vég. **20:** 187–228.

———. 1960. Étude de l'inflorescence de *Nicotiana glutinosa* L. Comp. Rend. Acad. des Sci. (Paris) **250:** 1531–1533.

BROWN, R. 1951. The effects of temperature on the durations of the different stages of cell division in the root-tip. Jour. Exptl. Bot. **2:** 96–110.

BROWN, W. V., C. HEIMSCH, & W. H. P. EMERY. 1957. The organization of the grass shoot apex and systematics. Amer. Jour. Bot. **44:** 590–595.

BRULFERT, J. 1965a. Étude expérimentale du développement végétatif et floral chez *Anagallis arvensis* L., ssp. *phoenicea* Scop. Formation de fleurs prolifères chez cette même espèce. Thèsis: L'Université de Paris. 62 p.

———. 1965b. Physiologie de la mise à fleurs d'*Anagallis arvensis* L., ssp. *phoenicea* Scop., et du développement végétatif en retour des méristèmes floraux. Bull. Soc. Franç. Physiol. Vég. **11:** 247–251.

BURG, S. P. & E. A. BURG. 1966. Auxin-induced ethylene formation: its relation to flowering in the pineapple. Science **152:** 1269.

BUVAT, R. 1950. Observations cytologiques sur l'évolution et le fonctionnement du point végétatif de la Giroflée (*Cheiranthus cheiri* L., Crucifères). Comp. Rend. Acad. des Sci. (Paris) **230:** 1968–1969.

———. 1951. Transformation du point végétatif de *Myosurus minimus* L. en méristème floral. Comp. Rend. Acad. des Sci. (Paris) **232:** 2466–2468.

———. 1952a. Structure, évolution et fonctionnement du méristème apical de quelques dicotylédones. Ann. des Sci. Nat., Bot. **11**, **13:** 199–300.

———. 1952b. L'organisation des méristèmes apicaux chez les végétaux vasculaires. Union des Nat., Bull. **40:** 54–66.

———. 1953. L'apex de *Triticum vulgare*; modalités de reprise des mitoses lors de la germination et du fonctionnement végétatif. Comp. Rend. Acad. des Sci. (Paris) **236:** 1989–1991.

———. 1955. Le méristème apical de la tige. Ann. Biol. **31:** 596–656.

——— & L. GENÈVES. 1951. Sur l'inexistence des initiales axiales dans la racine d'*Allium cepa* L. (Liliacées). Comp. Rend. Acad. des Sci. (Paris) **232:** 1579–1581.

CALDECOTT, R. S. & L. SMITH. 1952. A study of X-ray-induced chromosomal aberrations in barley. Cytologia **17:** 224–242.

CAMEFORT, H. 1950. Structure du point végétatif de *Picea excelsa*. Comp. Rend. Acad. des Sci. (Paris) **231:** 65–66.

———. 1956. Étude de la structure du point végétatif et des variations phyllotaxiques chez quelques Gymnospermes. Ann. des Sci. Nat., Bot. **11**, **17:** 1–185.

CATESSON, A.-M. 1953. Structure, évolution et fonctionnement du point végétatif d'une Monocotylédone: *Luzula pedemontana* Boiss. et Reut. (Joncacées). Ann. Sci. Nat., Bot. **11**, **14:** 253–291.

———. 1960. Zonation apicale et origine du procambium dans les rameaux d'*Acer pseudoplatanus*. Comp. Rend. Acad. des Sci. (Paris) **250:** 1709–1711.

———. 1964. Modifications cytochimiques saisonnières des points végétatifs dans les bourgeons de l'*Acer pseudoplatanus* L. Comp. Rend. Acad. des Sci. (Paris) **258**: 5709–5712.

CHAKRAVARTI, S. C. 1953. Organization of shoot apex during ontogeny of *Brassica campestris* L. Nature **171**: 223–224.

CHAMBERLAIN, C. J. 1916. Periodicity in mitosis. Bot. Gaz. **61**: 442–443.

CHOUARD, P. & M. TRÂN. 1962. Nouvelles recherches sur l'analyse des mécanismes de la vernalisation d'une plante vivace, le *Geum urbanum* L. (Rosacées). Bull. Soc. Bot., France **109**: 145–147.

——— & M. TRÂN THANH VÂN. 1965. Remplacement du froid vernalisant par l'ablation de l'apex chez la *Geum urbanum* L.; signification commune des divers processus vernalisants. Comp. Rend. Acad. des Sci. (Paris) **260**: 274–277.

CHOUINARD, L. 1959. I. Structure et fonctionnement de l'apex caulinaire de *Pinus banksiana* Lamb. au cours de la germination. Fonds de Rech. For. de l'Univ. Laval, Contrib. **4**: 1–26.

CLOWES, F. A. L. 1957. Chimeras and meristems. I. Shoot apical meristems. Heredity **11**: 141–148.

———. 1959. Adenine incorporation and cell division in shoot apices. New Phytol. **58**: 16–19.

———. 1961a. Apical meristems. Blackwell, Oxford. 217 p.

———. 1961b. Duration of the mitotic cycle in a meristem. Jour. Exptl. Bot. **12**: 283–293.

———. 1963. Micronuclei in irradiated meristems. Radiation Bot. **3**: 223–229.

CODACCIONI, M. 1962. Recherches morphologiques et ontogéniques sur quelques Cupulifères. Rev. Cytol. et Biol. Vég. **25**: 1–208.

CORSON, G. E., JR. 1969. Cell division studies in the shoot apex of *Datura stramonium* during transition to flowering. Amer. Jour. Bot. **56**: 1127–1134.

——— & E. M. GIFFORD, JR. 1969. Histochemical studies of the shoot apex of *Datura stramonium* during transition to flowering. Phytomorph. **19**: 189–196.

CUMMING, B. G. 1969. *Chenopodium rubrum* L. and related species. *In*: The induction of flowering. (L. T. Evans, Ed.) Cornell Univ. Press.

CUTTER, E. G. 1959. On a theory of phyllotaxis and histogenesis. Biol. Rev. **34**: 243–263.

———. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. Bot. Rev. **31**: 7–113.

DATH, M. T. 1963. Technique d'application de thymidine tritiée au niveau de méristèmes caulinaires. Bull. Soc. Royale Sci., Liège **9**: 697–703.

