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THE EFFECTS OF ACUTE GAMMA RADIATION ON
CHRYSANTHEMUM INFLORESCENCE MORPHOGENESIS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Richard John Naskali, B. Sc., M. Sc.

The Ohio State University
1969

Approved by

Richard A. Popham
Adviser
Department of Botany
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VITA

December 11, 1935
Born, Jefferson, Ohio

1957
B. Sc., Agriculture, The Ohio State University, Columbus, Ohio

1958-1959
Research Assistant, Department of Botany and Plant Pathology, The Ohio State University, Columbus, Ohio

1959-1960
Assistant, Department of Botany and Plant Pathology, The Ohio State University, Columbus, Ohio

1960-1967
Assistant Instructor or Instructor, Department of Botany and Plant Pathology, The Ohio State University, Columbus, Ohio

1964
Research Assistant, Summer Student Program, Brookhaven National Laboratory, Upton, N. Y.

1967-present
Acting Assistant Professor of Botany, Department of Biological Sciences, University of Idaho, Moscow, Idaho

PUBLICATIONS


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FIELDS OF STUDY

Major Field: Botany


Studies in Plant Genetics: Professor Elton F. Paddock.


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INTRODUCTION

Since W. C. Röntgen's discovery of X rays in 1895 and Henri Becquerel's discovery of radioactivity during the subsequent year, the study of effects of ionizing radiation on plants has proliferated at an approximately logarithmic rate. Between 1896 and 1955, over 2500 papers concerned with the effects of ionizing radiation on plants were published (Sparrow, et al., 1958). In subsequent years hundreds of papers have appeared annually in this subject.

A major portion of studies concerning irradiation of living organisms has been restricted to heritable genetic changes (bibliography of Sparrow, et al., 1958; Kuehnert, 1962). Many radiation effects investigations have been directed specifically toward induction of desirable mutants (Sparrow and Konzak, 1958; Nybom, 1960; Gustafsson, 1961; Gaul, 1964). Scores of other experiments have dealt with ascertaining the role of the plant cell nucleus in determining radiosensitivity (Sparrow, 1962). Most of these latter studies have involved comparison studies among species rather than among different stages in ontogeny of plants of a uniform genotype. In addition to these genetic and nuclear emphases, a not insignificant number of investigations have been concerned directly with the effects of ionizing irradiation on vascular plant morphogenesis. The effects of these high-energy radiation treatments have been reviewed extensively by Breslavets (1960), Gunckel (1957, 1965), Gunckel and Sparrow (1953, 1961), and others. From these reviews it is clear that ionizing irradiations may induce many morphological variations from the "normal" (non-irradiated) in stems, leaves, roots,
flowers, fruits, and seeds of plants.

In stems, radiation treatments are often followed by the development of shoots which have altered vascular patterns and phyllotaxis, bifurcations or fasciations, and changes in degree of "twisting". While low doses (usually less than 500 roentgens) of ionizing irradiation sometimes may stimulate stem elongation at certain stages of development (Sax, 1963), higher total dose exposure of stems may result in the formation of aerial roots (Christensen, 1954), tumors and adventitious buds (Pratt, 1959; Pratt, et al., 1959; Hagen, et al., 1961; Meiselman, et al., 1961 a,b; Miksche, et al., 1962; Haccius and Reichert, 1963; Bankes and Sparrow, 1969), or death of apical meristems. As a result of higher energy irradiation (X-ray, gamma-ray, and, to some extent, high energy particle bombardment) the usual morphogenesis of vegetative shoot apices, leaf primordia, and leaves is disrupted to the extent that leaf shape, thickness, size, color, pubescence, venation patterns, and/or plastochron duration frequently vary significantly from those of unirradiated individuals.

In roots and other underground organs, stunting of growth, inception of lesions and tumors, color changes, and growth stimulation are reported. In many cases, however, it is evident that growth stimulation phenomena in plant parts do not occur consistently (Skok, et al., 1965) in subsequent experimental replications.

If reproduction of angiosperms is considered, gamma- and X-irradiations may cause abscission, color changes, dwarfing, and fasciation of inflorescences or their parts and retardation of peduncle or pedicel growth as well as reduction in numbers of flowers which mature per stem. Relative sizes and numbers of parts in each flower may vary from the usual following irradiation. In some cases, embryo sac-like structures
form in stamens (Gunckel and Sparrow, 1953), pollen sacs appear in gynoecia (Singh and Gunckel, 1965), and open gynoecia (Singh and Gunckel, 1965) develop following irradiation treatment. Other deviations from usual patterns of androecium and gynoecium development, induced by high energy irradiation, are known in angiosperms (Gunckel and Sparrow, 1953; Singh and Gunckel, 1965; Steinman, 1961; Gupta and Samata, 1967).

Treatment with X- or gamma-irradiation can result in the induction of chimeras (Nybom, 1960; Pratt, 1967; Gaul, 1964, p 211) and even the "breaking" of chimeras (Sagawa and Mehlquist, 1957; Heiken and Ewertson, 1962; Howard, 1964; Nybom, 1960; Pratt, 1960; Weaver, 1963; Bowen, et al., 1962; Pereau-Leroy, 1969). In Sagawa and Mehlquist's (1957) experiments, irradiation of chimeral shoots often resulted in the formation of shoots in which there was only one genotype of cells in surviving or reconstituted apical meristems. Similar results appear probable in the experiments of Pereau-Leroy (1969).

In all variations in the biological world, correct explanations and interpretations result when the cause or causes of each phenomenon are known. After Gupta and Samata (1967) studied induction of somatic mutations in Cosmos, they concluded, "... it is difficult to determine definitely whether the observed changes are due to some true mutation or to some tempering physiological effect. The value of the method used in tests for somatic mutations is severely limited by the fact that the presumed mutant cannot be tested genetically."

Earlier, Gunckel and Sparrow (1953, p 272), while reviewing radiation-induced aberrant growth, concluded, "... most if not all of the morphogenetic abnormalities described above result from the induced physiological
or biochemical disturbances. Mutations, of course, probably result from the primary changes in the genes or chromosomes and are, therefore, an exception." Later, Gunckel and Sparrow (1961, p 595) further strengthened their earlier hypothesis when they stated, "... that plants with abnormal growth patterns nearly all revert to normal growth during a recovery period certainly suggests the basic cause to be non-genetic physiological disturbances induced by the radiation. Mutation changes would not be expected to revert to normal as reported above..."

Supporting the physiological disturbance approach exclusive of gene change, Gunckel, et al. (1953) pointed out that radiation effects were not qualitatively different than many aberrant changes observed in non-irradiated plants. Indeed, physiological bases can be found to explain many teratological phenomena which occur 'spontaneously' (Heslop-Harrison, 1952).

Support for Gunckel and Sparrow's 1953 conclusion may be inferred from many additional experiments. Gordon (1957), for example, found that free auxin concentrations immediately decreased in X-irradiated (25 r to 1 kr dose) kidney bean shoots. Free auxin levels in the plant returned to control plant levels about two weeks after the treatment. In relatively simple, non-living systems, ionizing irradiation can result in destruction or modification of molecules, e.g., sucrose (Holsten and Steward, 1965), adenine (Ponnamperuma, et al., 1963), ATP (Vaisey and Thatcher, 1962) papain (Pihl and Sanner, 1963) and indole acetic acid (Skoog, 1935).

Ionizing radiation damage can be simulated by manipulation of the plant's environment. Ionizing radiation (higher than 'background') or genotype change is not essential to modification of morphogenesis. Exogenously
applied natural or synthetic growth substances can promote or inhibit growth and alter differentiation in both vegetative and reproductive shoots (van Overbeek, 1966; Cathey and Stuart, 1958; Tepfer, et al., 1966; Meyer, 1966; Plack, 1958; Searle, 1965; Weijer, 1959; Letham, 1967; Nitsch, 1952, 1965; Heslop-Harrison, 1959; Schwabe, 1959; Wardlaw, 1965a; Lang, 1965).

Tumors and adventitious buds form spontaneously in plants of certain genotypes (Meiselman, et al., 1961a, b; Hagen, et al., 1961; Smith, et al., 1969) or may be induced by total debudding (Skok, 1967).

Whereas variations in development of a vascular system may accompany surgically caused bifurcations or fasciations (Loiseau, 1959), vegetative shoot, inflorescence, or floral bifurcation can be caused by microsurgical incisions of shoot apices (Ball, 1950a, 1955; Cusick, 1956, 1959; Soma, 1958; Loiseau, 1959; Wardlaw, 1963, 1965a,b,c; Soetiarto and Ball, 1969) or by chemical treatment (Powell and Andreasen, 1957; Gorter, 1951; Wardlaw, 1953; Applegate and Hamner, 1957; Soma, 1968). Shoot fasciation may happen spontaneously (Kundu and Rao, 1960; White, 1948; Astie, 1962) or may be enhanced by photoperiodic treatment (Piringer and Borthwick, 1961).

Radiation-induced changes in floral development, including changes in numbers of floral parts and modification of sexual expression sometimes may be induced by physical or biochemical treatments without ionizing irradiation. Hartsema (1961) noted that certain temperature treatments of hyacinth, tulip, and other bulbs caused variations in the usual numbers of floral appendages. So-called male and female sterility and "sex reversals" can be caused by manipulation of one or more environmental factors around intact, non-irradiated plants (e.g., Rehm, 1952; Rick and Boynton, 1967; Jain, 1959; Catarino, 1964; Galun, 1961; Joshi, 1939; Heslop-Harrison, 1957a,b, 1959, 1960; Negi and Olmo, 1966; Schaffner, 1931, 1933, 1935) or,
in vitro (e.g., Porath and Galun, 1967; Galun, et al., 1963), or, as a result of certain pathogens (Fischer and Holton, 1957, p 111-131; Heslop-Harrison, 1959; Wardlaw, 1960; Nunes, 1964). Photoperiodic treatment alone, or in certain combination treatments with other variable environmental factors, may also have pronounced effects on patterns of floral morphogenesis (e.g., Schwabe, 1959; Biddulph, 1935; Greulach, 1942; Arnal, 1966; Okada and Hiraki, 1954; Okada and Harada, 1954; Tsukamoto, 1957; Tsukamoto and Harada, 1957; Nitsch, 1952, 1965; Galun, 1961; Heslop-Harrison, 1957b, 1960; Meyer, 1966).

Chimeras, which sometimes are induced by ionizing irradiation, often originate spontaneously (e.g., Cramer, 1954; Tilney-Bassett, 1963; Nybom, 1960; Derman, 1960; Brabeck, 1965). From chimeral plants, shoots of a single, uniform genotype occasionally arise spontaneously (Derman, 1960; Weaver, 1963; Dowrick, 1953; Nybom, 1960; Cramer, 1954). Plants of a uniform single genotype can be induced from periclinal chimeras ("breaking" the chimer) by "eye excision" experiments (Howard, 1959) or by inducing vegetative bud formation on endogenously originated adventitious roots (Zimmerman and Hitchcock, 1951; Tilney-Bassett, 1963; Chittenden, 1927; Derman, 1960; Stewart and Arisumi, 1966; Robinson and Darrow, 1929; Stewart and Pryor, 1961).

Thus, many irradiation-induced variations in vascular plant morphogenesis may be similar, or even identical, to those variations which may be induced by other experimental means, including those physical and biochemical treatments which are not known to be mutagenic. It is not unreasonable, then, to consider that many of the radiation-induced morphological syndromes have physiological causes which are not contingent upon, or a result of, nuclear genotype changes. Variant color sectors, similar to those induced by
ionizing irradiation, have not been caused by non-mutagenic treatments, however.

Whereas many irradiation-caused morphological variations are reported, few investigators emphasize that gene-induced processes, underlying morphogenesis, are modified. It appears that most radiation-induced variations occur in growing plant parts. Similar changes could not occur in fully developed plant organs. In this context, the author intends to refer to morphological variations which are the end products of environmentally variable processes and gene actions which are possible even though genotype remains constant. Deemphasis of heredity is not intended. In several angiosperm families, there are genera in which a single gene, or a few pairs of genes can induce extreme variations from the typical flowers of the species (McCollum, 1966; Weijer, 1959; Bhatia and Swaminathan, 1963; Fejer, 1966; Keep, 1964; Aalders and Hall, 1963; Jain, et al., 1961; Rana, et al., 1963; Rana, 1965; Samata, 1962; Miyake, et al., 1927; Gupta and Samata, 1963).

In view of the recent emphasis on the ontogenetic approaches to solving problems concerning plant structure, it is indeed surprising that relatively few studies concerning the effects of ionizing irradiation on apical meristem ontogeny have been conducted. Shoot apical meristems have been most frequently investigated to acquire nuclear and chromosomal volume data in order to make interspecific comparisons of radiosensitivity (Sparrow, 1962; Yamakawa and Sparrow, 1966).

Ionizing irradiation effects on vegetative shoot apical meristems have been studied in a relatively small number of species, including ferns (Kuehnert and Miksche, 1964), pine (Mergen and Thielges, 1966; Donini,
1967), yew (Miksche, et al., 1962), apple (Pratt, et al., 1959; Pratt, 1960, 1967), grape (Pratt, 1959), pear (Pratt, 1967), tomato (Kuehnert, 1962), tobacco (Haccius and Reichert, 1963; Crockett, 1957), potato (Heiken and Ewerson, 1962; Howard, 1959, 1964), Tradescantia (Gunckel and Sparrow, 1953; Gunckel, et al., 1953), jute (Kundu and Rao, 1960), carnation (Sagawa and Mehlquist, 1957), corn (Stein and Steffensen, 1959a,b), barley (Mericle and Mericle, 1961), and Kalanchoe" (Stein and Sparrow, 1963, 1966). Microscopic studies of shoot apex dynamics have accompanied gross morphological examinations in the majority of these cases. In spite of the valuable results derived from these investigations, many of them were conducted during dormant stages, or throughout the growing season under chronic irradiation. Thus, from such experiments, it would be difficult to ascertain specific changes in radiosensitivity, or to pinpoint specific sites of radiodamage such as might result if acute irradiation treatment were used during different ontogenetic stages of growth to individuals of one clone.

Information concerning ionizing irradiation effects upon reproductive shoot morphogenesis is fragmentary. Whereas effects of high-energy irradiation upon inflorescence or flowers are frequently reported, there are only a few studies relating anatomical data with gross morphology. For the most part, in recent years, only additional gross morphological facts have been added in this area since Johnson's (1939) early survey gave a broad view of ionizing irradiations as they relate to gross floral morphology. Indeed, presumably in reference to radiosensitivity, Gunckel and Sparrow (1961) stated that "we know little or nothing of the basic differences of vegetative versus floral development."

Shoot apical meristems appear to be highly radiosensitive during flower
bud initiation in *Lycopersicon esculentum* (Gunckel, 1957; Brody, 1953, cited by Gunckel and Sparrow, 1961, p 593), *Tradescantia paludosa* (Yamakawa and Sparrow, 1966), *Dianthus caryophyllus* (Sagawa and Mehlquist, 1957), and *Antirrhinum majus* (Yamakawa and Sparrow, 1966). Similar radiosensitivity during flower bud initiation may be inferred from data reported for *Cosmos bipinnatus* (Gupta and Samata, 1967), *Xanthium pennsylvanicum* (Brown and Taylor, 1967), and several other species (Johnson, 1939).

In other investigations, the value of conducting studies relating ontogeny with ionizing irradiation treatments occasionally has been expressed. Numerous investigators suggest, either by direct statement, or by implication, that studies relating size, shape, and distribution of ionizing irradiation-induced mutant sectors could provide valuable insights into patterns of ontogeny in intact plants (e.g., Cuany, et al., 1958; Ichikawa and Sparrow, 1968; Kaukis and Reitz, 1955; Gupta and Samata, 1967, p 226; Mericle and Mericle, 1969; Bulatti, et al., 1969).

Since many varieties of *Chrysanthemum morifolium* Ramat. are obligate short-day plants with respect to flowering, timed development of inflorescences can be controlled. Flower bud initiation soon ensues when plants of a short-day clone of this species are placed in inductive conditions. Usually there are few anatomical variations in each stage of ontogeny among those plants of a clone which have been subjected to the same number of inductive photoperiods (Popham and Chan, 1952). Consequently, it is possible to obtain large populations of plants having synchronous inflorescence ontogeny. Furthermore, inflorescences of chrysanthemum each have a number of distinct structures which form sequentially. During inflorescence ontogeny, there are a number of distinctly discernable and sequentially occurring
phases which culminate in a complex inflorescence (Popham, 1963). After the onset of photoinduction, leaf initiation ceases, and a series of involucral bracts, ray florets, and disc florets usually are initiated until inflorescence apices form florets at their summits. Ray florets are strictly pistillate and disc florets are perfect in these chrysanthemums. Because of this combination of advantages, *Chrysanthemum morifolium* Ramat. is an ideal species for investigating the effects of single, acute-exposure radiation treatments of vegetative and inflorescence apices at different ontogenetic stages.

The purposes of this investigation were threefold: first, to ascertain the effects of acute irradiation treatments, given during various stages of inflorescence ontogeny, on mature inflorescence anatomy and morphology; second, to ascertain if vegetative and inflorescence apices in various developmental stages are differentially radiosensitive; and, third, to correlate irradiation-induced morphological variations among mature inflorescences at anthesis with anatomical events occurring during ontogeny.
MATERIALS AND METHODS

A. Irradiation Experiments

In order to minimize genetic variation among plants, 3,000 rooted cuttings of clonal Chrysanthemum morifolium Ramat. 'Criterion' (Bailey, 1949, p 989) were obtained from Yoder Brothers, Inc., Barberton, Ohio, for irradiation at Brookhaven National Laboratory, Upton, New York. 'Criterion', chosen because it has a minimum of 'spontaneous' mutations (William Duffett of Yoder Brothers, Inc., personal communication) is classed as having a large pink 'decorative' inflorescence. Plants are in peak flower, as judged by commercial salability, following 10 weeks of continuous short (nine hr photoperiod) days and culture at 23.5°C (day) and 20°C (night).

Rooted cuttings were planted individually in 10.2 cm (diameter) standard clay pots in a steam-pasteurized 1:1:2 (volume) mixture of sand, peat, and loam, and were placed in greenhouse benches April 11, 1964. Greenhouse temperatures were controlled at 20°C (nights) and 23.5°C (days). Standard horticultural practices of pest control, watering, and regular fertilization with a water solution of Kapco 20-20-20 (30 g/10 liters), containing trace elements, were followed during the experiments. During four nights after potting, supplemental illumination (10 PM to 12 midnight) provided non-inductive photoperiods.

For irradiation studies, the 3,000 plants were subdivided into ten consecutively numbered blocks of 300 plants each--designated as I, II, III,
etc. -- to allow sufficient numbers of plants for microscopic studies as well as for gross morphological observation and scoring evaluation of plants in flower. In each of the 10 blocks of 300 plants, labels of the first 150 plants were marked with an \( i \) for irradiation treatment and the remainder of the labels were designated with a \( c \) for no gamma irradiation. Thus, there were 150 consecutively numbered plants of \( i_i \), and of \( c_c \), \( i_ii \), \( ii_c \), etc. Beginning the night of Wednesday, April 15, standard horticultural black sateen cloth was drawn over all 3,000 plants at 5 PM and removed at 8 AM the following day to start the photoinductive cycles. This long night photoperiod control was continued daily for 10 weeks, thus providing a series of 15-hour photoinductive nights.

Beginning April 14, the block of 150 \( c_c \) (control) plants was taken from the Brookhaven greenhouses to the Control Field, where the individual pots were buried in soil to their rims, and where, during the subsequent 20 hours, they received no ionizing irradiation other than 'natural' background levels. At the same time, the 150 \( i_i \) plants were placed in the Brookhaven Gamma Field (Fig. 1), (Sparrow, 1966), where the pots were arranged along the arc of a circle the radius of which was 6 m from the irradiation source point. The \( i_i \) plant pots were buried to their rims in the native soil to keep them upright. Subsequently, the \( i_i \) plants received 2500 r gamma irradiation from a kilocurie cobalt-60 source. The \( i_i \) designated plants received an acute total gamma ray dose of 2500 r, at the rate of 125 r/hr, during 20 hours of their last long day which preceded photoinduction. During the same 20 hours, the 150 \( c_c \) plants received no additional gamma irradiation in the earth-shielded control field. At the end of the 20 hour period, irradiated (\( i_i \)) and control (\( c_c \)) plants were returned to their original greenhouse positions.
Figure 1. The Brookhaven National Laboratory Gamma Field.
On the following April 21, the 150 IIc (control) plants were taken to the Control Field and placed as previously described for Ic. At the same time, the 150 IIi plants were placed in the Gamma Field along the arc of the same radius as was done with II plants. Subsequently, IIi plants were irradiated at the 2500 r acute gamma dose. All IIc and IIi plants were returned to their original greenhouse positions April 22.

Weekly thereafter, the plants in the respective IIIc, IIIi, IVc, IVi, Vc, VI, VIC, VIi, VIIc, VIII, VIIIc, and VIIIi blocks were taken to their respective fields for the subsequent 20 hours and treated as designated by their predetermined labels. Thus, each week, a different group of 150 control (c) and 150 irradiation treatment (i) plants were manipulated. In this manner, different plants, in weekly stages of inflorescence ontogeny, were irradiated while comparable control plants were not exposed to additional irradiation beyond 'natural' background levels. Thus, plants were irradiated just prior to their 1st, 8th, 15th, 22nd, 29th, 36th, 43rd, or 50th photoinductive periods.

A total dose of 2500 r was chosen for these acute gamma irradiation experiments because Steinman (1961), William Duffett (personal communication), and Bowen (mimeo, Wantage Research Laboratory) had found optimum mutation induction with 2500 r ionizing irradiation in chrysanthemums. Jank (1957) reported optimum mutation induction after irradiating vegetative Chrysanthemum indicum plants with 2000 r X-rays.

By June 9th, after 55 consecutive daily photoinductive nights, all IX and X plants had such large terminal inflorescence buds that serial sectioning of terminal heads would have been difficult. Furthermore, because floral ontogeny had progressed through microsporocyte meiosis in the terminal florets of each terminal head, inflorescence development had progressed
through all early stages. For these reasons, irradiation treatments were discontinued June 9th and plants previously designated IXc, IXi, Xc, and Xi were discarded.

Histological study specimens were collected weekly from previously irradiated and control plants through the VIII treatment. Immediately upon return of the I plants to the greenhouse April 15, 10 stem tips\(^1\) each were collected from Ic and Ii plants and fixed in FPA (10 ml formaldehyde solution, 5 ml propionic acid, 50 ml 95% ethanol, and 35 ml water). After each subsequent weekly irradiation treatment, individual ten-shoot tip samples from the most recently treated \(\_\) and \(\_\) plants as well as from each block of previously treated \(\_\) and \(\_\) plants were harvested and fixed. In this manner, weekly histological developmental details, after irradiation, could be related to ontogenetic events in control plants and to gross morphological variations among flowering, intact irradiated and non-irradiated plants. Histological collection and irradiation treatment times are summarized in Table 1. As may be observed from the data in this table, block I (vegetative) plants were irradiated April 14-15, before photoinduction; Ic 1 and Ii 1 shoot samples were harvested April 15; block II plants were irradiated after seven inductive photoperiods. Histological samples Iic 1 and IIi 1 were collected April 22; Ic 2 and Ii 2 histological samples were collected April 22, one week after the I plants were irradiated, etc.

Samples were aspirated (Sass, 1958, p 13-14). Following 24 hours of fixation in FPA, histological specimens were processed in a standard ethanol-

\(^1\)Stem tips, especially in early collections, usually included the terminal apex as well as several leaf primordia and axillary apices.
Table 1. Schedule of irradiation treatments and histological sample collection times in relation to number of inductive photoperiods completed before irradiation treatments of respective blocks.

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toluene dehydration series and embedded in Summer Formula Embedding Mass [18.2 kg Sohio Parowax, 2 kg bayberry wax, and 4 kg stock rubber paraffin (240 g crude rubber added to 11 kg of 56–58°C paraffin)]. Toluene was the paraffin infiltration vehicle. Serial sections, cut at eight micra, were counterstained with safranin O and fast green FCF. Malfunction of the Lipshaw Automatic Tissue Processor occurred during dehydration-infiltration when histological specimens, harvested April 29 and May 20, were being infiltrated. Resultant tissue damage precluded detailed cytological observations and measurements.

In each of the experimental blocks of control and irradiation plants, 34 consecutively numbered plants remained intact throughout handling and flowering. Although all I through VIII mature (flowering) i and c plants were scored by a number of criteria, all results reported here are based only upon the first 34 intact individuals of any one block unless otherwise noted.

Where quantitative data, other than arbitrary scoring values, were used to evaluate irradiated and control plant raw data, statistical evaluation followed Steel and Torrie's (1960) orthogonal polynomial, factorial design, analysis of variance and covariance. Data for number of lateral heads, length of stems, and terminal head diameter variables were examined by this method (Appendix). In this manner, method (irradiation or no irradiation) and time (0, 7, 14, 21, 28, 35, 42, 49 short days) results and interactions were evaluated for significant linear, quadratic, cubic, and quartic relationships.

B. Ancillary Experiments

During the examination of irradiation experiment data, many results suggested supplementary experiments which might furnish insight into the mode of gamma-ray action in chrysanthemum inflorescence apices. As a
result, small scale preliminary investigations were conducted in a Sherer-Gillett controlled environment chamber at The Ohio State University. For these experiments, rooted cuttings of 'Criterion' were obtained from Yoder Brothers, Inc., Barberton, Ohio. They were planted in 10.2 cm (diameter) standard plastic pots in a 1:1:2 (volume) mixture of steam-pasteurized sand, peat, and loam. Plants were cultured in a growth chamber in which light intensity, at the soil surface, initially varied from 1500 to 1900 foot candles (fluorescent plus incandescent) at different bench positions in the chamber. Temperatures were maintained at 18°C (night) and 21°C (day).

Newly potted plants were cultured under non-inductive 16-hour (6 AM to 10 PM) photoperiods before the regime of nine-hour (lights on 8 AM to 5 PM) continuous daily inductive photoperiods began. Usual horticultural practices of watering and periodic fertilization with a solution of water-soluble Kapco 20-20-20 (30 g/10 liters), containing trace elements, were followed.

In these preliminary experiments, conducted with 'Criterion' only, a series of microsurgical punctures or incisions were made in shoot apices at various stages of inflorescence ontogeny. Bard-Parker knives, #11, 12, and 15, were used to fold back or remove just enough leaf or bract primordia to expose vegetative or reproductive shoot apices. Many of these 'operated' shoots were otherwise untreated, to serve as controls. Subsequently, some exposed apices were punctured or slit immediately prior to their 1st, 10th, 12th, 13th, 15th, 20th, 22nd, or 29th inductive photoperiods, and allowed to grow to anthesis for evaluation.

Johnson and Johnson, Dental Division, exodontia suture needles, #14, were hand-held to make single punctures in individual terminal apices or
primordial inflorescences. Single longitudinal slits in individual shoot apices or inflorescence primordia were made with hand-held small segments of double-edged Gillett Super Blue razor blades. Punctures or longitudinal slits were made in positions as close to the apex summit or inflorescence centers as could be approximated under the 40 X dissecting stereo microscope. Equivalent magnifications were employed to expose individual apices.

Except for the interval from just prior to the first inductive night through the 30th short day, growth chamber atmospheric relative humidity was maintained at 70 to 75% for the duration of microsurgical experiments. During the excepted period, growth chamber relative humidity was maintained at 90 to 95% in order to prevent desiccation of exposed and microsurgically treated apices.
RESULTS

A. General Development of 'Criterion' Terminal Inflorescences Without Irradiation

General ontogenetic details of 'Criterion' terminal inflorescence development and timing were similar to those reported earlier for Chrysanthemum morifolium 'Bittersweet' (Popham, 1963). Whereas 'Criterion' (a 10-week clone) decorative inflorescences have a majority of ray florets, mature capitula of 'Bittersweet' (a 10-week clone) are daisy-like with a majority of disc florets, all of which are centripetal to approximately four cycles of ray florets.

'Criterion' vegetative shoot apices (Fig. 2) have three or four mantle layers and the general zonation reported earlier for other chrysanthemum clones (Popham and Chan, 1950; Popham, 1963). Vegetative apices of rooted cuttings averaged 196 (range = 160 to 248) micra in diameter and 61 (range = 44 to 88) micra in height. Mean height/width ratio was 0.31. Maximum diameters and heights were attained immediately before leaf initiation; minimum dimensions were observed immediately after leaf initiation. On the average, individual shoot apex cells were isodiametric with cell mean widths and heights of 10.1 and 10.2 micra, respectively.

During the five non-inductive days which followed potting, mean cell and apex dimensions were as noted earlier for rooted cuttings. Possibly, leaf initiation occurred in some plants during these five days. Apex mean height/width ratio, 0.33 immediately before photoinduction, essentially did not
Figure 2. Median longitudinal section of a terminal vegetative shoot apex. X550.
In the summit portions of vegetative shoot apices, especially in the mantle, nuclei stained lightly but nucleoli were heavily stained. Nuclei in the apex flanks frequently stained more intensely and, consequently, had less prominent nucleoli. In part, staining differences were attributed to nuclear volume variation which was clearly evident in vegetative shoot apices (Fig. 2). Similarly, the "central zone" of Helianthus vegetative apices has larger, lighter staining nuclei than the "peripheral zones" (Steeves, et al., 1969).

Constant magnification, shoot apex projections were made with a Zeiss Large Camera Lucida (No. 47-46-05). On drawn images, shoot apex nuclear diameter measurements were restricted to those nuclei above the uppermost opposing leaf primordia. Once drawn, the generally hemispherical median sections of apices were delimited into two peripheral 45° sectors subjacent to one central, 90° sector. Nuclear volumes were calculated from diameter data: for the central 90° sector, including the central mantle and a portion of the subapical initials or central mother cells, and for the combined two, 45°, peripheral sectors. For purposes of making calculations, nuclei were assumed to be spheres.

In the central sector, interphase nuclei averaged 364 cubic micra in volume (mean diameter = 8.86 micra). In the peripheral zones of vegetative apices, mean interphase nuclear volume was 253 cubic micra (mean diameter = 7.85 micra). Because some tangential slices of nuclei probably were measured, the actual interphase nuclear diameters undoubtedly were larger than calculated values. Since volume measurements were made for 494 cells in the median section of 11 different apices, it is likely that representative sampling reflected real interphase nuclear volume.
variations between the two apex portions examined. Similar size-staining variations can be noted in the illustrations of Popham and Chan (1950).

Comparable staining and size variations were observed between the summit and peripheral portions of 'Criterion' axillary vegetative apices.

After one week of photoinductive nights, terminal apices averaged 140 micra (range = 128 to 160) in width and 37 micra (range = 24 to 44) in height. Apex height/width ratios averaged 0.26 at that time. After seven short days, mean cell height remained at 10.2 micra, but mean cell width was only 9.0 micra. By the end of the first week of short photoperiods mean interphase nuclear volume had increased to 388 cubic micra in the central sector and 338 cubic micra in the peripheral (flank) sectors of terminal apices.

At the end of the first photoinductive week, few cell divisions were observed in shoot apices, but bract initiation and early growth were occurring in many stem tips (Fig. 3). Thus, it appeared that involucral bract initiation commenced before completion of the seventh inductive photoperiod. Decreases in mean shoot apex dimensions, therefore, probably were attributable to the synchronous onset of involucral bract initiation in many plants, without significant mitosis and cell elongation in the remaining shoot apex. This inference was supported by the observation that shoot apices, after seven short days, averaged sixteen cells in width and four cells in height, compared with characteristic means of nineteen cells (width) and six cells (height) in vegetative apices. Thus, cell elongation, but not growth in cell width, accompanied shoot apex plastochronic events of the first seven inductive photoperiods.

Between the seventh and the fourteenth short days, intensification of
Figure 3. Median longitudinal section of a terminal apex after seven inductive photoperiods. X550.
mitosis and concomitant cell enlargement occurred in all shoots. After 14 short days, apices averaged 275 micra (range = 172 to 544) in width and 133 micra (range = 52 to 292) in height. At that time, individual apex cells averaged 10.9 micra and 9.9 micra in width and height, respectively. The mean height/width ratio of apices then was 0.48, essentially a two fold increase from the mean 0.26 value of the previous week. Thus, phasic changes in height/width ratios appeared to be diagnostically valuable in characterizing early inflorescence ontogeny in chrysanthemum.