DAYES-DUJEU, M. 1957. Étude du point végétatif et des méristèmes intercalaires de l'*Ephedra monostachya*. Rev. Gén. Bot. **64**: 41–75.

DENNE, M. P. 1966a. Morphological changes in the shoot apex of *Trifolium repens* L. 1. Changes in the vegetative apex during the plastochron. New Zealand Jour. Bot. **4**: 300–314.

———. 1966b. Morphological changes in the shoot apex of *Trifolium repens* L. 2. Diurnal changes in the vegetative apex. New Zealand Jour. Bot. **4**: 434–443.

———. 1966c. Diurnal and plastochronal changes in the shoot apex of *Tradescantia fluminensis* Vell. New Zealand Jour. Bot. **4**: 444–454.

DERMAN, H. & H. F. BAIN. 1944. A general cytological study of colchicine polyploidy in cranberry. Amer. Jour. Bot. **31**: 451–463.

DIOMAIUTO-BONNAND, J. 1962. Étude de quelques cymes unipares scorpioides anormales chez *Nicotiana glutinosa* L.: possibilité de passage à un état végétatif. Comp. Rend. Acad. des Sci. (Paris) **254**: 1140–1142.

———. 1966. Morphologie et ontogénie de quelques espèces de *Nicotiana*. Etude de l'inflorescence. Rev. Cytol. et Biol. Vég. **29**: 1–172.

DUFF, G. H. & N. J. NOLAN. 1958. Growth and morphogenesis in the Canadian

forest species. III. The time scale of morphogenesis at the stem apex of *Pinus resinosa* Ait. Canadian Jour. Bot. **36**: 687–706.

EDGAR, E. 1961. Fluctuations in mitotic index in the shoot apex of *Lonicera nitida*. Univ. Cant., Publ. 1, 91 pp.

ENGLAND, W. H. & R. J. TOLBERT. 1964. A seasonal study of the vegetative shoot apex of *Myriophyllum heterophyllum*. Amer. Jour. Bot. **51**: 349–353.

EVANS, H. J., G. J. NEARY, & S. M. TONKINSON. 1957. The use of colchicine as an indicator of mitotic rate in broad bean root meristems. Jour. Genet. **55**: 487–502.

EVANS, L. T. & I. F. WARDLAW. 1966. Independent translocation of ^{14}C-labelled assimilates and of the floral stimulus in *Lolium temulentum*. Planta **68**: 310–326.

FAHN, A. 1953. The origin of the banana inflorescence. Kew Bull. **8**: 299–306.

———, S. STOLER, & T. FIRST. 1963. Vegetative shoot apex in banana and zonal changes as it becomes reproductive. Bot. Gaz. **124**: 246–250.

FOSKET, D. E. & J. P. MIKSCHE. 1966. A histochemical study of the seedling shoot apical meristem of *Pinus lambertiana*. Amer. Jour. Bot. **53**: 694–702.

FOSTER, A. S. 1938. Structure and growth of the shoot apex in *Ginkgo biloba*. Bull. Torrey Bot. Club **65**: 531–556.

FREEBERG, J. A. & R. H. WETMORE. 1967. The Lycopsida—a study in development. Phytomorph. **17**: 78–91.

FREDERICQ, H. 1960. The change of the apex of *Kalanchoë blossfeldiana* towards inflorescence formation. Biol. Jaarboek **28**: 76–87.

GALUN, E., J. GRESSEL, & A. KEYNAN. 1964. Suppression of floral induction by actinomycin D—an inhibitor of 'messenger' RNA synthesis. Life Sci. **3**: 911–915.

GAUL, H. 1959. Über die chimärenbildung in Gerstenpflanzen nach Röntgenbestrahlung von Samen. Flora **147**: 207–241.

———. 1961. Studies on diplontic selection after X-irradiation of barley seeds. *In*: Effects of ionizing radiations on seeds. Proc. International Atomic Energy Agency. Vienna. pp. 117–138.

GIFFORD, E. M., JR. 1943. The structure and development of the shoot apex of *Ephedra altissima* Desf. Bull. Torrey Bot. Club **70**: 15–25.

———. 1950. The structure and development of the shoot apex in certain woody Ranales. Amer. Jour. Bot. **37**: 595–611.

———. 1954. The shoot apex in angiosperms. Bot. Rev. **20**: 477–529.

———. 1960. Incorporation of tritiated thymidine into nuclei of shoot apical meristems. Science **131**: 360.

———. 1961. Histology of vegetative and strobilate apices in certain gymnosperms. *In*: Recent Advances in Botany. Univ. of Toronto Press. pp. 750–754.

———. 1963. Developmental studies of vegetative and floral meristems. *In*: Meristems and Differentiation. Brookhaven Symposia in Biology **16**: 126–137.

———. 1969. Initiation and early development of the inflorescence in pineapple (*Ananas comosus*, 'Smooth Cayenne') treated with acetylene. Amer. Jour. Bot. **56**: 892–897.

———, S. KUPILA, & S. YAMAGUCHI. 1963. Experiments in the application of H^3-thymidine and adenine-8-C^{14} to shoot tips. Phytomorph. **13**: 14–22.

——— & N. T. MIROV. 1960. Initiation and ontogeny of the ovulate strobilus in ponderosa pine. Forest Sci. **6**: 19–25.

——— & K. D. STEWART. 1965. Ultrastructure of vegetative and reproductive apices of *Chenopodium album*. Science **149**: 75–77.

——— & H. B. TEPPER. 1961. Ontogeny of the inflorescence in *Chenopodium album*. Amer. Jour. Bot. **48**: 657–667.

——— & ———. 1962a. Histochemical and autoradiographic studies of floral induction in *Chenopodium album*. Amer. Jour. Bot. **49**: 706–714.

———— & ————. 1962b. Ontogenetic and histochemical changes in the vegetative shoot tip of *Chenopodium album*. Amer. Jour. Bot. **49**: 902–911.

———— & R. H. WETMORE. 1957. Apical meristems of vegetative shoots and strobili in certain gymnosperms. Proc. Natl. Acad. Sci. (U.S.) **43**: 571–576.

GRAY, L. H. & M. E. SCHOLES. 1951. The effect of ionizing radiations on the broad bean root. VIII. Growth rate studies and histological analyses. Brit. Jour. Radiol. N.S. **24**: 348–352.

GRÉGOIRE, V. 1938. La morphogenèse et l'autonomie morphologique de l'appareil floral. I. Le carpelle. La Cellule **47**: 287–452.

GRIFFITH, M. M. 1952. The structure and growth of the shoot apex in *Araucaria*. Amer. Jour. Bot. **39**: 253–263.

GUTTENBERG, H. VON. 1960. Grundzüge der Histogenese höherer Pflanzen. I. Die Angiospermen. *In*: Handbuch der Pflanzenanatomie. Band VIII, Teil 3. Gebrüder Borntraeger, Berlin-Nikolassee.

————. 1961. Grundzüge der Histogenese höherer Pflanzen. II. Die Gymnospermen. *In*: Handbuch der Pflanzenanatomie. Band VIII, Teil 4. Gebrüder Borntraeger, Berlin-Nikolassee.

HADJ-MOUSTAPHA, M. 1957. Point végétatif et point inflorescentiel chez le *Cleome spinosa* Jacq. Comp. Rend. Acad. des Sci. (Paris) **245**: 710–712.

————. 1958. Organogénèse de la fleur axillaire chez quelques Capparidacées. Comp. Rend. Acad. des Sci. (Paris) **246**: 2390–2393.

HAGEMANN, W. 1960. Kritische Untersuchungen über die Organisation des Sprossscheitels dikotyler Pflanzen. Österr. Bot. Z. (Wein) **107**: 366–402.

————. 1963. Weitere Untersuchungen zur Organisation des Sprossscheitelmeristems. Der Vegetationspunkt traubiger Floreszenzen. Bot. Jahrb. **82**: 273–315.

HANAWA, J. 1966. Growth and development in the shoot apex of *Pinus densiflora*. I. Growth periodicity and structure of the terminal vegetative shoot apex. Bot. Mag., Tokyo **79**: 736–746.

————. 1967. Growth and development in the shoot apex of *Pinus densiflora*. II. Ontogeny of the dwarf shoot and the lateral branch. Bot. Mag., Tokyo **80**: 248–256.

HARA, N. 1958. Structure of the vegetative shoot apex and development of the leaf in the Ericaceae and their allies. Jour., Faculty of Science, Univ. of Tokyo, Sect. III, Bot. **7**: 367–450.

————. 1961. Apical views of vegetative shoot apex. Bot. Mag., Tokyo **74**: 267–268.

————. 1962. Structure and seasonal activity of the vegetative shoot apex of *Daphne pseudo-mezereum*. Bot. Gaz. **124**: 30–42.

HEALY, P. L. 1964. Histochemistry and ultrastructure in the shoot of *Pharbitis* before and after induction. Ph.D. dissertation, Univ. of Calif., Berkeley.

HEJNOWICZ, Z. 1957. Ontogenic studies on shoot apices in *Chamaecyparis pisifera* and an interpretation of the form *squarrosa*. Acta Soc. Bot. Polon. **26**: 413–466.

HILLMAN, W. S. 1962. The physiology of flowering. Holt, Rinehart & Winston, New York. 164 pp.

HOFFMAN, J. G. 1949. Theory of the mitotic index and its application to tissue growth measurement. Bull. Math. Biophys. **11**: 139–144.

HOLT, I. V. 1954. Initiation and development of the inflorescences of *Phalaris arundinaceae* L. and *Dactylis glomerata* L. Iowa State College Jour. Sci. **28**: 603–621.

HSÜ, J. 1944. Structure and growth of the shoot apex of *Sinocalamus Beechyana* McClure. Amer. Jour. Bot. **31**: 404–411.

IKENBERRY, G. J., JR. 1966. Reactivation of the dormant embryo, and initiation and development of buds and leaves on *Avena sativa*. Iowa State Jour. Sci. **41**: 7–23.

IM, H. B. 1961. The effect of gibberellin on the differentiation and development of barley spikes. Seoul Univ. Jour. **10D**: 55–67.

———. 1962. The effect of gibberellin on the leaf development and the cyto-histological zones of shoot apices in barley plants. Seoul Univ. Jour. **12D**: 25–34.

———. 1963. Study of gibberellin effects on the differentiation and development of barley spikes. Seoul Univ. Jour. **14B**: 47–92.

JACKMAN, V. H. 1960. The shoot apex of some New Zealand gymnosperms. Phytomorph. **10**: 145–157.

JACOBS, W. P. & I. B. MORROW. 1961. A quantitative study of mitotic figures in relation to development in the apical meristem of vegetative shoots of *Coleus*. Developmental Biology **3**: 569–587.

——— & V. RAGHAVEN. 1962. Studies on the floral histogenesis and physiology of *Perilla*. I. Quantitative analysis of flowering in *P. frutescens* (L.) Britt. Phytomorph. **12**: 144–167.

JACQMARD, A. 1964. Action comparée de la photopériode et de l'acide gibbérellique sur le méristème caulinaire de *Rudbeckia bicolor* Nutt. Bull. Acad. Roy. Belg. Cl. Sci. **50**: 174–188.

———. 1967. Étude cinétique de la stimulation de l'activité mitotique dans le bourgeon terminal de *Rudbeckia bicolor* traité par l'acide gibbérellique. Comp. Rend. Acad. des Sci. (Paris) **264D**: 1282–1285.

———. 1968. Early effects of gibberellic acid on mitotic activity and DNA synthesis in the apical bud of *Rudbeckia bicolor*. Physiol. Vég. **6**: 409–416.

JENTSCH, R. 1956. Untersuchungen an den Sprossvegetationspunkten einiger Saxifragaceen. Flora **144**: 251–289.

———. 1960. Zur Kenntnis des Sprossvegetationspunktes von *Hippuris* und *Myriophyllum*. Flora **149**: 307–319.

JOHNSON, M. A. & R. J. TOLBERT. 1960. The shoot apex in *Bombax*. Bull. Torrey Bot. Club **87**: 173–186.

KALBE, L. 1962. Histogenetische Untersuchungen an Sprossvegetationspunkten dikotyler Holzpflanzen. Flora **152**: 279–314.

KAUFMAN, P. B. 1959a. Development of the shoot of *Oryza sativa* L. I. The shoot apex. Phytomorph. **9**: 228–242.