The theoretical close synchrony of development among apices did not appear to continue after seven days, however. Although most incipient inflorescence apices were hemispherical (Fig. 4) after 14 short days, approximately 25% of the apices already had become distinctly capitate (Fig. 5) in a form generally characteristic of the mature 'Criterion' compound receptacle. In spite of variations in shape and volume among apices, the pronounced elongation of pith cells, noted earlier by Popham and Chan (1952), occurred in all 'Criterion' terminal apices after two weeks of photoinduction (Figs. 4, 5). By this time, approximately one half of the involucral bract primordia had formed in two or more imbricate layers in the most advanced inflorescence apices.

Through the 14th short day, all terminal 'Criterion' inflorescence apices continued to have the three or four mantle layers which also characterized vegetative shoot apices. Because mean cell heights and widths were 9.9 micra and 10.9 micra, respectively, cell elongation usually continued after mitosis in the 7- to 14-day interval after photoinduction was initiated.

During the third week of short days, rapid growth and pronounced
Figure 4. Median longitudinal section of an incipient inflorescence apex after 14 short days. X220.
Figure 5. Median longitudinal section of a capitate, terminal inflorescence apex after 14 short days. X220.
differentiation continued in all primordial inflorescences. In terminal\(^1\) apices these steps included: (a) completed initiation of the characteristically 37- to 38-bracted involucre; (b) onset of rapid floret initiation; and (c) the extremely frequent mitoses by anticlinal wall formation in the now four-to seven-layered mantle of the remaining inflorescence apex. In seven days, mitosis and concomitant cell enlargement were so rapid that receptacle primordia, exclusive of florets and involucral bracts, average 510 micra and 1039 micra in height and width, respectively, on the 21st short day. Thus, dimensions of the combined receptacle-apex complex had increased four fold. Height/width ratios, which averaged 0.49, essentially did not change from the 14th to the 21st short day.

After 21 short days a minimum of three floret cycles were present (Fig. 6). Rapid growth of involucral bracts resulted in the closely-imbricate covering of the terminal inflorescence apex. After three cycles of florets formed, all terminal receptacles had clearly identifiable procambium (Figs. 6, 7). In the most advanced primordial inflorescences (Fig. 7), in which at least nine cycles of florets were initiated, procambium had differentiated to the bases of oldest florets. By the 21st short day, corolla initiation had occurred in the most advanced two cycles of florets of some capitula (Fig. 7). No procambium was identifiable within florets at that time.

After approximately three cycles of floret primordia formed on any

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\(^{1}\)Although all chrysanthemum capitula are terminal in respect to their peduncles, terminal capitulum in this paper will refer to the inflorescence at the summit of the main plant axis; lateral capitula will refer to those at the summit of axillary branches.
Figure 6. Median longitudinal section of a primordial, terminal inflorescence and two subjacent lateral inflorescence apices after 21 short days. X90.
Figure 7. Median longitudinal section of an advanced stage of terminal inflorescence ontogeny after 21 short days. X90.
inflorescence apex, pronounced periclinal divisions began in the receptacular protoderm between adjacent older florets. In this manner, and by subsequent periclinal divisions and cell enlargement, uniseriate papillose trichomes, about four to six cells in length, formed. No other periclinal divisions of the L-1, or its derivatives, were observed in any control plant.

Loss of apical dominance was a characteristic manifestation of early stages of photoinduction by the 21st short day. Rapid growth of uppermost, formerly vegetative, axillary buds had progressed through all early stages of inflorescence induction and initiation, including the strictly acropetal initiation of involucral bracts (Fig. 6). Although somewhat smaller, the most advanced axillary inflorescence apices were in developmental stages which were fully equivalent to those of terminal apices only one week earlier (compare Figs. 5, 6). Thus, the third week of inflorescence ontogeny probably was one of ever quickening physiological and anatomical change.

At 28 short days, floret initiation continued in some terminal apices (Fig. 8). In the most advanced capitula all floret primordia had formed by the 28th short day (Fig. 9). While papillose trichome initiation continued on some receptacles, it had already occurred between all florets over the summit of the most advanced receptacles. In all terminal capitula, continued laminar growth resulted in the tighter interdigitation of the one to four cell-thick involucral bract tips (Figs. 8, 9).

Between the 21st and 28th short days, continued growth of the receptacle essentially doubled its width and height to means of 2421 micra and 1235 micra, respectively. Height/width ratio of the receptacle, now 0.51, essentially had not changed during the past three weeks.

Corolla initiation had commenced on all terminal, and even some
Figure 8. Median longitudinal section of a primordial terminal inflorescence with portions of two subjacent lateral capitula after 28 short days. X35.
Figure 9. Median longitudinal section of an advanced terminal capitulum after 28 short days. X35.
lateral, inflorescences (Fig. 8). Stamen initiation, which had begun in only the oldest florets of some terminal capitula (Fig. 8), was completed in all florets of the most advanced heads. Carpel initiation, which was complete in the oldest 11 to 13 cycles of florets on advanced capitula (Fig. 9), was only beginning in the florets of some primordial terminal heads (Fig. 8). Ovule primordia were observed only in the florets of the oldest three or four cycles of a few receptacles. In some terminal capitula, procambium was observable in all florets.

Although continued slow growth of corollas and stamens was evident in all older florets, morphological differences of ray and disc florets were not evident in any capitula at the 28th short day. During the next week of continued photoinduction, however, remarkable morphological differences occurred.

By the 35th short day, initiation of all florets, papillose trichomes, corollas, stamens and carpels was completed in terminal capitula. Procambium had differentiated into all florets of terminal heads. In many terminal heads, ovules without megasporocytes were found in florets of the oldest four to six cycles. Rapid sympetalous corolla growth, evident in the peripheral three to seven cycles of florets, frequently resulted in corolla curvature against the tightly imbricate involucre. Within the oldest four or five cycles of florets, there was rapid carpel and corolla elongation and slow stamen enlargement. In the clearly identifiable incipient disc florets, however, individual corollas had petal tips which overarched the androecium and the relatively blunt, short pistil primordium. Primordial disc florets had clavate stamen primordia which were about fourteen cells long and six to eight cells wide at their midsection. In the oldest primordial ray florets, by
contrast, the columnar stamen primordia commonly were about eight cells long and four cells wide.

Epigyny, evident in only the oldest ray florets, was marked by an obvious constriction at the junction between a primordial ovulary and its respective corolla. Although stamens of some disc florets appeared to have sporogenous tissue, meiosis had not occurred in any ovules or anthers observed at the 35th short day.

Continued enlargement of receptacles contributed to their mean widths of 4734 micra and heights of 3133 micra by the 42nd short day. In contrast with mean height/width ratios of the 14th through the 28th short days (0.48 to 0.51), the mean ratio was 0.66 on the 42nd inductive day.

In median longitudinal section, the ovate-conical receptacles were clearly vascularized and had numerous, schizogenously derived, cavities (Fig. 10). At the periphery of receptacles many canals, similar to those of 

_Helianthus annuus_, were evident in the receptacle.

By the 42nd short day, all florets had become epigynous. Although ovule initiation was completed in ray and disc florets, continued development through meiotic prophase I had occurred in only a few of the anatropous ovules of some older ray florets. In many ray floret primordia stigmatic surfaces stained intensely, reminiscent of secretory structure differentiation. Seemingly arrested androecium development characterized all rays (Fig. 10). Stamen primordia were identifiable in all rays, however. Microsporocytes were present in disc florets.

Whereas corollas of the disc florets remained tightly closed, the dorsi-ventral ray floret corollas were open (Fig. 10). By the 42nd short day, even
Figure 10. Median longitudinal section of a terminal primordial inflorescence after 42 short days. X19.
the youngest incipient disc florets elongated more rapidly than adjacent, and older, ray florets. In many capitula, the massive growth of receptacle, rays, and discs contributed to the tearing, crushing, and separation of the enveloping, thin involucral bracts.

At approximately 44 short days, extremely rapid floret elongation commenced on typical 'Criterion' terminal capitula. This onset was marked by floret emergence by tearing and protruding through the drying, thin involucral bracts. Upon emergence, living ray florets were extremely pale, and faintly greenish-pink. At the same time, however, disc florets were deep green, again indicating that pronounced physiological differentiation preceded and accompanied morphological differentiation of florets into either rays or discs.

At about the 46th short day, accelerated elongation of peripheral ray florets frequently obscured the small, centrally located group of disc florets. Subsequent ray floret elongation was accompanied by unfurling of the laterally rolled and folded, open-ended ray florets. After 65 to 70 short days, the oldest ray florets were pink-pigmented. Although unfolding of the bilabiate stigmas soon ensued in the oldest peripheral ray florets, anthesis and stigmatic maturation in disc florets did not commence until several days later. Whereas intensification of anthocyanins was the pronounced pigmentation change that occurred in ray florets between the 46 and 70th short days, chlorophyll disappearance and simultaneous yellowing occurred during the pre-anthesis maturation of disc florets.

At maturity, typical ligulate (ray) florets were bilaterally symmetrical with entire corolla tips. Uniform elongation around the entire corolla
periphery only occurred in extremely young ray primordia. Subsequently, sympetalous corolla elongation ceased at a point near the base of the corolla. That point, closest to the receptacle summit, eventually became the open side of the dorsiventral mature ray. At maturity, each elongated ray reflexed away from the compound receptacle summit, at which time the divergent stigma lobes could be macroscopically observed without dissection. Dorsiventrality of ray florets may have resulted from a marked growth inhibition, or failure of promoted elongation, at the one basal corolla point on the epigynous ovulary. No rudimentary stamens were macroscopically visible in ray florets.

By contrast, all typical disc florets were actinomorphic with four or five distinct short petal lobes at the summit of the otherwise tubular disc corolla. At anthesis, anthers, stigmas, and the uppermost portion of the style extended above the disc floret corolla. In contrast with ray florets, there was uniform circumferential corolla tube growth in disc florets, all of which were located at the summit of the ovate-conical receptacle. Typically, the disc region was approximately four to six florets in diameter (Fig. 10).

Occasionally, in control plant terminal heads, individual corolla-less florets were found among otherwise typical ligulate florets. These simple 'florets' consisted only of stigma, style, and ovulary.

During the development of disc and ray florets, continued stamen growth and maturation were associated with limited growth of a tubular corolla. Arrested stamen growth was characteristically correlated with the extensive elongation of the open ligulate corolla of the male sterile ray florets.
In the fully developed 'Criterion' terminal inflorescence, absolute numbers of disc and ray florets and disc/ray ratios appeared to vary greatly with cultural conditions. Although only one typical 'Criterion' terminal head, from the Brookhaven National Laboratory greenhouse (brightly illuminated, full sunlight April to June), was dissected, it had 243 ray florets and 93 centrally located disc florets. It had a disc/ray ratio of 0.38. In contrast, typical 'Criterion' terminal heads, from growth chamber experiments (fluorescent plus incandescent light, 1500 to 1900 foot candles) had different values. Of four terminal capitula dissected, the following mean numbers of parts were found: 37.5 involucral bracts (range = 35 to 39); 350 ray florets (range = 342 to 365) and 17 disc florets (range = 13 to 21). The mean disc/ray ratio was 0.05.

By gross inspection, similar variations have been observed for many chrysanthemum clones cultured in greenhouses throughout the year at prevailing natural light intensities and only partially controlled temperatures. William Skou (Yoder Brothers, Inc., Barberton, Ohio, personal communication) noted that "any environmental factor that will delay the development of a flower head produces a greater proportion of developed ray florets to disc florets". Moreover, he noted that 'Yellow Delaware' is "open-centered" when flowering in winter and "fully petalled" and commercially desirable when flowering during summer months. Furthermore, Skou added, "'Delight' is a good example of a duplex daisy that becomes semi-double in the summer flowering period and almost a true single if flowered under short days and low light of winter".

As an additional consequence of floral induction and subsequent
Initiation, extreme variations in plastochron were evident. As a comparison base, plastochron duration was ascertained by comparing total leaf and leaf primordium numbers for five 'Criterion' rooted cuttings, at potting time, and five 'Criterion' plants which were cultured under long photoperiods for 14 days. Culture conditions were previously described under ancillary experiment methods. At potting time, five rooted cuttings had a mean of 17 (range = 15 - 19) leaf and leaf primordia. After 14 non-inductive days, the five other established plants had a mean of 23.6 (range = 22 to 25) leaves and leaf primordia. Although the apparent mean foliage leaf plastochron of 2.1 days appeared short for a dicotyledon, plants of Helianthus tuberosus have been reported to have a 2.0 day foliage leaf plastochron (Popham, 1966, p 156-157).

Involucral bract initiation was complete on the 21st short day. If it is assumed that involucral bract initiation commenced on the sixth short day in 'Criterion', and if the mean value of 37.5 involucral bracts is considered typical, these would indicate a 0.4 day plastochron for involucral bracts. Similarly, mean plastochrons were calculated for floret primordia. Initiation of floret primordia commenced no earlier than the 14th short day and was completed on all terminal heads by the 35th short day. If the observed means of 350 rays and 17 discs were typical, then mean floret initiation plastochron would be 0.057 day, or about 82.4 minutes.

In 'Criterion' inflorescences, involucral bracts and florets formed in a complex, strictly acropetal, spiral sequence. Once floret initiation commenced, it appeared that progressively fewer florets formed in each successive cycle. Florets were alternately arranged in adjacent cycles.
Typical terminal capitula had 37.5 (mean), alternately arranged, involucral bracts in spiral sequence. Commonly involucral bracts formed in eight to ten parastichies. At the periphery of the receptacle a minimum of 22 ray floret parastichies were observed among numerous capitula. There may have been 30 to 45 parastichies present. Moreover, among equally numerous heads, a minimum of 22 contact parastichies was noted. Although involucral bracts appeared to be initiated individually, simultaneous floret initiation around the capitulum was common, if not universal, in any one cycle of florets.

If mean floret plastochron were recalculated on the basis of contact parastichies, independent of total numbers of florets, then the mean floret cycle plastochron would be 0.95 day, or 22.8 hours. Thus, regardless of methods of calculation, extreme shortening of the plastochron accompanied terminal inflorescence morphogenesis in 'Criterion'. Moreover, phyllotactic changes were additional consequences of inflorescence ontogeny. Whereas a 3/8 phyllotaxis was common among vegetative stems, the characteristic development of 22 or more parastichies always occurred during floret initiation.

In general, on any one short day, inter-plant variations appeared to be greater than the close synchrony in 'Bittersweet' chrysanthemums (Popham, 1963). For the irradiation experiments, recently potted 'Criterion' plants grew under non-inductive photoperiods for five days. Popham (1963), however, cultured 'Bittersweet' under long day conditions for about five weeks before he began his photoperiodism experiments. Because 'Criterion' plants may not have been well 'established' before irradiation experiments and photo-induction commenced, it tentatively was concluded that all, or most of the
inter-plant ontogenetic variation was attributable to variations in degree of 'establishment' of newly potted cuttings. To be sure, the financial successes of closely timed, commercial culturing practices attests to the remarkable photo-controllability of chrysanthemum inflorescence ontogeny. It is extremely clear that easily overlooked, and often ignored, small ontogenetic variations among plants subsequently can be magnified immensely during rapid growth and differentiation in a complex, dynamic system. Such phenomena may have been responsible for those variations noted among apices harvested at 14, 21, 28, and 35 short days.

B. Effects of Ionizing Irradiation on Gross Morphology

Irradiation at various stages in inflorescence ontogeny induced numerous variations from the usual development of 'Criterion' leaves, stems, capitula, and florets. Whereas leaf and stem morphogenesis is not emphasized in this investigation, these organs were affected by irradiation treatments, even though leaf initiation apparently ceased with the onset of photoinduction.

In early developmental stages acute gamma irradiation prevented lamina enlargement and appeared to enhance petiole elongation (Fig. 11) in the uppermost appendages of irradiated stems. Typical radiation-damaged foliage leaves had smaller laminae, longer petioles, extensive laminal mottling and irregular thickening, open sinuses and longer, acute lobe tips. In irradiated plants, reflexing of laminae appeared to be less extensive than that of control plant leaves which commonly had curved lobe tips and closed sinuses. Blade-petiole length/width ratios were not calculated.

On the main axis of irradiated and control plants, bracts subtending the
Figure 11. Radiation damaged leaves (right) in comparison with a control leaf from a comparable internode.
involucre were unlobed with entire margins and had progressively smaller laminae with increasing proximity to the terminal capitulum. Radiation-induced damage to these developing foliage bracts, however, was discernable by irregular thickening and mottling of foliage bract laminae.

As photoinduction and subsequent inflorescence development continued, progressively fewer foliage leaves and bracts were susceptible to damage by gamma-rays. When only those foliage leaves and bracts over 2 cm long were considered, no macroscopic damage could be observed in plants which were irradiated immediately prior to their 35th short day, or later (Fig. 12).

On their main axes, irradiated and control plants had essentially the same total number of foliage leaves and bracts between the terminal capitulum and the soil surface. Among all control (c) plant blocks, mean leaf and foliage bract number per plant varied from 15.97 to 16.71, while comparable means ranged from 15.74 to 16.82 among all irradiation (i) plant blocks. From immediately prior to the 1st inductive night, through the 7th, 14th, 21st, 28th, and 35th inductive nights respectively, means of 13.6, 11.4, 10.3, 6.7, 1.7, and 0.0 foliage leaves had observable radiation-induced damage.

Among treatment times, stem sensitivity to gamma-ray modification exhibited a distinctly different pattern from that of foliage leaf and foliage bract radiosensitivity. Whereas all control and irradiated plants had essentially the same number of nodes on the main axis, maximum stem length from the soil surface to the involucre varied greatly among treatments (Fig. 13). Analysis of variance and covariance (Appendix) revealed highly significant (F test, p = 0.005) stem length variations between control and irradiated plants for the following parameters: method and time of treatment,
Figure 12. Mean percentage of gamma-ray-damaged foliage leaves and bracts, over 2 cm long, in relation to time of irradiation treatment.
Radiation damaged leaves per plant as % of controls vs. number of inductive photoperiods completed.

The graph shows a linear relationship with the equation:

\[ Y = 87.8414 - 2.4963X \]
Figure 13. Relationship of irradiation time to mean stem length on the 57th short day.
MEAN STEM LENGTH IN CM

NUMBER OF INDUCTIVE PHOTOPERIODS COMPLETED

IRRADIATED •
CONTROL △
including time, time linear, and time cubic. Equally significant interactions were found between method and time, including time linear, time cubic, and time quartic. Because there were highly significant (F test, p < 0.005) residual variances attributable to time, and time-method interactions, it is clear that other unknown factors also contributed to stem length variations between control and irradiated plants.

Internode elongation was most inhibited (36%) by irradiation immediately after 14 inductive photoperiods were completed (Fig. 14). Whereas it is possible that internode elongation was slightly (1.0%) enhanced by irradiation during the 41st to 42nd short day, there is little evidence that internode elongation was affected greatly by irradiation after the 35th short day.

Gamma-radiation, at weekly intervals of inflorescence ontogeny, subsequently induced numerous variations from typical (control) 'Criterion' terminal inflorescences (Figs. 15, 16, 17, 18). Modified morphogenesis resulted in variations of: (1) symmetry, distribution, and numbers of ray and disc florets, (2) degree of fasciation, (3) terminal head maximum diameter, (4) number of axillary flowering capitula on peduncles over one centimeter in length, (5) number and sizes of differently colored, presumably 'mutant', sectors, involving more than one ray floret, (6) 'mutant' color sector length and distribution within individual ray florets, and (7) degree of incision, or lobing, of individual corolla tips in ray florets.

In each of the seven criteria, maximum variation from controls was achieved by irradiation of apices and inflorescences only in certain ontogenetic stages. These optimum irradiation times appeared to be specific, and often different, for each variation type considered. Thus, with respect to inflorescence variations, the differing optimum induction times could be
Figure 14. Mean inhibition (-) or promotion (+) of stem elongation, expressed as percentage of controls, induced by irradiation in relation to number of inductive photoperiods completed before treatment.
INHIBITION (-) OR PROMOTION (+) OF STEM ELONGATION AS % OF CONTROLS

PROMOTION | INHIBITION

NUMBER OF INDUCTIVE PHOTOPERIODS COMPLETED

0 \( \rightarrow \) 49
Figure 15. Typical mature terminal inflorescences of control (c) plants in comparison with representative mature terminal capitula from plants irradiated after 0 short days (1) or 7 short days (2).
Figure 16. Typical mature terminal inflorescences of control (c) plants in comparison with representative mature terminal capitula from plants irradiated after 14 short days (3) or 21 short days (4).
Figure 17. Typical mature terminal inflorescences of control (c) plants in comparison with representative mature terminal capitula from plants irradiated after 28 short days (5) or 35 short days (6).
Figure 18. Typical mature terminal inflorescences of control (c) plants in comparison with representative mature terminal capitula from plants irradiated after 42 short days (7) or 49 short days (8).
considered variations in shoot and inflorescence apex differential radiosensitivity.

For evaluation of terminal head symmetry, distribution and frequency of ray and disc florets, four score values were defined: score zero, heads with a small, centrally located circular disc floret region which had no ray florets (Fig. 19); score one, heads with a small, centrally located, disc region in which a few ray florets were located centripetal to the outermost disc florets (Fig. 20); and score two, heads with a large-diametered disc region with numerous disc florets and a minority of seemingly randomly distributed ray florets (Fig. 21). Score three was used to designate heads in which: (a) outermost disc florets formed the periphery of a large-diametered hypothetical circle, (b) a second series of ray florets formed centripetal to the outer disc region, and (c) a small, centrally located disc region frequently was obscured by innermost ray florets (Fig. 22).

In flowers, "sex reversal" results when certain patterns of stamen and pistil development are modified from the usual. Modifications of ray-disc floret numbers and positions were induced by irradiation before 28 short photoperiods were completed (Table 2). Although a score value of zero usually characterized control plants, up to 23.5% of unirradiated plants had the slight head variations defined for score one. By gross inspection alone, heads of score two had the highest absolute number of disc florets, all of which appeared to be typical.

In the development of typical 'Criterion' terminal capitula (score zero, Table 2) there appears to have been a long period of physiological conditions conducive to the differentiation of the numerous ray florets. Subsequently, there appears to have been a short period of physiological conditions
Figure 19. Terminal head, typical of controls, with a ray-disc floret distribution and symmetry score of zero.
Figure 20. Terminal inflorescence with a ray-disc floret distribution and symmetry score of one.
Figure 21. Terminal head with a ray-disc floret distribution and symmetry score of two.
Figure 22. Terminal inflorescence with a ray-disc floret distribution and symmetry score of three.
Table 2. Distribution patterns of ray and disc florets in irradiated (i) and control (c) plant heads, expressed as percentage of plants with each score value. Plants evaluated after 70 photoinductive nights.

<table>
<thead>
<tr>
<th>Plant Block No.</th>
<th>Number of Inductive Photoperiods Completed at Irradiation Time</th>
<th>PERCENTAGE OF PLANTS WITH TERMINAL HEAD SCORE VALUE</th>
<th></th>
<th></th>
<th></th>
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<td>1 (Fig. 20)</td>
<td>2 (Fig. 21)</td>
<td>3 (Fig. 22)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I c</td>
<td>0</td>
<td>76.5</td>
<td>23.5</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I i</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>II c</td>
<td>7</td>
<td>91.2</td>
<td>8.8</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II i</td>
<td>7</td>
<td>0.0</td>
<td>0.0</td>
<td>2.9</td>
<td>97.1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III c</td>
<td>14</td>
<td>88.2</td>
<td>11.8</td>
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<td>0.0</td>
<td>100</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>III i</td>
<td>14</td>
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<td>IV c</td>
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<td>76.5</td>
<td>23.5</td>
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<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV i</td>
<td>21</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V c</td>
<td>28</td>
<td>85.3</td>
<td>14.7</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V i</td>
<td>28</td>
<td>91.2</td>
<td>8.8</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI c</td>
<td>35</td>
<td>91.2</td>
<td>8.8</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI i</td>
<td>35</td>
<td>85.3</td>
<td>14.7</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII c</td>
<td>42</td>
<td>85.3</td>
<td>14.7</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII i</td>
<td>42</td>
<td>91.2</td>
<td>8.8</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII c</td>
<td>49</td>
<td>94.1</td>
<td>5.9</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII i</td>
<td>49</td>
<td>85.3</td>
<td>14.7</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
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<td></td>
</tr>
</tbody>
</table>

a Three terminal heads (8.8%) in this block had no macroscopically visible disc floret region above their small involucres.
conducive to the differentiation of youngest floret primordia into disc florets. Thus, hypothetically, a small, centrally located disc floret region resulted in the formation of typical 'Criterion' inflorescences.

Inflorescence apices at the 21st to the 22nd short days were at critical morphogenetic stages. At that time, primordial inflorescences were most susceptible to extreme physiological diversion from the usual sequences of corolla and stamen ontogeny which underlie ray and disc floret differentiation. A terminal head with a score of two (Table 2) appears to have had a short period of physiological conditions necessary to ray floret differentiation. Then, hypothetically, there was a relatively long period of physiological conditions conducive to the differentiation of an unusually large number of disc florets.

Irradiations at zero through 14 short days also modified the usual sequences of ray and disc floret differentiation (Table 2). Conceivably, the unusual alternate arrangement of ray and disc floret regions (Fig. 22; Score 3, Table 2) may have resulted from alternating periods of conditions promotive to ray and disc floret differentiation. This, hypothetically, resulted in a large percentage of heads with score three values observed at zero, seven, and 14 short days. Among all irradiated and control plants, no typical ray florets were observed with mature, pollen-bearing anthers.

Morphogenetic modifications innate to the assignment of score values two or three to terminal capitula would seem to justify the application of the term "sex reversal". That is, irradiation induced the differentiation of perfect (disc) florets in the usual positions of the strictly pistillate (ray) florets. Apparently, those physiological conditions which underlie ray and
disc floret differentiation are not modified by irradiation on the 28th short day, or later (Table 2).

Fasciation of terminal inflorescences was common among plants irradiated prior to their 21st inductive photoperiod. Degree of fasciation was evaluated with a zero through seven scoring system. Perfect, radially symmetrical heads (Fig. 23) were assigned score zero. Inflorescences with slightly ovoid centers (Fig. 24) were evaluated one and ovoid terminal capitula which were partially bifurcated by an included secondary inflorescence, complete with subtending involucre (Fig. 25), were assigned a two score value. Additional scoring values were defined as follows: three, terminal heads partially subdivided into three closely appressed secondary heads, each with a partial involucre, but all subtended by a common involucre and peduncle (Fig. 26); four, a terminal 'head' consisted of two closely appressed, but nearly distinct heads, each with a complete involucre but both attached to a common peduncle (Fig. 27); five, terminal inflorescences were trifurcated into three tightly appressed involucrate heads on a common peduncle (Fig. 28); and six, the terminal 'inflorescence' seemingly was comprised of two, distinctly involucrate, heads on a common peduncle (Fig. 29). Occasionally a small spot, appearing necrotic, was found at the summit of the common peduncle. Score seven was applied to those 'single' terminal inflorescences which appeared to be comprised of three involucrate heads formed on a common peduncle (Fig. 30).

Fasciation score types six and seven were found only among plants which were irradiated immediately after their 14th inductive photoperiod. Together they comprised 32.3% of the evaluated population (Table 3). Among
Figure 23. Terminal inflorescence with a fasciation score of zero.
Figure 24. Terminal inflorescence with a fasciation score of one.
Figure 25. Terminal inflorescence with a fasciation score of two.
Figure 26. Terminal inflorescence with a fasciation score of three.
Figure 27. Terminal inflorescence with a fasciation score of four.
Figure 28. Terminal inflorescence with a fasciation score of five.
Figure 29. Terminal inflorescence with a fasciation score of six.
Figure 30. Terminal inflorescence with a fasciation score of seven.
Table 3. Distribution patterns of terminal head fasciation types among control (c) and irradiated (i) plant blocks expressed as percentage of plants with each score value. Plants evaluated after 70 photoinductive nights.

<table>
<thead>
<tr>
<th>Block No.</th>
<th>Number of inductive Photo-periods Completed at Irradiation Time</th>
<th>PERCENTAGE OF PLANTS WITH TERMINAL HEAD SCORE VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Fig. 23)</td>
<td>1 (Fig. 24)</td>
</tr>
<tr>
<td>I c</td>
<td>0</td>
<td>94.1</td>
</tr>
<tr>
<td>I i</td>
<td>0</td>
<td>52.9</td>
</tr>
<tr>
<td>II c</td>
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<td>49</td>
<td>94.1</td>
</tr>
<tr>
<td>VIII i</td>
<td>49</td>
<td>94.1</td>
</tr>
</tbody>
</table>

a Three terminal heads (8.8%) in this block had no macroscopically visible florets above their respective small involucres.
terminal heads which were assigned score values of six and seven, one might question whether true bifurcation or trifurcation of an inflorescence apex actually occurred. Among IIIi apices studied histologically, no ontogenetic stages were found to support the conjecture that heads scored six and seven were actually products of fasciation.

During histological sampling, it was not obvious how the terminal heads of score types six and seven originated. Thus, stem tip harvests of advanced stages might have been insufficient to demonstrate the true origin of these alleged fasciation types. It is equally possible that both of these controversial types are, in reality, typical single terminal heads which are closely appressed to one or two axillary inflorescences. Typically, in chrysanthemum, the uppermost one to three foliage bracts have no lateral inflorescences. To be sure, the remote attachment of the uppermost foliage bracts in Figures 29 and 30 is of no aid in evaluating the validity of score values six and seven. The validity of fasciation scores zero through five is histologically verifiable, as will be noted later.

Although radial symmetry (score zero) characterized 88.2 to 100% of all non-irradiated control plants, slightly ovoid (score one) terminal capitula sometimes can develop without ionizing irradiation (Table 3). Fasciation types two through five were gamma-ray induced. In each of the terminal inflorescences which were assigned scores of two through five, unusually located bracts were observable (Figs. 25, 26, 27, 28). In each of these four types, additional involucral bracts were initiated centripetal to the oldest incipient ray florets.

Whereas plants were most susceptible to irradiation-induced "sex
reversal" on the 21st to 22nd short day (Table 2), the most extreme
fasciation (Table 3) and the greatest inhibition of stem elongation (Fig. 14)
occurred as a result of irradiation on the 14th to 15th day of photoinduction.
By the 21st short day, terminal inflorescence primordia had passed
morphogenetically critical stages and fasciation no longer could be gamma-
ray induced.

Terminal head maximum diameter, ray tip to ray tip, was measured
to the nearest one-half centimeter on the 72nd short day. At this time, mean
terminal head diameter varied considerably among control and irradiated
plants (Table 4). These data provided a partially quantitative assessment of
facts evident in Figs. 15, 16, 17, 18. In many cases, intra-inflorescence
irregularities were such that many irradiated plants had ragged-appearing
terminal capitula. Gamma-rays modified ray floret growth through the 49th
short day (Table 4) at which time treatments ceased.

Gamma-ray-induced enhancement of terminal head growth, apparent in
the treatments at zero through seven short days, may be attributable to any
or all of several factors. Plants of Ii and III (Table 4) appeared to mature to
showy inflorescence stages before any other plants, including respective
controls. This may be observed in the degree of maturity of capitula centers
(Fig. 15). Furthermore, casual observations suggested that terminal
capitula of the Ii and III plants senesced before those of any other plants.

In chrysanthemum, manual removal of very young lateral inflorescences
("disbudding") results in marked enlargement of terminal heads (Kiplinger,
1954, p 18). Furthermore, Kiplinger (p 66) stated that early disbudding of
chrysanthemum shoots "leads to their rapid development" and earlier
Table 4. Mean terminal head diameters and ranges among control and irradiated plants, with mean enhancement (+) or inhibition (-) of growth resulting from irradiation.