———. 1959b. Development of the shoot of *Oryza sativa* L. II. Leaf histogenesis. Phytomorph. **9**: 277–311.

———. 1959c. Development of the shoot of *Oryza sativa* L. III. Early stages in histogenesis of the stem and ontogeny of the adventitious root. Phytomorph. **9**: 382–404.

KAUSSMANN, B. 1955. Histogenetische Untersuchungen zum Flachsprossproblem. Botanische Studien, Heft **3**: 1–136.

KEMP, M. 1959. Morphological and ontogenetic studies on *Torreya californica*. II. Development of the megasporangiate shoot prior to pollination. Amer. Jour. Bot. **46**: 249–261.

KLAUS, H. 1966. Ontogenetische und histogenetische Untersuchungen an der Gerste (*Hordeum distichon* L.). Bot. Jahrb. **85**: 45–79.

KNOX, R. B. & L. T. EVANS. 1966. Inflorescence initiation in *Lolium temulentum* L. VIII. Histochemical changes at the shoot apex during induction. Austral. Jour. Biol. Sci. **19**: 233–245.

——— & ———. 1968. Inflorescence initiation in *Lolium temulentum* L. XII. An autoradiographic study of evocation in the shoot apex. Austral. Jour. Biol. Sci. **21**: 1083–1094.

KUNDU, B. C. & N. S. RAO. 1957. The shoot apex of *Boehmeria nivea* during morphogenesis. Cellule **58**: 217–228.

KUPILA, S. & E. M. GIFFORD, JR. 1963. Shoot apex of *Pseudolarix amabilis*. Bot. Gaz. **124**: 241–245.

LANCE, A. 1952. Sur la structure et le fonctionnement du point végétatif de *Vicia faba* L. Ann. Sci. Nat., Bot. **11, 13:** 301–339.

————. 1953. Sur l'absence d'initiales apicales et la configuration de l'anneau initial chez *Vicia faba* L. Comp. Rend. Acad. des Sci. (Paris) **26:** 510–512.

————. 1957. Recherches cytologiques sur l'évolution de quelques méristèmes apicaux et sur ses variations provoquées par des traitements photopériodiques. Ann. Sci. Nat., Bot. **11, 18:** 91–422.

LANCE-NOUGARÈDE, A. 1961a. Sur l'incorporation de l'adénine marquée au tritium (^3H) dans les noyaux et le cytoplasme des cellules de deux méristèmes caulinaires: *Lupinus albus* (Papilionacées) et *Teucrium scorodonia* (Labiées). Compt. Rend. Acad. des Sci. (Paris) **252:** 1504–1506.

————. 1961b. Comparison du fonctionnement reproducteur chez deux plantes vivaces construisant des inflorescences en grappe indéfinie sans fleur terminale, *Teucrium scorodonia* L. (Labiées) et *Alyssum maritimum* Lamk. (Crucifères). Comp. Rend. Acad. des Sci. (Paris) **252:** 924–926.

———— & R. BRONCHART. 1965. Métabolisme des acides nucléiques dans le méristème apical du *perilla nankinensis* au cours des diverses phases du développement. Comp. Rend. Acad. des Sci. (Paris) **260:** 3140–3143.

———— & J.-E. LOISEAU. 1960. Sur la structure et le fonctionnement du méristème végétatif de quelques Angiospermes aquatiques ou semi-aquatiques dépourvues de moelle. Comp. Rend. Acad. des Sci. (Paris) **250:** 4438–4440.

LANCE, A. & P. RONDET. 1957. Évolution du méristème apical de *Beta vulgaris* L. (variété Cérès sucrière) de la germination à l'inflorescence. Comp. Rend. Acad. des Sci. (Paris) **245:** 712–715.

———— & ————. 1958. Sur le fonctionnement du méristème apical de *Beta vulgaris* L. (variété Cérès sucrière) depuis la phase adulte jusqu' à la fleur terminale. Compt. Rend. Acad. des Sci. (Paris) **246:** 3177–3180.

LANG, A. 1956. Induction of flower formation in biennial *Hyoscyamus niger* by treatment with gibberellin. Naturwiss. **43:** 284–285.

LAUGHLIN, H. H. 1919. Duration of the several mitotic stages in the dividing root-tip cells of the common onion. Carnegie Inst. Wash., Publ. No. 265.

LEDIN, R. B. 1954. The vegetative shoot apex of *Zea mays*. Amer. Jour. Bot. **41:** 11–17.

LOISEAU, J.-E. 1959. Observations et expérimentations sur la phyllotaxie et le fonctionnement du sommet végétatif chez quelques Balsaminacées. Ann. Sci. Nat., Bot. **11, 20:** 1–214.

————. 1962. Activité mitotique des cellules superficielles du sommet végétatif caulinaire. Mém., Soc. Bot., France, pp. 14–23.

LOZE, J.-C. 1965. Etude de l'ontogenèse de l'appareil reproducteur femelle de l'If. *Taxus baccata* L. Rev. Cytol. et Biol. Vég. **28:** 211–256.

LYNDON, R. F. 1970a. DNA, RNA, and protein in the pea shoot apex in relation to leaf initiation. Jour. Exptl. Bot. **21:** 286–291.

————. 1970b. Rates of cell division in the shoot apical meristem of *Pisum*. Ann. Bot. **34:** 1–17.

MAKSYMOWYCH, R. & R. O. ERICKSON. 1960. Development of the lamina in *Xanthium italicum* represented by the plastochron index. Amer. Jour. Bot. **47:** 451–459.

MARUSHIGE, Y. 1965a. Ontogeny of the vegetative and the reproductive apices in *Pharbitis nil* Chois. I. Development of the vegetative apex. The Bot. Mag., Tokyo **78:** 353–359.

————. 1965b. Ontogeny of the vegetative and the reproductive apices in *Pharbitis nil* Chois. II. Development of the terminal flower bud. The Bot. Mag., Tokyo **78:** 397–406.

————. 1965c. Ontogeny of the vegetative and the reproductive apices in *Pharbitis nil* Chois. III. Development of axillary buds. The Bot. Mag., Tokyo **78:** 407–411.