<table>
<thead>
<tr>
<th>Treatment Block No.</th>
<th>No. of Short Photoperiods Completed at Irradiation Time</th>
<th>Mean Terminal Head Diameter in Cm</th>
<th>Range of Terminal Head Diameters, in Cm</th>
<th>(i-c) Diameters Expressed as Irradiation Enhancement (+) or Inhibition (-) of Terminal Head Growth, in Cm</th>
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</thead>
<tbody>
<tr>
<td>I c</td>
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<td>7.7</td>
<td>7.5-8.0</td>
<td>+0.6</td>
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<tr>
<td>I i</td>
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<td>8.3</td>
<td>7.5-9.0</td>
<td>+0.4</td>
</tr>
<tr>
<td>II c</td>
<td>7</td>
<td>7.7</td>
<td>6.5-8.5</td>
<td>-1.4</td>
</tr>
<tr>
<td>II i</td>
<td>7</td>
<td>8.1</td>
<td>6.0-9.0</td>
<td>-1.2</td>
</tr>
<tr>
<td>III c&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
<td>7.6</td>
<td>0.5-8.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>III i</td>
<td>14</td>
<td>6.2</td>
<td>7.0-8.0</td>
<td>-0.9</td>
</tr>
<tr>
<td>IV c</td>
<td>21</td>
<td>7.7</td>
<td>6.0-8.0</td>
<td>-0.9</td>
</tr>
<tr>
<td>IV i</td>
<td>21</td>
<td>6.5</td>
<td>6.0-7.0</td>
<td>-0.9</td>
</tr>
<tr>
<td>V c</td>
<td>28</td>
<td>7.6</td>
<td>7.0-8.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>V i</td>
<td>28</td>
<td>7.5</td>
<td>6.0-8.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>VI c</td>
<td>35</td>
<td>7.5</td>
<td>6.0-7.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>VI i</td>
<td>35</td>
<td>6.6</td>
<td>6.0-7.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>VII c</td>
<td>42</td>
<td>7.6</td>
<td>6.5-8.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>VII i</td>
<td>42</td>
<td>6.7</td>
<td>6.0-7.0</td>
<td>-0.9</td>
</tr>
<tr>
<td>VIII c</td>
<td>49</td>
<td>7.5</td>
<td>7.0-8.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>VIII i</td>
<td>49</td>
<td>6.8</td>
<td>5.0-7.5</td>
<td>-0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data include three heads with sparse ray florets.
senescence of flowers than occurs in comparable shoots which have not been disbudded.

Larger than usual terminal inflorescences (Il and Iii, Table 4) might have resulted from an irradiation-promoted "disbudding" effect. Alternatively, the promoted enlargement may have been a case of gamma-ray-induced growth stimulation by other mechanisms. In any event, the real cause or causes, may be ascertained by additional investigations to relate critical effects of irradiation and manual disbudding upon earliness in all modifiable stages of floret growth.

One may infer that factors which underlie ligulate (ray) floret growth are complex from statistical analysis of terminal head diameter data shown graphically in Figure 31. No single critical stage of pronounced radiosensitivity was detected.

One may infer that highly significant (F test, p < 0.005) variations in terminal head diameter are attributable to method (control vs. irradiation) and time of treatment, including time linear, quadratic, cubic, and quartic, from factorial design analysis of variance and covariance (Appendix). No equally significant variations were attributable to method-time interactions. Because there are also highly significant time, residual and method-time, residual, interactions, other unknown factors undoubtedly played decisive roles in determining terminal head diameters.

On the 71st short day, all terminal inflorescences were evaluated for size and distribution of variant color segments. Terminal head variant color sectors or spots, interpreted here as 'mutants', were common among plants of all irradiation treatment blocks. For heads in which an individual sector was wider than one ray floret (a multiple floret sector) (Fig. 32), sizes were
Figure 31. Relationships between mean terminal head diameter and time of irradiation (number of short days completed before treatment).
MEAN TERMINAL HEAD DIAMETER IN CM

NUMBER OF INDUCTIVE PHOTOPERIODS COMPLETED

IRRADIATED

CONTROLS

IRRADIATED CONTROLS

0 7 14 21 28 35 42 49
Figure 32. Lateral inflorescence with a 155 to 160 degree multiple floret white 'mutant' sector.
measured by five degree increments with a transparent protractor.

Presumably, each color sector was derived from a single cell which 'mutated' during treatment.

Because no 'mutant' spots, streaks, or multiple floret sectors were found in any terminal or lateral heads of control plants, qualitative and quantitative data of variant color sectoring did not obviate statistical analysis.

In mature 'Criterion' inflorescences, the imbricate nature of ray florets (Fig. 19) presented problems with respect to ascertaining the exact centrifugal extent of 'mutant' sectors. The older ray florets not only senesced before the inner, younger ray florets, but also the older ray florets were partially shaded by overlapping younger rays. Commonly, anthocyanin development has been reported to be directly associated with light intensity (Miller, et al., 1967). Therefore, 'mutant', multiple floret, sector sizes (Table 5) are better indications of minimum arcs of 'mutant' cell distribution than indications of maximum acropetal-basipetal 'mutant' cell distributions on capitula.

Terminal head multiple floret 'mutant' sectors were found only in those plants which were irradiated prior to their 28th short day (Table 5). Means of 67.6%, 47.0%, 38.2%, and 88.2% of plants irradiated prior to the 1st, 8th, 15th, or 22nd short day, respectively, had no multiple floret terminal head sectors. Thus, the number of plants which had 'mutant' terminal capitum sectors increased from zero through 14 short days (32.4%, 52.9%, and 61.7%, respectively), then decreased to 11.8% on the 21st short day treatment.

Bronze and white were the only terminal head 'mutant' colors observed (Table 5), with bronze 'mutants' generally occurring four times more frequently than white sectors (i.e. 60 bronze: 15 white). According to
Table 5. Distribution of 'mutant' color sectors involving more than one ray floret number of inductive photoperiods completed before treatment.

<table>
<thead>
<tr>
<th>Treatment Block</th>
<th>No. of Short Days Completed at Irradiation Time</th>
<th>No. of 'Non-mutant' Typical Heads</th>
<th>DISTRIBUTION OF 'MUTANT' Color</th>
<th>0-5°</th>
<th>5-10°</th>
<th>10-15°</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0</td>
<td>23</td>
<td>Bronze</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>16</td>
<td>Bronze</td>
<td>8</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>IIIi</td>
<td>14</td>
<td>13</td>
<td>Bronze</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>IVi</td>
<td>21</td>
<td>30</td>
<td>Bronze</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VI-VIIIi</td>
<td>28-49</td>
<td>34</td>
<td>Bronze</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>
than one ray floret, among terminal inflorescences of irradiated plants in relation to treatment.

<table>
<thead>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
William Duffett (personal communication) bronze in chrysanthemums results when reddish pigment(s) is (are) present in cells which also have yellow pigment(s).

Microscopic examination of whole mount, living peals from petals revealed that chrysanthemum ray floret pigments were present only in epidermal cells, a usual phenomenon among angiosperm flowers (Pereau-Leroy, 1969).

The post-irradiation occurrence of 'mutant' color sectors probably was indicative of only a small percentage of the actual number of irradiation-induced genotype changes and other chromosome aberrations. Undoubtedly, many inflorescence parts, including those heads with 'mutant' color sectors, probably were crypto-chimeras in which methods of observation did not reveal other genotype changes.

In spite of limitations in methodology, one may infer that terminal inflorescence development had progressed so far by the 28th short day that thereafter no one cell could contribute to the ontogeny of more than one ray floret. This inference is in agreement with histological data presented earlier.

Two or more distinct 'mutant' sectors were found in some individual terminal capitula of plants in blocks II, III, IIIi, and IVi; thus, summations in Table 5 do not equal 34. Separate bronze and white sectors were found in some individual terminal heads, e.g., in plants irradiated after their 7th or 14th short day. Three-sectored terminal heads, in all 'mutant' color combinations, were found only among those plants irradiated after their 14th or 21st photoinductive days.

'Mutant' color streaks or spots, less than one ray floret wide, were common among plants of all irradiation times. Terminal heads of plants in
irradiation blocks from vegetative (zero short days) through the 28th photoinductive day had floret 'mutant' streaks which extended from the tip of the ligule to the base of the corolla tube. Plants which were irradiated after their 35th, 42nd, or 49th short days never had individual ray floret 'mutant' streaks which extended from the tip of the ligule to the base of the corolla. This was not unexpected, however, because all ray florets were initiated and had begun elongating by the 35th short day.

Among all plants irradiated after their 49th short day, dozens to hundreds of short, often uniseriate, 'mutant' streaks were distributed evenly over the entire pigmented adaxial surface of ray florets. The entire adaxial surfaces of ray florets in irradiation blocks VIIi and VIIIi were "salt and pepper" mosaics of linearly oriented rows of 'mutant' and 'non-mutant' cells.

Although typical 'Criterion' ray floret tips usually were entire (Fig. 19), some irradiation treatments resulted in extreme incision of ray tips (Figs. 21, 22, 27; compare Figs. 15, 16, 17, 18). No ray florets were incised among control plants or among plants irradiated after the 42nd short day. At the extreme, ray floret tips were incised into four or five lobes, many of which were over one mm long. By approximation, less than 50% of the ray florets were lobed in terminal capitula of plants irradiated after zero or seven short days. The degree of ray floret incision was highly variable in plants irradiated after their 14th or 21st inductive night; i.e., many entire-tipped rays were distributed among highly incised forms. In plants irradiated after 28 or 35 short days, however, the majority of ligulate florets were two to five lobed. Irradiation of later ontogenetic stages did not result in terminal head ray floret incision. Because of their small size and the fact that 'Criterion' disc florets are usually four- to five-lobed, a detailed examination of them
for 'mutant' sectoring or increased incision was not pursued.

Gamma-ray-induced variations of lateral head numbers provided another criterion for assessment of radiation-caused inhibition or promotion as well as differential radiosensitivity. Irradiation of vegetative plants (zero inductive photoperiods, II), or of plants through their seventh short photoperiod, significantly (t test, \( p < 0.005 \)) decreased the mean number of lateral inflorescences per plant by 2.3 to 2.4 axillary heads (Table 6).

Subsequent irradiation, after 14 or 21 short days, was progressively less effective in inhibiting lateral inflorescence development (Table 6; Fig. 33). Although irradiation of plants preconditioned with 28 or more inductive photoperiods appeared to result in a slight increase in mean numbers of lateral heads, promotive effects were not as great as inhibitory effects elicited by early treatments.

Factorial design analysis of variance and covariance (Appendix) supported the inference that complex interactions of time and method, portrayed in Fig. 33, were valid. Highly significant (F test, \( p < 0.005 \)) variations were induced by method of treatment (irradiation versus controls) as well as by time of treatment, including considerations of time linear, time quadratic, and method-time interactions. There were, however, equally significant residual effects for time of treatment as well as method-time interactions. Cubic and quartic analyses of treatment times, and linear, quadratic, cubic, or quartic analyses of method-time interactions were not significant (Appendix).

The influences of gamma irradiation are of significant importance to lateral head development. Not only was time of irradiation significant, but also, other unknown factors had significant roles. The possibly invalid
Table 6. Mean number, and ranges, of flowering lateral shoots over one cm long in relation to number of photoinductive nights completed before irradiation time.

<table>
<thead>
<tr>
<th>Treatment Block No.</th>
<th>Number of Short Days Completed before Irradiation</th>
<th>Mean No. of Flowering Lateral Shoots over 1 Cm Long</th>
<th>Range</th>
<th>(i-c) Approximate Values of Irradiation Induced Promotion (+) or Inhibition (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I c</td>
<td>0</td>
<td>5.7</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td>I i</td>
<td>0</td>
<td>3.4</td>
<td>3-5</td>
<td>-2.3</td>
</tr>
<tr>
<td>II c</td>
<td>7</td>
<td>6.1</td>
<td>5-8</td>
<td>-2.4</td>
</tr>
<tr>
<td>II i</td>
<td>7</td>
<td>3.7</td>
<td>2-6</td>
<td>-2.4</td>
</tr>
<tr>
<td>III c</td>
<td>14</td>
<td>6.4</td>
<td>5-8</td>
<td>-1.1</td>
</tr>
<tr>
<td>III i</td>
<td>14</td>
<td>5.3</td>
<td>2-9</td>
<td></td>
</tr>
<tr>
<td>IV c</td>
<td>21</td>
<td>7.1</td>
<td>5-8</td>
<td></td>
</tr>
<tr>
<td>IV i</td>
<td>21</td>
<td>6.5</td>
<td>3-9</td>
<td>-0.6</td>
</tr>
<tr>
<td>V c</td>
<td>28</td>
<td>6.8</td>
<td>5-9</td>
<td></td>
</tr>
<tr>
<td>V i</td>
<td>28</td>
<td>7.3</td>
<td>5-11</td>
<td>+0.5</td>
</tr>
<tr>
<td>VI c</td>
<td>35</td>
<td>6.2</td>
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<tr>
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<td>6.5</td>
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<td>+0.3</td>
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<td>VII i</td>
<td>42</td>
<td>6.6</td>
<td>5-8</td>
<td>+0.4</td>
</tr>
<tr>
<td>VIII c</td>
<td>49</td>
<td>6.3</td>
<td>5-8</td>
<td></td>
</tr>
<tr>
<td>VIII i</td>
<td>49</td>
<td>6.5</td>
<td>4-9</td>
<td>+0.2</td>
</tr>
</tbody>
</table>
Figure 33. Relationships between mean number of flowering lateral shoots, over one cm long, and time of irradiation (number of short days completed before treatment).
Number of lateral heads vs. number of short days completed.

- Control (△)
- Irradiated (○)

Graph shows a peak at around 28 days for both control and irradiated conditions.
fasciation scores six and seven, mentioned earlier, might account for some of the highly significant residual effects noted. This possibility is unlikely, however, because mean number of lateral heads on plants irradiated after their 14th short day (IIIi) did not disrupt trends which were evident (Fig. 33). Furthermore, fasciation scoring presented no inconsistencies for the other seven irradiation times.

Maximum inhibition of lateral head development was associated with maximum terminal head diameters (compare Figs. 31, 33). Thus, it appears probable that a disbudding effect of gamma irradiation was a cause of promotion of terminal head growth in diameter.

Although the study of lateral heads is not emphasized in this investigation, lateral heads were scored for number and sizes of multiple floret 'mutant' sectors. Of all plants evaluated in each irradiation block, absolute numbers and maximum sizes of variant color sectors were greater among lateral heads than among terminal inflorescences. Some lateral heads, which were complete (360°) ray floret color 'mutants', were found among individual plants irradiated after 0, 7, 14, or 21 short days. Whether or not the outermost, oldest, ray florets of these lateral heads were 'mutant', was not ascertained.

Lateral head median sizes of 'mutant' color sectors were 50-55°, 120-125°, 80-85°, 15-20°, 5-10°, and 0° for plants irradiated after 0, 7, 14, 21, 28, or 35 short days, respectively. Relative times of susceptibility to multiple floret 'mutant' sectoring differed among terminal and lateral heads. Multiple floret 'mutant' sectors were not induced in terminal heads on the 28th short day; comparable sectoring was not induced in lateral heads on the 35th short day. This apparent ontogenetic delay of one week indicates that
all ray floret primordia were probably initiated, or individually delimited, by the 35th short day.

There were averages of 0.38, 0.76, 0.85, 0.21, and 0.0 multiple floret 'mutant' sectors in terminal heads of plants irradiated after 0, 7, 14, 21, or 28 short days respectively. Means of 0.12, 0.15, 0.36, 0.58, 0.27, and 0.0 'mutant' sectors were found among individual lateral heads of plants irradiated after 0, 7, 14, 21, 28, or 35 short days, respectively. If sector sizes are ignored, individual terminal heads had, on the average, more multiple floret 'mutant' sectors than did individual lateral heads.

Included in the above data for lateral head multiple floret sectors were 20 yellow sectors found among the 816 sectors of all 801 irradiated plants which grew to maturity. No comparable yellow sectors were found among terminal capitula. In addition, one complexly striped lateral head was observed on a plant irradiated after 21 short days. In this variant, in which all 360° were affected, each ray floret usually had a central bronze stripe and pink adjacent stripes which extended full-length in individual ray floret corollas. This one inflorescence was not dissimilar to ones pictured earlier by Offerijns (1926) for Dahlia 'Helvetia' (Compositae) and by Chittenden (1927) for Dahlia 'Union Jack' and Tagetes 'Star of India' (Compositae). Whether the unusual 'Criterion' inflorescence grew from a complex chimeral shoot apex, such as Pereau-Leroy (1969) suggested for the orange and red striped flowers of 'Jacqueline Sim' carnation, was not ascertained.

C. Histological Variations Induced by Gamma-Irradiation

Gamma-irradiation induced numerous microscopically visible effects. In some instances, effects were evident immediately after cessation of irradiation. In others, effects were not observable until 14 to 35 days had
elapsed. Moreover, it is notable that all inflorescence apices survived and grew, no matter at what ontogenetic stage irradiation was given. Because not all plants were at precisely the same ontogenetic stage at any one irradiation time, not all plants were affected uniformly by gamma irradiation. Specific examples will be given as individual treatment times are considered. Unless otherwise noted, overall apex sizes, and height/width ratios were essentially the same as controls.

Plants irradiated at zero short days - Within two hours after cessation of irradiation, after zero short days, marked variations in nuclear volume were evident (compare Figs. 34, 2). When compared with developmentally equivalent controls, mean interphase nuclear volume had decreased in central as well as peripheral sectors of irradiated apices (Fig. 34). In the central-most 90° sector of irradiated apices, mean interphase nuclear volume was only 65% of that of control plants (235 vs 364 cubic micra). Smaller decreases were evident in the peripheral flanks of apices of irradiated plants, in which mean interphase nuclear volume was 83% of control plant values (211 vs 253 cubic micra). Moreover, shrinkage of nuclei continued throughout the first week of photoinduction. After seven short days, irradiated apices had many small nuclei (Fig. 35), especially in the central 90° apex sector. In the central sector, mean interphase nuclear volume was 45% of control values (177 vs 388 cubic micra). In the peripheral portion of the apex, interphase nuclei had, on the average, 61% of the volume of comparable control nuclei (206 vs 388 cubic micra). Irradiated nuclei decreased in mean volume through the first photoinductive week during which time mean volume of interphase nuclei usually increased, especially in the flanks, in control plant apices.
Figure 34. Median longitudinal section of a terminal vegetative shoot apex two hours after cessation of irradiation. Irradiated at zero short days. X550.
Figure 35. Median longitudinal section of an irradiated terminal apex after seven inductive photoperiods. Irradiated at zero short days. X550.
Terminal apices of plants irradiated after zero short days changed neither in height or width nor in numbers of cells in height and width during the week following irradiation. That is, after seven short days, and the elapse of seven to eight days since irradiation treatment, apices averaged 192 micra (19 cells) in width and 57 micra (5 cells) in height. The reader is reminded that, during the same period, dimensions of control apices noticeably decreased in width and height, most probably due to onset of involucral bract initiation. Popham and Chan (1952) found that a detectable increase in mitoses, and growth in width and height, began after six short days in 'Bittersweet' chrysanthemums. If these observations are related, then onset of involucral bract initiation occurred between the sixth and seventh short day in 'Criterion' control plants. Apparently, these events were inhibited by irradiation given one week earlier.

The swelled condition of interphase nuclei in control plants, on the seventh short day, indicated the onset of marked metabolic changes associated with inflorescence induction. If mean apex dimensions were 343 micra (width) and 130 micra (height) on the 14th short day. Thus, irradiation-caused inhibition of mitoses and cell enlargement appeared to decline 14 days after treatment.

During subsequent weeks, with few exceptions, time and sequence of differentiation essentially duplicated events noted earlier for control plants. If apices were increasing in width, height, and cell numbers at a rapid rate on the 14th short day, at which time about 25 to 50% of the involucral bracts were already initiated. All involucral bracts were initiated before 21 short days, in most cases. Rapid, strictly acropetal, floret initiation and early
stages of papillose trichome development were evident in the majority of li apices on the 21st short day.

By the 28 short days, florets covered most of the receptacle. Ray and disc floret differences were obvious by the 42nd short day at which time all floret primordia were present. Rapid corolla elongation was occurring in peripheral florets on the 42nd short day.

During the period from seven through 14 short days after li irradiation, apical summit portions frequently had minutely wavy contours, suggestive of cell shrinkage which might have accompanied the massive decline in inter-phase nuclear volume. As early as the 14th short day, two weeks after irradiation, necrotic lesions (Fig. 36) were noted at the periphery of some apices. Numerous mitotic figures were present immediately adjacent to those wounds. In some cases, apparently living cells were seemingly isolated from the remainder of the plant by a mass of disintegrating cell fragments. Frequency and location of lesions led to the conclusion that irradiation might have killed incipient leaf or involucral bract primordia which were beginning to differentiate at treatment time.

In about 50% of li apices examined at 14 short days, many necrotic-appearing cells were found in mantle layers, as well as in subjacent layers (Fig. 37). Their often small nuclei stained darkly and did not have readily discernable nucleoli. Most commonly, the necrotic-appearing cells were located in the central portion of apices. One week later, those necrotic-appearing cells could not be identified in any primordial capitula. That the necrotic-appearing cells, observable at 14 short days, could not be seen at 21 short days was attributable to three possible phenomena. Necrotic-appearing individual cells could have been "lost" among those rapidly
Figure 36. Tangential longitudinal section through a peripheral gamma-ray induced lesion, 14 days after irradiation. Irradiated after zero short days. Fourteen short days completed. X550.
Figure 37. Median longitudinal section of an incipient terminal inflorescence apex after 14 short days. Irradiated after zero short days. Note necrotic appearing cells in mantle and subjacent area. X220.
mitosing cells of the large receptacle. "Repair" of necrotic-appearing nuclei may have occurred, followed by mitosis of many of the previously damaged cells. A third possibility is that apparently damaged cells were "resorbed" (Soetiarto and Ball, 1969) by digestion of cell parts.

At the 28th short day, when over one half of the florets were initiated, the remaining summit portions of some inflorescence apices stained intensely (Fig. 38) and appeared to be incipiently necrotic. Not only were the nuclei small, intensely red-stained, and with obscure nucleoli, but also, the usual smooth continuity of the five to eight mantle layers was not evident in median sections (Fig. 39). Frequently the apparently damaged cells were more elongated, parallel to the surface, than comparable control plant cells.

In some of the more advanced capitula, also on the 28th short day, super-numerary involucral bracts were present high on the receptacular flanks (Fig. 40). Lesions, consisting of disintegrating cells, were usually found immediately adjacent to the extra involucral bracts (Fig. 41). Cell arrangement and tabular shape of adjacent living cells suggested a cambium-like activity which commonly occurs during wound healing. Moreover, the general wound areas in these irradiated 'Criterion' apices closely resembled wound areas 14 days after microsurgery in Portulaca floral apices (Soetiarto and Ball, 1969, Fig. 9). In addition to lesions on receptacles, necrotic cells were noted in some floret primordia 28 days after irradiation (Fig. 41). Some capitula (Fig. 40), at 28 short days had characteristics which later might have been scored at fasciation values of two, three, four, five, six, or seven (Table 3).

Just as in control heads, ray-disc morphological differentiation was evident in li capitula on the 35th short day. Some florets on the receptacle
Figure 38. Median longitudinal section of a terminal capitulum after 28 short days. Irradiated at zero short days. Note incipiently necrotic summit of capitulum. X35.
Figure 39. Median longitudinal section of the summit portion of an irradiated terminal capitulum after 28 short days. Irradiated at zero short days. Note disrupted continuity of mantle and apparently necrotic cells. X145.
Figure 40. Median longitudinal section of an irradiated terminal capitulum after 28 short days. Irradiated after zero short days. Note supernumerary involucral bracts on receptacle flanks. X35.
Figure 41. Median longitudinal section through a lesion on the flanks of a terminal receptacle after 28 short days. Irradiated after zero short days. Note supernumerary bracts, wound healing and flap of living cells over necrotic area. X145.
flanks were morphologically differentiated as young disc florets. By the 42nd short day, disc and ray florets were readily identifiable in some terminal capitula (Fig. 42): pollen grains were present in anthers of the unusually located peripheral disc florets but terminal disc florets still had microsporocytes. In other li terminal heads at 42 short days, meiosis was occurring in terminal disc floret stamens. Thus, at maturity, the terminal head, portrayed in Figure 42, would have been scored three in ray-disc floret distribution and frequency (Table 2).

Terminal receptacles of li plants averaged 3775 micra in diameter and 1759 micra in height on the 28th short day. All li terminal heads were larger than those of control plants at that time. By the 42nd short day, li receptacles, exclusive of florets, averaged 6167 micra in width and 4101 micra in height, much larger than control plants. li receptacles then had a mean height/width ratio of 0.67, essentially that of controls.

Although data were limited, it is tempting to conclude that precocious maturation of extra disc florets greatly enhanced the growth of li receptacles before the 42nd short day. Simultaneous with the usual differentiation of florets into either discs or rays, mean receptacle height/width ratios changed from approximately 0.51 (28th short day) to 0.66 (42nd short day). Whether or not these events are causally related is, of course, unknown. Conceivably, the precocious development of extra disc florets may have caused the apparent earliness of flowering of li plants.

The fact that li plants had mean receptacle diameters which were 1.4 mm larger than controls, is insufficient to be the direct and only cause of larger terminal inflorescences in li than in controls. Terminal heads of flowering
Figure 42. Median longitudinal section of an irradiated terminal capitulum after 42 short days. Irradiated after zero short days. Note incipient disc florets on receptacular flanks and ray floret primordia near summit. X13.
Ii plants were, on the average, 6.0 mm larger than respective controls. 

Plants irradiated after seven short days - -Immediately after the Iii plants were irradiated following the seventh short day, mean cell number and apex dimensions were essentially identical to those of vegetative plants. Therefore, it is certain that involucral bract initiation began in control plants while Iii plants were being irradiated. Furthermore, it follows that irradiation after the seventh short day delayed first bract initiation and the concomitant usual plastochronic decline of apex dimensions. One week later, however, mean sizes of Iii terminal apices were greater than those of controls. At that time, the 14th short day, the ranges of apex widths were 276 micra to 436 micra in irradiated plants and 172 to 544 micra in control plants. That would appear to confirm that rapid involucral bract formation was occurring in control plants but not in irradiated ones.

Radiation-induced nuclear shrinkage was common in sub-protodermal, central portions of some apices, but not in others on the 14th short day, one week after treatment. If the Iii plants were at differing stages of the plastochron when irradiation treatment began, some of the inter-plant variation might be explained. From the data of Soma (1968), there are intra-plastochronic variations in sensitivity to exogenous auxin applications. Likewise, intra-plastochronic variations in radiosensitivity might exist during chrysanthemum reproductive morphogenesis.

On the 21st short day, two weeks after treatment, wide variations in ontogenetic stage were noted among Iii plants. These variations were essentially as great as noted for Ii plants. Whereas involucral bract initiation continued in a few apices, one to four cycles of florets were initiated in other Iii plants on the 21st short day.
Evidence of differential cell death was especially clear within some IlI apices on the 21st short day. In some cases, mantle discontinuities were apparent at the inflorescence apex summit. Elsewhere, often in the same capitulum, protodermal discontinuities were caused by L-1 and/or L-2 cell death at the summit of newly initiated florets. Post-irradiation cell necrosis often directly coincided with initiation of structures on capitula. That is, certain cells did not begin necrosing until they were intimately involved with bract or floret initiation.

At the periphery of some capitate inflorescence apices, presumptive sites of last bracts, or first florets, were necrotic on the 21st short day. Adjacent to disintegrating cells, there were files of living cells which were radially oriented toward the wound site. Whether the apparent wound healing was due to cambium-like mitosis, or whether cell pattern was due to mere centrifugal elongation of survivors, was not clarified. None the less, those peripheral necrotic areas appeared to have significant effects upon subsequent terminal head symmetry. Localized lesions on receptacular flanks commonly were adjacent to supernumerary bracts. Furthermore, localized cell necrosis resulted in a disruption of usual ray and disc floret distribution (Fig. 43). Thus, differential necrosis of capitulum cells caused fasciation of inflorescences.

In general, IlI heads developed as those of IlI. In both groups, final floret initiation was delayed approximately one week over that of controls, in most of which the last florets were initiated by the 28th short day. In some IlI apices, not all florets were formed over the receptacular summit until the 35th or 42nd short day. Unusually located disc florets, on the flanks of the
Figure 43. Transverse section through an irradiated terminal capitulum after 35 short days. Irradiated after seven short days. (Hollow appearing florets are incipient rays; recently differentiated disc florets have five stamen primordia each). X30.
receptacle, were well differentiated from incipient rays on the 35th short day in II and III capitula.

At advanced stages of development, receptacle dimensions varied so greatly among II and also among III plants that mean values were meaningless. It is tempting to attribute large receptacles sizes, which exceeded control plant values, to radiation-induced growth stimulation. None the less, receptacle dimensions may have varied greatly because of (a) small populations for microscopic examination, (b) inter-plant variation within a block, (c) innate difficulties in ascertaining median sections, and (d) the uncertainty of longitudinal cutting planes in material such as that portrayed in Figure 43.

Plants irradiated after 14 short days - -just as one might anticipate from gross morphology data, irradiation after 14 short days induced the most extensive cell necrosis and greatest variations from the usual 'Criterion' terminal inflorescence form. Cessation of irradiation was followed by differences in apex dimensions, a phenomenon which confirmed that incipient inflorescence apices undoubtedly were in their most rapid growth phase at 14 short days. Following irradiation, apices averaged 192 micra (22 cells) in width and 83 micra (9 cells) in height, compared with control values of 275 micra (25 cells) in width and 133 micra (14 cells) in height. Moreover, respective irradiated cell mean heights and widths were 8.9 micra and 8.7 micra, compared with 9.9 and 10.9 micra in controls. Thus, irradiation after 14 photoinductive nights greatly inhibited mitosis and concomitant cell enlargement. Immediately following irradiation there was no obvious nuclear shrinkage such as that which immediately followed irradiation at zero and seven short days.
Throughout the remainder of the observation periods, including the 42nd short day, apices and/or receptacles of plants irradiated at 14 short days were smaller than those of respective controls or those of any other irradiated plants at the same number of short days.

By seven short days following irradiation, at 21 short days, involucral bract initiation had not ceased in treated plants. Floret initiation had not commenced (Fig. 44) even though abundant mitoses were noted. Development of the involucre was greatly inhibited in treated plants (compare Figs. 6, 7, 44). Irradiated inflorescence apices were small. They averaged 685 micra in width and 319 micra in height. Height and width of receptacles were essentially 60% of those of controls.

Among typical apex cells, there were numerous light-stained cells which frequently were spindle-shaped (Fig. 44). In many apices, there were randomly located necrotic cells in various stages of disintegration. Patterns of cell necrosis can be seen in transverse sections (Fig. 45) but cannot be readily identified in longitudinal sections (Fig. 44). Elongated, light-staining, and frequently spindle-shaped cells formed a general reticulum which encompassed cylindrical to spherical groups of smaller meristematic cells. In some cases, cell orientations suggested cambium-like, isolated growth centers surrounded by the distinctly mitosis-free, elongated cells of the reticulum. This reticulum pattern is reminiscent of early stages of procambium differentiation. Mitosis in the numerous isolated centers contributed to the highly irregular profile of the inflorescence apex above the youngest involucral bracts.

Individual cells or small groups of spindle-shaped cells were disintegrating in the mantle and in the corpus. Cell necrosis had no apparent
Figure 44. Median longitudinal section of an irradiated terminal capitulum after 21 short days. Irradiated after 14 short days. X90.
Figure 45. Transverse section of an inflorescence apex, above youngest bract primordia, after 21 short days. Irradiated after 14 short days. Note reticulum of elongated, light-staining cells.
correlation with apex zonation. Necrosis may have resulted from irradiation- 
killing of cells which were at some critical phase in the mitotic cycle at 
treatment time.

Popham and Chan (1952) speculated that procambium differentiation might 
be related to floret initiation in 'Bittersweet' chrysanthemums. At the time of 
irradiation of Illi plants (14 short days), there were no observable clues that 
procambium differentiation was occurring and no evidence that floral 
initiation was occurring. Within one week, however, the reticulum of spindle-
shaped, light-staining cells appeared (Fig. 45) and still there was no evidence 
of floret initiation.

By the 28th short day, two weeks after irradiation, terminal capitula 
averaged 1581 micra in width and 692 micra in height, essentially 70% of 
control plant values. Terminal capitula frequently were bifurcated (Fig. 46), 
or trifurcated, and were deeply channelled with necrotic cells. Elsewhere, in 
the apparently unaffected portions of terminal capitula, floret initiation 
commenced and usual procambial strands were clearly identifiable. Continued 
inhibition of involucre ontogeny was so extensive that only the oldest few bracts 
overarched the developing terminal capitulum (Fig. 46; compare with Figs. 
8, 9).

A few necrotic lesions were present at the periphery of some capitula on 
the 28th short day. More commonly, deep fissures, comprised of disinte-
grated cells and air spaces, extended from the summit region of the 
original apex into the pith of the receptacle. Although cell necrosis was 
usually limited to the central core of terminal receptacles, necrotic channels 
ocasionally developed in the peripheral third of some receptacles.