MIA, A. J. 1960. Structure of the shoot apex of *Rauwolfia vomitoria*. Bot. Gaz. **122**: 121–124.

MICHAUX, N. 1964. Structure et évolution du méristème apical, du *Jussieua grandiflora* Michx., durant les phases végétative et reproductrice. Rev. Gén. Bot. **71**: 91–170.

MILLINGTON, W. F. & E. L. FISK. 1956. Shoot development in *Xanthium pennsylvanicum*. I. The vegetative plant. Amer. Jour. Bot. **43**: 655–665.

MOH, C. C. 1961. Does a coffee plant develop from one initial cell in the shoot apex of an embryo? Rad. Bot. **1**: 97–99.

MURAKAMI, K. 1960. Studies on morphological and physiological changes in shoot apex of rice plant during floral differentiation. I. Distribution of polysaccharides in shoot apex of a light-sensitive variety observed under short-day treatment. Jap. Jour. Breeding **10**: 75–80 (with English summary).

———. 1962. Studies on morphological and physiological changes in shoot apex of rice plant during floral differentiation. II. Changes in quantity of RNA, protein, amino acids and soluble sugar. Jap. Jour. Breeding **12**: 85–92. (with English summary).

NEWMAN, I. V. 1956. Pattern in meristems of vascular plants. I. Cell partition in living apices and in the cambial zone in relation to the concepts of initial cells and apical cells. Phytomorph. **6**: 1–19.

———. 1961. Pattern in the meristems of vascular plants. II. A review of shoot apical meristems of gymnosperms, with comments on apical biology and taxonomy, and a statement of some fundamental concepts. Proc. Linn. Soc. (New So. Wales) **86**: 9–59.

———. 1965. Pattern in the meristems of vascular plants. III. Pursuing the patterns in the apical meristem where no cell is a permanent cell. Jour. Linn. Soc. (Bot.) **59**: 185–214.

NITSCH, J. P. & C. NITSCH. 1965. The induction of flowering in *Nicotiana*. III. Variations in the level of endogenous growth substances. Amer. Jour. Bot. **52**: 591–598.

NOUGARÈDE, A. 1965a. Le méristème caulinaire des angiosperms: problèmes posés par le passage à la phase reproductrice. Bull. Soc. Franç. Physiol. Vég. **11**: 105–138.

———. 1965b. Organisation et fonctionnement du méristème apical des végétaux vasculaires. *In*: Travaux dédiés au Lucien Plantefol. Masson et Cie. Paris. 520 pp.

———. 1967. Experimental cytology of the shoot apical cells during vegetative growth and flowering. Intl. Rev. Cytol. **21**: 203–351.

——— & G. BERNIER. 1966. The intermediate phase in photoperiodic and cold-requiring plants; its signification. *In*: Differentiation of apical meristems and some problems of ecological regulation of development of plants. Proceedings of a symposium, Praha-Nitra, 1964. pp. 25–30.

——— & R. BRONCHART. 1965. Le métabolisme des acides nucléiques au cours des diverses phases du développement dans le méristème du *Perilla nankinensis*. Bull. Soc. Franç. Physiol. Vég. **11**: 149–154.

———, ———, G. BERNIER, & P. RONDET. 1964. Comportement du méristème apical du *Perilla nankinensis* (Lour.) Decne. en relation avec les conditions photopériodiques. Rev. Gén. Bot. **71**: 205–238.

———, E. M. GIFFORD, JR., & P. RONDET. 1965. Cytohistological studies of the apical meristem of *Amaranthus retroflexus* under various photoperiodic regimes. Bot. Gaz. **126**: 281–298.

——— & P. RONDET. 1962. Variations morphologiques et histologiques du développement de l'*Alyssum maritimum* Lamk. en relation avec les conditions de culture; essai de réversion de la phase reproductrice à la phase végétative. Rev. Cytol. et Biol. Vég. **25**: 209–240.

OPATRNÁ, J., F. SEIDLOVÁ, & K. BENEŠ. 1964. The anatomy of the shoot apex of wheat (*Triticum aestivum* L.) during transition from the vegetative to the reproductive state and the determination of the primordia. Biol. Plant. Acad. Sci. Bohemoslov. **6**: 219–225.

OWENS, J. N. & R. P. PHARIS. 1967. Initiation and ontogeny of the microsporangiate cone in *Cupressus arizonica* in response to gibberellin. Amer. Jour. Bot. **54**: 1260–1272.

———— & F. H. SMITH. 1964. The initiation and early development of the seed cone of Douglas fir (*Pseudotsuga douglasii*). Canad. Jour. Bot. **42**: 1031–1047.

PAOLILLO, D. J., JR. & E. M. GIFFORD, JR. 1961. Plastochronic changes and the concept of apical initials in *Ephedra altissima*. Amer. Jour. Bot. **48**: 8–16.

PARKE, R. V. 1959. Growth periodicity and the shoot tip of *Abies concolor*. Amer. Jour. Bot. **46**: 110–118.

PARTANEN, C. R. & E. M. GIFFORD, JR. 1958. Application of autoradiographic techniques to studies of shoot apices. Nature **182**: 1747–1748.

PHELOUZAT, R. 1957. Le méristème apical d'*Agrimonia eupatoria* L. dans l'ontogénie de la pousse végétative et de l'inflorescence. Comp. Rend. Acad. des Sci. (Paris) **245**: 2525–2528.

————. 1964a. Présence d'un anneau initial fonctionnel dans l'apex de quelques inflorescences en grappes. Mém. Soc. Bot., France, pp. 160–167.

————. 1964b. Le système inflorescentiel du *Geum urbanum* L. Mém. Soc. Bot., France, 1964, pp. 168–172.

PHILIPSON, W. R. 1947. Some observations on the apical meristems of leafy and flowering shoots. Jour. Linn. Soc. (Bot.) **53**: 187–193.

————. 1966. Introduction to session on the development of the shoot apex. *In*: Differentiation of apical meristems and some problems of ecological regulation of plants. Proceedings of a symposium, Praha-Nitra, 1964. pp. 13–19.

PILLAI, S. K. 1963a. Zonal structure and seasonal variations in the shoot apex of *Podocarpus gracilior* Pilger. Proc. Indian Acad. Sci. Sect. B. **57**: 58–67.