In most cases, all mantle layers at the summit of the inflorescence
Figure 46. Median longitudinal section of an irradiated terminal inflorescence after 28 short days. Irradiated after 14 short days. Broken branch on right caused in tissue fixation. X35.
apex died. At the outer limits of the necrotic mantle area, mantle layer continuity was further disrupted by small extrusive outgrowths of second or subjacent mantle layers. No totally reconstituted apices, comparable to those figured by Sagawa and Mehlquist (1957), were observed in any gamma-ray-damaged chrysanthemum apices.

Precocious development of papilllose trichomes appeared to be a common consequence of irradiation treatment after the 14th short day. In many cases, trichomes developed between the youngest two cycles of florets. Moreover, biseriate trichomes, four to five cells long, were present on the receptacle at the 28th short day.

Periclinal divisions of the outermost mantle layer have occasionally been observed in irradiated angiosperm apices (Crockett, 1957, 1968; Haccius and Reichert, 1963). In general, periclinal divisions of the outermost mantle layer are rare in dicotyledons (Popham, 1966, p 90, 208, 209) but appear to occur more frequently in monocotyledons (Popham, 1966, p 90). In irradiated chrysanthemum apices, however, no definitive, comparable periclinal divisions were observed in any irradiated material. It would have been extremely difficult to distinguish between an unusual L-1 periclinal division in an apex and precocious trichome development. Moreover, the marked disruption of mantle continuity, coupled with the occasional death of small groups of L-1 cells and the extrusive growth of subjacent cells through the outer mantle breaks, strongly precluded the certainty of observing L-1 periclinal divisions.

Compared with terminal capitula, the less advanced lateral inflorescence apices were relatively insensitive to the otherwise highly destructive gamma-ray treatment after the 14th short day (Fig. 46). In some cases, lateral
capitula were ontogenetically equal, or more advanced than irradiated terminal capitula (Fig. 46; compare with Figs. 6, 8).

At 35 short days, three weeks after treatment, superficially it appeared that all florets had been initiated in terminal capitula. Carpels had been initiated only in the oldest three or four floret cycles. Papillose trichomes abounded, and occasionally were found in unusual positions, e.g., in the corolla tubes of some florets. Floret symmetry mechanisms were extremely disoriented. There was no consistent pattern of numbers and/or kinds of floret parts such as were observed in control plant capitula. Necrosing lesions were present at the tips of some developing corollas.

By the 42nd short day, four weeks after irradiation, terminal inflorescences were highly variable among plants. In some, the broad receptacle was bifurcated (Fig. 47), or trifurcated with a distinctly necrotic core. In others, receptacles were exceptionally elongated (Fig. 48; compare with Fig. 10). Final floret initiation had just occurred at the summits of some extrusively enlarging growth centers. Supernumerary involucral bracts were common on all capitula.

Although meaningful dimensions of fasciated inflorescence buds were difficult to ascertain, mean receptacle height was 2556 micra and mean width was 3518 micra on the 42nd short day. Essentially, dimensions were approximately 80% of those of controls. Equally difficult was the ascertaining of structures in the highly variable florets, few of which were typical (Fig. 47). Unusual number and distribution of stamens, incompletely developed corollas, and orthotropous ovules were common. Some stamens and ovules had sporocytes, or were in initial phases of meiosis I, generally comparable to stages noted in controls. In the youngest florets, however, stamen
Figure 47. Median longitudinal section of an irradiated terminal inflorescence after 42 short days. Irradiated after 14 short days. Note necrotic lesion at receptacle summit. X19.
Figure 48. Median longitudinal section of an irradiated inflorescence after 42 short days. Irradiated after 14 short days. X19.
initiation was just commencing on the 42nd short day. Occasionally, one to three florets, in a small 'subhead', were enveloped in a single involucre of supernumerary bracts. The bases of those bracts were adjoined to the base of some florets (Fig. 48). Among irradiated plants, no enveloping involucres were ever as extensively developed as those of controls (compare Figs. 10, 47, 48).

Plants irradiated after 21 short days - Irradiation immediately following the 21st short day (IVi), at stages equivalent to those of Figures six and seven, was markedly less destructive than gamma-ray treatment the previous week. At the 21st short day, it will be recalled, all capitula had a complete involucre and a minimum of three cycles of florets. Moreover, irradiation after the 21st short day was simultaneous with rapid involucral bract growth and interdigitation over the capitulum summit.

Immediately following the 21st short day irradiation, there was no clear evidence of nuclear shrinkage or cell necrosis. Capitular dimensions and height/width ratios were fully equal to those of controls. Thus, receptacles were growing less rapidly than during the 14th short day.

One week later, at the 28th short day, there were notable differences between irradiated and control plants. No irradiated inflorescence had completed floret initiation as was the case in most control plants. Only eight to eleven cycles of florets were present, and all irradiated plant capitula were comparable to those of the least advanced control plant terminal inflorescences (Fig. 8). Stamen initiation had begun in the oldest cycle of florets on one capitulum. No carpels were initiated. Irradiated inflorescences averaged 2115 micra in width and 1127 micra in height, or about 90% of control plant
values. Mean height/width ratios, 0.53, were generally the same as controls.

In contrast to plants which were irradiated one week earlier, there was little evidence of induced cell necrosis by irradiation at the 21st short day. One week after treatment, one to four necrosing cells were occasionally observed in the first mantle (L-1) layer of the remainder of the inflorescence apex. No necrotic floret cells were observed.

One week after the IVi irradiation, normal appearing procambium was present in receptacle flanks subjacent to floret primordia. Procambium was present immediately below the youngest florets, approximately 18-20 cells from the floret base. Subjacent to the mantle of the remaining inflorescence apex, however, there was a coarse reticulum of elongated, light-staining cells not unlike those portrayed in Figure 45. If the elongated cells of the reticulum were incipient procambium, there was additional evidence for:

(a) a higher radiosensitivity of chemical reactions which underlie procambium differentiation than processes in adjacent cells, and (b) procambium physiological differentiation preceding floret initiation.

By the 35th short day, floret initiation was completed in many capitula which were irradiated two weeks earlier. In some cases all florets were not initiated until the 42nd short day. On the 42nd short day, when ray florets were distinguishable from incipient disc florets, terminal capitula had large diametered disc floret regions, typical of that portrayed in Figure 21. The most notable effects of irradiation at 21 short days were: (a) delay in final floret initiation, (b) inhibition of receptacle enlargement, and (c) promotion of a higher disc/ray floret ratio.

Plants irradiated after 35 short days - Irradiation of capitula after 35 short days, when all florets were present and differentiated into either rays
or discs on most capitula, had no immediately detectable effect. The following week, however, differential radiosensitivity was evident within and among florets. No cell necrosis or damage was discernible in the oldest one to three cycles of ray florets. In the remainder of younger ray florets, there were frequent patches of disintegrating cells in the tips of corollas and in upper stylar portions. In disc florets, cell disintegration was prominent in corolla lobe tips and in upper stylar portions. Earlier (p 107, 108), the most rapidly growing apices were susceptible to the most extensive cell necrosis. Therefore, the localization of necrotic lesions near corolla tips lead to the inference that ray floret corollas were growing primarily by rapid apical growth between the 35th and the 42nd short days.

Elsewhere, radiation damage was not detectable. Presumably, the rate of apical floret growth had decreased, or ceased, in the oldest one to three ray floret cycles. Prophase I meiosis was occurring in the oldest ray floret ovules on the 42nd short day. All disc floret anthers had microsporocytes in early meiotic prophase. No ovule or anther necrosis was observed. Elsewhere, hundreds of randomly distributed mitotic figures were observed. Receptacle widths and heights were comparable to controls on the 42nd short day.

In review of histological data, two points are worthy of final note. Pronounced nuclear shrinkage was most evident following the irradiation treatments at zero and seven short days. Among those apices, which had low mitotic rates at treatment time, there was no extensive death of cells in subsequent weeks. Moreover, all of those apices survived and only a few small peripheral or apical lesions subsequently resulted. Thus, the precipitous decline in mean interphase nuclear volume was not a reliable,
long-range indication of irradiation-induced damage. When extremely rapid mitosis and concomitant growth in apex height and width were occurring, such as at 14 short days, scores to hundreds of cells died and disintegrated within two weeks after treatment. Thus, apices in early stages of rapid inflorescence growth were the most susceptible to irradiation damage as assayed by cell necrosis, and, therefore, were the most radiosensitive of all stages observed.

Irradiation-induced peripheral or terminal necrotic lesions were subsequently associated with supernumerary involucral bract initiation only when those lesions were caused by gamma-ray treatments at zero, seven or fourteen short days.

D. Microsurgical Replication of Gamma-Ray Induced Variations

Microsurgical dissections and subsequent slits or punctures in 'Criterion' apices duplicated many effects of gamma-ray treatment. When microsurgical slits or punctures were made at various ontogenetic stages of apices, variations in ray and disc floret distribution, supernumerary involucral bract distribution, terminal head symmetry, and degree of fasciation were induced without ionizing irradiation.

As a check for effects of varying light intensities in the Sherer-Gillett chamber, two rows of plants, used as controls, were not subjected to dissection or subsequent microsurgical treatment. One row of five plants, which were placed along the chamber end wall, grew at lowest light intensities (average initial intensity = 1500 foot candles at soil surface). A second row of five control plants grew along a central transverse line in the chamber at highest light intensities (average initial intensity = 1900 foot candles at soil surface).
candles at soil surface). At the end of the experiment, terminal heads of the ten controls were evaluated for head symmetry and dissected for counts of involucral bracts, ray florets and disc florets.

At maturity, all ten controls had ray-disc symmetry scores of zero in terms of values reported for irradiated plants (Table 2; Fig. 19). Light intensity, however, played a measurable role in determining number of florets as well as mean disc/ray ratios. At the higher light intensities, control plant terminal heads averaged 8.3 cm in diameter (range = 7.8 to 8.7).

On the average, there were 39.4 (range = 38 to 40) involucral bracts, 368.6 (range = 351 to 385) ray florets, and 37.0 (range = 12 to 55) disc florets per terminal head.

At the lower light intensities, terminal heads were smaller (mean = 7.5 cm; range = 6.0 to 8.4 cm). On the average, terminal heads had 37.5 (range = 35 to 39) involucral bracts, 350 (range = 342 to 365) ray florets, and 17 (range = 13 to 21) disc florets. Thus, at higher light intensities, terminal heads grew larger and formed about 10% more florets than terminal heads of plants at the lower light intensities. There was no readily apparent relationship between absolute number of ray florets and number of disc florets within, or among, the two groups of control plants. Likewise, no meaningful relationship was detected between absolute numbers of involucral bracts and numbers of disc florets. There was a general two-fold increase in absolute number of disc florets in brighter light than at lower light intensities. Disc/ray floret ratios averaged 0.10 in brighter light and 0.05 in lower light intensities.

On each microsurgery date, additional measures were followed to
evaluate frequency of accidental damage to apices during the dissections which were necessary to exposure of apices. Several plants, whose apices were exposed by removing or folding back leaf and involucral bract primordia, were regularly left without slitting or puncturing their terminal meristems. During final evaluation of mature terminal heads and data, it was evident that accidental, unrecognized wounding of apices occurred in less than 20% of terminal shoots. In the majority of cases, there was no damage to apices; typical terminal capitula subsequently developed (Fig. 49). When all 89 plants in the microsurgical experiments were scored at maturity, it was evident that some upper leaf primordia were damaged in gaining access to terminal apices. Damaged leaves and foliage bracts, at maturity, usually had curled, incompletely developed laminae which often had necrotic tips. Development and mature sizes of stipules appeared to be typical, thus indicating either that they were initiated after the lamina-petiole primordium, or that they were untouched during microdissection procedures. Plants which were dissected and otherwise treated at zero through 20 short days had four to 11 foliage leaves and foliage bracts with some evidence of earlier damage. After 22 short days, foliage leaves and foliage bracts could merely be bent back to expose terminal capitula.

When raw data from 22 plants whose apices were exposed by removal of bracts but whose apices were not slit or punctured were examined, there was no evidence that damage to foliage leaf and bract primordia was effective in causing variation in results from those completely intact controls. That is, plants with dissection damage evident on mature leaves and foliage bracts had variations within the ranges present among the two groups (higher vs lower light intensities) of strictly intact control plants. Thus, it was
Figure 49. Typical terminal inflorescence bud following dissection just prior to the 10th short day. Note central, differentiated young disc florets. Photographed on 46th short day.
generally assumed that the damage to some previously initiated leaf primordia did not play a major role in varying the typical morphogenesis of involucral bracts, ray and disc florets, as well as disc/ray ratios.

Among the terminal apices microsurgically treated at zero short days, only one survived. Death of most apices so treated was attributed to desiccation in that initial microsurgery was attempted in an air-dry laboratory (ca. 50-60% relative humidity). When microsurgery was conducted in the humid (90-95% relative humidity) growth chamber, in which the plants remained after treatment, all terminal apices survived and grew to flowering. Soetiarto and Ball (1969), without reporting humidity conditions of microsurgery and subsequent growth, noted that desiccation was a problem throughout their research in slitting Portulaca flower primordia.

In order to ascertain whether or not the sequences of physiological and morphological changes, induced by ionizing irradiation, could be duplicated by mechanical means, 59 plants were treated at seven stages of inflorescence development. Twenty-two plants served as controls. The terminal apices of 37 plants were exposed by removal of leaves and involucral bracts. Twenty-three of them were given a single median longitudinal slit; 14 were given a single median summit puncture. During evaluation of mature heads of the 59 plants, all bracts and florets were sequentially removed from receptacles to note relative positions of involucral bracts and ray and disc florets.

After a vegetative shoot apex, equivalent to that of Fig. 2, was slit with a median longitudinal cut just prior to the first inductive night, each apex half subsequently grew into a complete terminal inflorescence (Figs. 50, 51; compare with Figs. 25, 27, 29). In each resulting inflorescence, terminal
Figure 50. Twin terminal inflorescence buds resulting from a median longitudinal apex bisection at zero short days. Photographed on 46th short day. Note microsurgically damaged foliage bract on right.
Figure 51. Mature twin terminal inflorescences resulting from a median longitudinal apex bisection at zero short days. Photographed on 74th short day.
head symmetry was perfectly radial, characteristic of controls. In one head, which was 7.8 cm in diameter at maturity, there were 35 involucral bracts, 321 ray florets and 12 disc florets. In the other, which was 8.0 cm in diameter at maturity, there were 36 involucral bracts, 344 ray florets and 18 disc florets. Disc/ray ratios were 0.04 and 0.05. The two portions of a bisected apex formed two separate involucres, and, together, they initiated 695 florets during the short photoperiods. By comparison, the most prolific control plant concurrently formed only 500 florets from a single terminal apex.

When single longitudinal incisions were made immediately after 12 short days (at a stage intermediate between those of Figs. 3 and 4) nearly complete terminal heads formed (Fig. 52; compare with Figs. 25, 27, 47). When they flowered, each 'subhead' had a complete involucre except immediately acropetal to the vertical scar of the bisected stem. At maturity (Fig. 53) one subhead was 7.6 cm in diameter and had 28 involucral bracts, 266 ray florets and 14 centrally located disc florets in a disc/ray ratio of 0.05. The other, 6.2 cm in diameter, had 22 involucral bracts, 215 ray florets and 6 centrally located disc florets in a disc/ray ratio of 0.03. Thus, 501 florets ultimately were derived from one original primordial inflorescence apex. Positional (Fig. 52) and size differences indicated that the apex bisection was not exactly median.

Among all mature inflorescences which grew from apices bisected immediately after the 12th short day, supernumerary involucral bracts were present on receptacle flanks. Moreover, solitary disc florets often were present among ray florets.

Depth of bisection was of utmost importance when inflorescence apices,
Figure 52. Two nearly complete terminal inflorescence buds resulting from a near-median longitudinal apex bisection made after 12 short days. Face view (left) and side view (right).
Figure 53. Two mature, nearly complete terminal inflorescences resulting from a near-median longitudinal apex bisection made after 12 short days. Note dark colored, trichome-free incision scars. Photographed on 74th short day.
equivalent to those of Figure four, were bisected after the 14th inductive
night. Subsequent to shallow cuts, a single terminal inflorescence (Fig. 54)
with a two-peaked receptacle, developed. Whenever one of these two peaked
receptacles formed, all disc florets were located only at the two cone summits
and no supernumerary bracts formed. Only ray florets were found in the
'valley' between the two receptacle peaks.