————. 1963b. Structure and seasonal study of the shoot apex of some *Cupressus* species. New Phytol. **62**: 335–341.

PLANTEFOL, L. 1947. Hélices foliaires, point végétatif et stèle chez les Dicotylédons. La notion d'anneau initial. Rev. Gén. Bot. **54**: 49–80.

————. 1957. Sur deux notes relatives à l'ontogénie d'inflorescences. Comp. Rend. Acad. des Sci. (Paris) **245**: 603–607.

POPHAM, R. A. 1951. Principal types of vegetative shoot apex organization in vascular plants. Ohio Jour. Sci. **51**: 249–270.

————. 1958. Cytogenesis and zonation in the shoot apex of *Chrysanthemum morifolium*. Amer. Jour. Bot. **45**: 198–206.

———— & A. P. CHAN. 1950. Zonation in the vegetative stem tip of *Chrysanthemum morifolium* Bailey. Amer. Jour. Bot. **37**: 476–484.

POUX, N. 1957. Comportement du méristème apical d'un Blé d'hiver (*Triticum sativum* Lamk.) semé au printemps. Comp. Rend. Acad. des Sci. (Paris) **245**: 2522–2525.

————. 1958. Comportement du méristème apical de deux variétés de Blé, *Triticum sativum* Lamk., L'une de printemps, l'autre d'automne, semées simultanément au printemps. Rev. de Cytol. et Biol. Vég. **19**: 273–305.

————. 1960a. Variations de la répartition des ribonucléoprotéines dans l'apex d'un Blé de printemps (*Triticum vulgare* Vill.) au cours du développement. Comp. Rend. Acad. des Sci. (Paris) **250**: 585–587.

————. 1960b. Évolution cytologique du méristème apical d'un Blé de printemps. (*Triticum vulgare* Vill., var. Vilmorin Werna). Comp. Rend. Acad. des Sci. (Paris) **250**: 1099–1101.

———. 1960c. Variations de la répartition des ribonucléoprotéines dans les apex d'un Blé d'hiver (*Triticum vulgare* Vill.) semé, soit au printemps, soit en automne. Comp. Rend. Acad. des Sci. (Paris) **251**: 1904–1906.

PRATT, C. 1959. Radiation damage in shoot apices of Concord grape. Amer. Jour. Bot. **46**: 103–109.

———. 1960. Changes in structure of a periclinal chromosomal chimera of apple following X-irradiation. Nature **186**: 255–256.

———. 1963. Radiation damage and recovery in diploid and cytochimeral varieties of apples. Rad. Bot. **3**: 193–206.

RAM, H. Y. M., M. RAM, & F. C. STEWARD. 1962. Growth and development of the banana plant. 3. A. The origin of the inflorescence and the development of the flowers. B. The structure and development of the fruit. Ann. Bot. N.S. **26**: 657–673.

RAMJI, M. V. 1960. The structure of the shoot apex and leaf initiation in *Polyalthia longifolia*. Proc. Indian Acad. Sci. Sect. B. **51**: 227–241.

———. 1961a. Ontogenetic studies in *Sarcandra irvingbaileyi* Swamy. I. The structure of the shoot apex and ontogeny of leaf. Proc. Indian Acad. Sci. Sect. B, **53**: 20–35.

———. 1961b. Cell divisions in the shoot apices of *Sarcandra irvingbaileyi*. Jour. Madras Univ. **31B**: 315–321.

RAUH, W. & H. REZNIK. 1953. Histogenetische Untersuchungen an Blüten- und Infloreszenzachsen. II. Die Histogenese der Achsen Köpfcheförmiger Infloreszenzen. Beitr. Biol. Pflanzen **29**: 233–296.

REMACLE-DATH, M. T. 1965. Contribution à la détermination au moyen de thymidine tritiée, de la durée de la mitose dans le méristème caulinaire. I. Comparaison des techniques. Bull. Soc. Royale Sci., Liège. **34**: 354–359.

RIJVEN, A. H. G. C. & L. T. EVANS. 1967a. Inflorescence initiation of *Lolium temulentum* L. IX. Some chemical changes in the shoot apex at induction. Austral. Jour. Biol. Sci. **20**: 1–12.

——— & ———. 1967b. Inflorescence initiation in *Lolium temulentum* L. X. Changes in ^{32}P incorporation into nucleic acids of the shoot apex at induction. Austral. Jour. Biol. Sci. **20**: 13–24.

ROHWEDER, O. 1963. Anatomische und histogenetische Untersuchungen an Laubsprossen und Blüten der Commelinaceen. Bot. Jahrb. **82**: 1–99.

ROMBERGER, J. A. 1963. Meristems, growth and development in woody plants. U.S. Dept. Agric. Tech. Bull. No. 1293. 214 p.

SAINT-CÔME, R. 1965. L'apex du *Coleus blumei* durant les phases végétative, préfloral et reproductrice. Bull. Soc. Franç. Physiol. Vég. **11**: 139–147.

———. 1966. Application des techniques histoautoradographiques et des méthodes statistiques à l'étude du fonctionnement apical chez le *Coleus blumei* Benth. Rev. Gén. Bot. **73**: 241–323.

SALISBURY, F. B. 1959. Growth regulators and flowering. II. The cobaltous ion. Plant Physiol. **34**: 598–604.

———. 1963. The flowering process. Pergamon Press, Macmillan Co., New York. 234 p.

SÁRKÁNY, S. & E. PERCS. 1957. Histogenetical observations in the stem tip of *Papaver somniferum* L. Acta Biologica Acad. Sci. Hungaricae **7**: 183–201.

SATINA, S. & A. F. BLAKESLEE. 1941. Periclinal chimeras in *Datura stramonium* in relation to the development of leaf and flower. Amer. Jour. Bot. **28**: 862–871.

——— & ———. 1943. Periclinal chimeras in *Datura* in relation to the development of the carpel. Amer. Jour. Bot. **30**: 453–462.

———, ———, & A. G. AVERY. 1940. Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. Amer. Jour. Bot. **27**: 895–905.

SAVELKOUL, R. M. H. 1957. Distribution of mitotic activity within the shoot apex of *Elodea densa*. Amer. Jour. Bot. **44**: 311–317.

SCHÜEPP, O. 1966. Meristeme. Experientia Supl. 11. Berkhaüser Verlag, Basel und Stuttgart. 253 pp.

SCHWABE, W. W. 1959. Some effects of environment and hormone treatment on reproductive morphogenesis in the *Chrysanthemum*. Jour. Linn. Soc. Bot. **56**: 254–261.

SEELIGER, I. 1954. Studien am Sprossvegetationskegel von *Ephedra fragilis* var. *campylopoda* (C.A.Mey.) Stapf. Flora **141**: 114–162.

———. 1955. Studien am Sprossvegetationskegel von *Thujopsis dolabrata* (L.f.) Sieb. et Zucc. Flora **142**: 183–212.

SEIDLOVÁ, F. & J. ŠTICHOVÁ. 1968. Development of the shoot apex of *Chenopodium rubrum* L. after photoperiodic induction in the cotyledon stage. Biol. Plant. Acad. Sci. Bohemoslov. **10**: 131–134.

SENGHAS, K. 1956a. Histogenetische Studien an Sprossvegetationspunkten dicotyler Pflanzen. I. Bau und Histogenese des Spross-Scheitelmeristems einiger Cruciferen. Beiträge zur Biol. Pflanzen **33**: 85–113.

———. 1956b. Histogenetische Studien an Sprossvegetationspunkten dicotyler Pflanzen. II. Gestalt und Architektonik des ruhenden, embryonalen Vegetationspunktes. Beiträge zur Biol. Pflanzen **33**: 325–370.

SHAH, J. J. & P. M. JANI. 1964. Shoot apex of *Euphorbia nerifolia* L. Proc. Natl. Inst. Sci., India **30B**: 81–91.

——— & K. S. THULASY. 1967. Structure and organization of the shoot apex of *Picea smithiana* (Wall.) Boiss. Proc. Indian Acad. Sci., Sec. B **65**: 175–180.

SHARMAN, B. C. 1942. Shoot apex in grasses and cereals. Nature **149**: 82–83.

SHIMIZU, M. 1959. Effects of nitrogen, phosphorus and potassium on the structure and physiology of the vegetative shoot apex in the rice plant. Jap. Jour. Breeding **9**: 79–86. (with English summary).

———. 1960a. Effects of nitrogen, phosphorus and potassium on the panicle differentiation and the time of heading in rice plants. Jap. Jour. Breeding **10**: 52–60 (with English summary).

———. 1960b. Effects of nitrogen, phosphorus and potassium on the spike differentiation and development in wheat plants. Jap. Jour. Breeding **10**: 179–187 (with English summary).

SINGH, H. 1961. Seasonal variations in the shoot apex of *Cephalotaxus drupacea* Sieb. et Zucc. Phytomorph. **11**: 146–153.

SMITH, C. A. 1963. Shoot apices in the family Moraceae with a seasonal study of *Maclura pomifera* (Raf.) Schneid. Bull. Torrey Bot. Club **90**: 237–258.

SOMA, K. 1958. Morphogenesis in the shoot apex of *Euphorbia lathyris* L. Jour. Fac. Sci., Univ. of Tokyo. Sec. III, Bot. **7**: 199–256.

——— & E. BALL. 1963. Studies of the surface growth of the shoot apex of *Lupinus album*. *In*: Meristems and Differentiation. Brookhaven Symposia in Biology **16**: 13–45.

STEFFENSEN, D. M. 1968. A reconstruction of cell development in the shoot apex of maize. Amer. Jour. Bot. **55**: 354–369.

STEIN, D. B. & O. L. STEIN. 1960. The growth of the stem tip of *Kalanchoë* cv. 'Brilliant Star.' Amer. Jour. Bot. **47**: 132–140.

STEIN, O. L. & A. H. SPARROW. 1963. The effects of chronic gamma irradiation on the growth of *Kalanchoë* cv. 'Brilliant Star.' Rad. Bot. **3**: 207–222.

STEWART, R. N. & H. DERMEN. 1970. Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Amer. Jour. Bot. **57**: 816–826.

SUNDERLAND, N. 1961. Cell division and expansion in the growth of the shoot apex. Jour. Exptl. Bot. **12**: 446–457.

——— & R. BROWN. 1956. Distribution of growth in the apical region of the shoot of *Lupinus albus*. Jour. Exptl. Bot. **7**: 127–145.

TAILLANDIER, J. 1965. Sur l'incorporation de thymidine tritiée dans l'apex végétatif du *Pinus pinea*. Comp. Rend. Acad. des Sci. (Paris) **260**: 4043–4045.

———. 1966. Ontogenèse du cône femelle chez le *Pinus maritima* Lam. Rev. Gén. Bot. **73**: 324–337.

———. 1967. Ontogenèse du cône mâle chez le *Pinus maritima* Lam. Comp. Rend. Acad. des Sci. Ser. D (Paris) **265**: 1479–1481.

TAKIMOTO, A. 1969. *Pharbitis nil* Chois. *In*: The induction of flowering. L. T. Evans (Ed.). Cornell Univ. Press. Ithaca, New York.

TAYAL, M. S., M. P. KAUSHIK, & K. K. NANDA. 1961. Growth and phasic development of oat. I. A developmental study of the shoot apex in two varieties of *Avena sativa* under differential photoperiodic treatments. Indian Jour. Plant Physiol. **4**: 132–149.

TEPFER, S. S. 1960. The shoot apex and early leaf development in *Clematis*. Amer. Jour. Bot. **47**: 655–664.

TEPPER, H. B. 1963. Dimensional and zonational variation in dormant shoot apices of *Pinus ponderosa*. Amer. Jour. Bot. **50**: 589–596.

———. 1964. Ontogeny of the shoot apex of seedlings of *Pinus ponderosa*. Amer. Jour. Bot. **51**: 859–865.

THIELKE, C. 1954. Die histologische Struktur des Sprossevegetationskegels einiger Commelinaceen unter Berücksichtigung panaschierter Formen. Planta **44**: 18–74.

———. 1955. Die Struktur des Vegetationskegels einer sektorial panaschierten *Hemerocallis fulva* L. Ber. Deut. Bot. Gesell. **68**: 233–238.

———. 1959. Der Sprossscheitel in der Gattung *Saccharum*. Naturwiss. **46**: 478–479.

———. 1960. Histologische Untersuchungen am Sprossscheitel von *Saccharum spontaneum* L. I. Der Sprossscheitel von *Saccharum spontaneum* L. Ber. Deut. Bot. Gesell. **73**: 147–154.

———. 1962. Histologische Untersuchungen am Sprossscheitel von *Saccharum*. II. Der Sprossscheitel von *Saccharum sinense*. Planta **58**: 175–192.

———. 1963. Der Strukturwechsel am vegetativen Sprossscheitel von *Erianthus* Michx. Planta **59**: 587–599.

———. 1964. Histologische Untersuchungen am Sprossscheitel von *Saccharum*. IV. Der Sprossscheitel von *Saccharum officinarum* L. Planta **62**: 332–349.

———. 1965. Strukturwechsel und Enzymmuster am Sprossscheitel einiger Gräser. Planta **66**: 310–319.

THOMAS, R. G. 1961. Correlations between growth and flowering in *Chenopodium amaranticolor*. I. Initiation of leaf and bud primordia. Ann. Bot. N.S. **25**: 138–151.

———. 1963. Floral induction and the stimulation of cell division in *Xanthium*. Science **140**: 54–56.

THOMPSON, M. M. & H. P. OLMO. 1963. Cytohistological studies of cytochimeric and tetraploid grapes. Amer. Jour. Bot. **50**: 901–906.

TOLBERT, R. J. 1961. A seasonal study of the vegetative shoot apex and the pattern of pith development in *Hibiscus syriacus*. Amer. Jour. Bot. **48**: 249–255.

——— & M. A. JOHNSON. 1966. A survey of the vegetative shoot apices in the family Malvaceae. Amer. Jour. Bot. **53**: 961–970.

TRÂN THANH VÂN, M. 1965a. La vernalisation du *Geum urbanum* L. Étude expérimentale de la mise à fleur chez une plante vivace en rosette exigeant le froid vernalisant pour fleurir. Ann. Sci. Nat., Bot., **12**, **6**: 373–594.

———. 1965b. Evolution des méristèmes gemmaires du *Geum urbanum* L. en fonction des traitements subis aux divers stades du developpement. Bull. Soc. Franç. de Physiol. Vég. **11**: 233–242.

TRIBOT, R. 1961. Le point végétatif du *Cryptomeria japonica* Don. Phyllotaxie et vascularisation des axes épicotylés. Rev. Cytol. et Biol. Vég. **23**: 1–48.

TUCKER, S. C. 1962. Ontogeny and phyllotaxis of the terminal vegetative shoots of *Michelia fuscata*. Amer. Jour. Bot. **49**: 722–737.

VANDEN BORN, W. H. 1963. Histochemical studies of enzyme distribution in shoot tips of white spruce (*Picea glauca* (Moench.) Voss). Canadian Jour. Bot. **41**: 1509–1527.

VAN OVERBEEK, J. & H. J. CRUZADO. 1948. Flower formation in the pineapple plant by geotropic stimulation. Amer. Jour. Bot. **35**: 410–412.

VAUGHAN, J. G. 1955. The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa-pastoris* (L.) Medic. and *Anagallis arvensis* L. Jour. Linn. Soc. Bot. **55**: 279–301.

WADA, K. 1967. Studies on the flower initiation in *Pharbitis* seedlings. I. Ages of shoot apices and initiation rates of leaf primordia at the shoot apex. Bot. Mag., Tokyo. **80**: 161–171.

————. 1968. Studies on the flower initiation in *Pharbitis* seedlings, with special reference to the early stimulation of cell division at the flower-induced shoot apex. Bot. Mag., Tokyo **81**: 46–47.

WARDLAW, C. W. 1957a. The floral meristem as a reaction system. Proc. Roy. Soc. Edinb. B. **66**: 394–408.

————. 1957b. The reactivity of the apical meristem as ascertained by cytological and other techniques. New Phytol. **56**: 221–229.

————. 1957c. On the organization and reactivity of the shoot apex in vascular plants. Amer. Jour. Bot. **44**: 176–185.

————. 1965a. The organization of the shoot apex. *In*: Encyclopedia of plant physiology. XV/1. pp. 966–1076. Springer-Verlag, Berlin.

————. 1965b. Organization and evolution in plants. Longmans, Green & Co., London.

————. 1968. Morphogenesis in plants: A contemporary study. Methuen & Co., London.

WATERKEYN, L., P. MARTENS, & J. P. NITSCH. 1965. The induction of flowering in *Nicotiana*. I. Morphological development of the apex. Amer. Jour. Bot. **52**: 264–270.

WATSON, J. D. & R. E. F. MATTHEWS. 1966. Effect of actinomycin D and 2-thiouracil on floral induction and nucleic acid synthesis in the bud in *Chenopodium amaranticolor*. Austral. Jour. Biol. Sci. **19**: 967–980.

WEILING, F. 1962. Lässt sich die Zahl der Initialzellen der embryonalen Sprossanlage höherer Pflanzen mit Hilfe von Röntgenmutanten ermitteln? Ber. der Deut. Bot. Gesell. **75**: 211–217.

WEST, W. C. & J. E. GUNCKEL. 1968a. Histochemical studies of the shoot of *Brachychiton*. I. Cellular growth and insoluble carbohydrates. Phytomorph. **18**: 269–282.

———— & ————. 1968b. Histochemical studies of the shoot of *Brachychiton*. II. RNA and protein. Phytomorph. **18**: 283–293.

WETMORE, R. H., E. M. GIFFORD, JR., & M. C. GREEN. 1959. Development of vegetative and floral buds. *In*: Photoperiodism and Related Phenomena in Plants and Animals. Washington. pp. 255–273. (R. B. Withrow, Ed.).

WILLIS, J. C. 1966. A dictionary of the flowering plants and ferns. 7th ed. Cambridge.

ZAMORA, P. M. 1959. Zonal structure of the apex of *Oryza sativa* L. Philippine Agr. **42**: 391–403.

ZEEVAART, J. A. D. 1962. DNA multiplication as a requirement for expression of floral stimulus in *Pharbitis nil*. Plant Physiol. **37**: 296–304.

————. 1969. *Perilla*. *In*: The induction of flowering. (L. T. Evans, Ed.) Cornell Univ. Press. Ithaca, New York. pp. 116–155.