Whenever single longitudinal cuts were sufficiently deep that the
receptacle was completely bisected immediately after the 14th short day, two
separated incomplete 'inflorescences' developed (Fig. 55; compare with Fig.
54). Frequently, dark green, trichome-free wound scars were observable on
peduncles. Supernumerary involucral bracts were common and were attached
immediately acropetal to scarred areas of the two opposing, distinctly
separated 'receptacles'. Although disc florets were present on the summit
of each separate receptacle, random formation of a few individual disc florets
occurred among ray florets.

Supernumerary involucral bracts were not induced by inflorescence apex
bisections made after 19 short photoperiods. When shallow inflorescence apex
bisections were made immediately before the 20th short day (at a stage
intermediate between those of Figs. 5, 6), only slight external indications of
microsurgery were noted in terminal inflorescence buds on the 46th short
day (Fig. 56; compare with Fig. 52). After ray florets were removed,
however, receptacles were generally ovoid in cross section, with a slight
(ca. 1.0 mm) trough at the zenith. In some cases, disc florets formed only
at the summits of the two raised opposing peaks; in other cases, a few ray
florets were intermixed in the common disc region which included both
receptacle peaks. After all florets were removed, a small, macroscopically
Figure 54. Bifurcated terminal inflorescence resulting from a shallow median longitudinal apex bisection made after 14 short days. Note the two separate disc floret regions. Photographed after 46 short days (upper) and 74 short days (lower).
Figure 55. Bifurcated terminal inflorescence resulting from a deep, near-median longitudinal apex bisection made after 14 short days. Note typical stipules. Face view (left) and side view (right).
Figure 56. Terminal inflorescence buds resulting from a single median longitudinal incision made after 19 short days. Original incision positions indicated by arrows.
visible pore, opening into the partially hollow receptacle, was observed at the zenith of the original incision site (Fig. 57).

Longitudinal inflorescence bisections, made after 21 or 28 short days (comparable to stages portrayed in Figs. 7, 8), induced the same general results as longitudinal slits made after 19 days. Although disc floret zones sometimes were irregularly shaped, or with an occasional included ray floret, inflorescences from the later bisection times each had one disc floret region which encompassed both contiguous high points of the receptacle.

Among median longitudinally bisected apices, especially those slit after 21 short days, apparent receptacle grafting occurred (Fig. 57). There was no evidence that grafting occurred in the earliest bisections (Figs. 50, 51, 52, 53). Similarly, Soetiarto and Ball (1969) noted that grafting occurred more rapidly in older bisected *Portulaca* flower primordia than in bisected meristematic floral apices.

When single median punctures were made in inflorescence apices immediately prior to the 13th short day, mature receptacles were prominently three or four peaked. At maturity, disc florets were only found at the summits of peaks. In one case, 13 supernumerary involucral bracts differentiated in the intrazonal 'valley' around the base of one of four peaks.

When median apical punctures were made after 12, 14, 19, or 21 short days, the resulting mature receptacle bases were circular in outline (Fig. 58). Those receptacles always were three peaked, undoubtedly because the minute point of the exodontia needle, employed in punctures, was microscopically triangular. As noted for mature receptacles from the later bisection times, some of the later punctured receptacles were partially hollow with a centrally located pore (Fig. 58).
Figure 57. Mature receptacle resulting from a single median longitudinal bissection made after 21 short days. Note original incision site and terminal pore. (Black lines, lower left, are extraneous debris.)
Figure 58. Mature receptacle after a single median longitudinal puncture made at 21 short days.
Consistent with apex bisections and irradiation treatments, apical punctures, made before the 15th short day, caused the differentiation of supernumerary involucral bracts on the flanks of receptacles. Supernumerary involucral bract initiation was not induced by microsurgical wounds or irradiation after 15 short days. Only the microsurgical bisection at zero short days resulted in the formation of more florets per original apex than any control plant terminal inflorescence.

In addition to median longitudinal bisections and apical punctures, two other types of incisions were made among 15 other terminal apices. In the first type, two parallel, tangential longitudinal incisions were made to remove the opposing peripheral sections of a primordial inflorescence without disrupting the apex summit. When such incisions were made on an apex immediately after 14 short days (stage equivalent to that of Fig. 5), the resulting terminal inflorescence had an elongated disc floret region comprised of 25 contiguous disc florets. The disc floret region was elongated at a right angle to the planes of the original parallel incisions. Moreover, six distinct disc florets and one additional floret, intermediate between disc and ray, were interspersed among typical ray florets. These seven florets were distributed along an imaginary extension of the elongated central disc floret zone. Involutural bracts were not well developed immediately subjacent to the cutting planes. Other involucral bracts, in a plane parallel to that of the original cuts, appeared to be typical.

Similar results were noted in another terminal inflorescence, which was previously slit with two comparable parallel incisions after the 21st short day. In this type, the main disc zone was generally circular, but had one imaginary extension, comprised of four disc florets. That hypothetical
extension of the central disc floret zone was oriented toward one original incision site. On the opposing flank of the same receptacle were four rudimentary florets which consisted only of an ovulary, stigma and style. Those four 'florets' were attached on the receptacle immediately acropetal to the incision scar.

In the third variation of microsurgical slitting, three longitudinal incisions were made in any one primordial inflorescence. In these cases, the three tangential longitudinal incisions removed peripheral portions of primordial terminal inflorescences immediately preceding the 20th of the 22nd short day. After cutting, the intact summit of the inflorescence apex was at the center of a three sided body. The oldest floret primordia were located at each of the three corners. At maturity, the terminal inflorescences were dissected and evaluated. Results were entirely consistent with those of the parallel incisions. In addition to a summit zone of contiguous disc florets, there were three imaginary extensions of the disc floret zone. Those three extensions were oriented toward original cutting planes. Moreover, florets which were intermediate between ray and disc types, were part of those extensions. Typical 'Criterion' ray florets were interspersed among those disc and intermediate florets of the imaginary extensions. In two of these three terminal inflorescences, there was limited involucral bract growth subjacent to the cutting planes. That is, involucres appeared to be comprised of roughly three ranks of bracts.

During dissection to expose primordial capitula, some apices were accidentally wounded. In one case, a peripheral flank was damaged at nine short days. Near maturity, the disc floret zone was markedly elongated into a crescent (Fig. 59; compare with Figs. 24, 25). In the mature head,
Figure 59. Terminal inflorescence bud resulting from peripheral injury to shoot apex made after the 9th short day. Original wound site at arrow.
supernumerary bracts were attached on the receptacle flanks immediately acropetal to the original wound site. The original wound was at the hypothetical focus of the concave shaped disc floret zone. Wardlaw (1965a, Plate 20, C), without full explanation, illustrated a strikingly similar case of an elongated "male" floret zone at the summit of a "fasciated terminal capitulum" of *Petasites hybridus* (Compositae).

In general, from the microsurgical studies, it appears that any group of contiguous disc florets were the last florets initiated on any one isolated growth center. Moreover, it generally appears that any group of contiguous disc florets develop at the maximum distance from the usual and the supernumerary involucral bracts. Occasionally, individual disc florets were adjacent to supernumerary bracts. More commonly, corolla-less, rudimentary florets comprised only of ovulary, stigma and style, were associated with those bracts.
DISCUSSION

A. General Development of Chrysanthemum Inflorescences

Vegetative and reproductive growth in chrysanthemums is susceptible to innumerable physiological alterations which, in turn, determine morphology. Not only do seasonal physiological changes occur among vegetative plants under non-inductive photoperiods, but also, myriads of sequential physiological changes undoubtedly ensue immediately after photo-inductive cycles commence. A synthesis of some usual events in shoot and inflorescence development will, therefore, serve as a point of departure for clarifying some deviations induced by microsurgical treatments and gamma-ray treatments at weekly intervals in terminal inflorescence ontogeny.

As chrysanthemums grow vegetatively, marked variations in shoot growth, as reflected in plastochron duration and leaf shape, are induced by temperature variations of only 10°C (Schwabe, 1959). For instance, as temperatures increase from 17°C to 27°C, the degree of leaf dissection and depth of sinuses decrease markedly. Within the same temperature range, plastochron duration varies by more than 1.25 days, depending upon day and night temperature regimes (Schwabe, 1959). Overall foliage leaf mean plastochron duration, 2.2 to 3.2 days in the low-temperature vernalizable chrysanthemum 'Sunbeam' (Schwabe, 1959), was somewhat longer than the mean 2.1 day plastochron duration found in 'Criterion'.

Since auxin generally causes initiation of adventitious roots on cuttings (Salisbury and Ross, 1969, p 455; Dore, 1965, p 71), it may be suggested
that quantitative variations in endogenous auxins might subsequently be reflected in variations in rooting time among standard-sized cuttings. If, in actuality, that is the case, then endogenous auxin concentrations vary seasonally. William Skou (personal communication) states that, within a variety, rooting time varies with season of the year.

In a number of short day species, extractable free auxin levels decline after photoinduction commences (Cooke, 1954). By indirect evidence, endogenous auxin supplies in chrysanthemum appear to decline precipitously immediately after the onset of short days. For chrysanthemums commencing photoinduction, Nitsch (1965) related several facts to support his auxin decline hypothesis when he pointed out that the onset of short days was associated with: (a) loss of apical dominance, (b) failure of internode elongation in the involucre, and (c) decline in number of adventitious roots initiated on standard-sized cuttings taken after seven short days.

That the onset of short days causes declines in endogenous auxin levels appears probable from other indirect evidence. There is considerable evidence that auxin stimulates cambial mitosis (Wareing et al., 1963). There is a marked decrease in cambial activity following onset of photoinduction in short day species of *Soja*, *Xanthium*, and *Cosmos* (Roberts and Struckmeyer, 1948; Struckmeyer, 1950).

Chrysanthemum inflorescence development is markedly delayed by exogenous auxins in an aqueous spray every third day (Tsukamoto and Harada, 1957) or in a lanolin-auxin paste (Schwabe, 1959). Recently, Lindstrom and Asen (1967) prevented the formation of visible flower buds for a minimum of 105 days by daily exogenous auxins applied to chrysanthemum
growing under inductive photoperiods. Since flowering in many chrysanthemums is a phytochrome-controllable phenomenon (Salisbury and Ross, 1969, p 599; Cathey, 1969), Hillman and Galston (1957) may have discovered a tenable mechanism for explaining at least part of the auxin decline. In peas, they noted that indoleacetic acid oxidase activity was greatly inhibited by red light (ca. 660nm) but the inhibition was, under some circumstances, reversible by far red ("near infrared") light (ca. 730nm).

Recently, Chailakhyan (1967) reported that a general decline in auxin and gibberellin content occurs in short day plants shortly after onset of photoinduction. Lona (1962) suggested that gibberellin concentration progressively declined in chicory (Compositae), commencing before inflorescence initiation. Moreover, these workers corroborated prior findings of Cathey and Stuart (1958) who reported that chrysanthemum plants contain an abundance of natural gibberellic acid-like substance during long photoperiods or at the start of short photoperiods. Furthermore, Cathey and Stuart noted that the maximum promotion of stem elongation was induced by exogenous gibberellic acid applications on the 21st short day in the 10 week chrysanthemum 'Shasta'. Exogenously applied gibberellins were less effective at other weekly intervals. In view of "the failure of GA to increase cell division activity in the subapical meristem of untreated Chrysanthemum plants is perhaps an indication that in many caulescent plants these substances are present at optimum levels for the function of this meristem" (Sachs et al., 1960), Cathey and Stuart's (1958) data may be preliminary approximations of endogenous gibberellin levels.

Assuming, in reality, that Cathey and Stuart's data concerning weekly variations in promoted stem elongation reflect endogenous gibberellin status
within a chrysanthemum, then it may be inferred that endogenous gibberellins are in their lowest level on the 21st short day (Fig. 60).

Similarly then, Nitsch's (1965, Fig. 4) data concerning number of adventitious roots which form on stem tip cuttings, taken at weekly intervals during photoinduction, may reflect weekly variations in total auxin supply in stem tips. If so, Nitsch's data would suggest that total endogenous auxin is at the lowest level on the 14th short day. If that interpretation of Nitsch's (1965) and Cathey and Stuart's (1958) data is correct, then the period of marked auxin and gibberellin declines completely encompasses the period of minimal mitotic activity (zero to six short days) reported earlier by Popham and Chan (1952).

During the period of minimum mitotic activity and assumed declines in auxin and gibberellin content, the "receptacle induction period" is completed. If only five short days are provided to 'Bittersweet' chrysanthemums, a receptacle will form and differentiate "to the floret initiation stage" in long days (Popham and Chan, 1952). Presumably, that differentiation of a "crown bud" includes the initiation of at least part of the involucre. Differentiation of the receptacle does not, however, induce the development of a typical complete involucre (compare Figs. 6, 8 of Popham and Chan, 1952).

Because a "crown bud", induced by only five short photoperiods, is subsequently subtended by the typical foliage bracts (Nitsch, 1965, Fig. 3A), it is evident that: (a) five inductive photoperiods change the morphogenetic pattern from that of foliage leaf to that of a typical sub-inflorescence foliage bract, and (b) foliage bract development appears to be unrelated to floret initiation and development. Indeed, the formation of unlobed, simple foliage bracts may be a concomitant result of mitotic arrest at the start of
Figure 60. Inferred weekly changes in endogenous levels of gibberellins (G) and auxin-like substances A (A), B (B), and C (C) in relation to morphological events in ten-week chrysanthemum varieties. Gibberelin levels based upon data of Cathey and Stuart (1958) for 'Indianapolis Yellow'. Levels of auxin-like substances A, B, and C based upon data of Harada and Nitsch (1959) for 'Shasta'.
RELATIVE ENDOGENOUS LEVELS

NUMBER OF INDUCTIVE PHOTOPERIODS COMPLETED
photoinduction. Beach and Leopold (1953) reported that foliar applications of a mitosis inhibitor, maleic hydrazide, resulted in loss of apical dominance, suppressed new leaf growth and was followed by formation of "flowering bract-like leaves instead of the normal vegetative ones".

Comparison with regular lobed foliage leaves, the unlobed foliage bracts of chrysanthemum strikingly resemble variations in leaf forms on "juvenile" and "adult" Hedera branches. Since exogenous gibberellin applications result in renewed juvenility, and result in the formation of lobed leaves on "adult" Hedera branches (Robbins, 1960), it is tempting to relate chrysanthemum foliage bract growth to the apparent declining endogenous gibberellin supplies which accompany receptacle induction. Moreover, exogenous gibberellins can promote leaf expansion (Stowe and Yamaki, 1959).

Immediately after six to seven short days, mitosis resumes in chrysanthemum apices (Popham and Chan, 1952). By the seventh short day, mean interphase nuclear volume had increased in 'Criterion' apices, especially in the apical flanks, to an overall average of 114% of vegetative apices (p 22, 23). With the onset of rapid shoot growth in many woody plants, mean interphase nuclear volume increases to about 165% of dormant stage values (Sparrow, et al., 1968b). Nuclear volume increase, before spring growth, is not universal in trees, however, (Taylor, 1965). Similar nuclear volume increases occur concomitantly with differentiation in vertebrates (Harris, 1968). Moreover, Harris (1968, p 103) has noted that "there are grounds for supposing that changes in nuclear volume may be part of a general mechanism which permits large genetic areas to be opened up or closed down in an ordered sequence". During embryonic development in Vanda, Alvarez (1968) inferred that nuclear volume increases were
attributable to increases in the amounts of DNA and nonhistone nuclear protein. Most of the nuclear volume increase was due to an increase in DNA.

Marked physiological changes occur between the fifth and eighth short days in that floret initiation can proceed in long days, once eight consecutive short photoperiods have been completed (Popham and Chan, 1952). Perhaps the increased nuclear volume in 'Criterion' apices is a consequence of the arrival of the hypothetical florigen and renewed auxin synthesis, the latter of which is thought to promote protein synthesis (Fan and Maclachlan, 1967).

Plastochron duration also provides evidence that inflorescence apices have a remarkably different physiology than vegetative apices. Whereas foliage leaf mean plastochron was about 2.1 days in 'Criterion', mean involucral bract plastochron was 9.6 hours (0.4 day), a value highly comparable to the 9.0 hours reported for a low temperature vernalizable chrysanthemum (Schwabe, 1959). Thus, there is a 5.24 fold decrease in mean plastochron before involucral bract initiation commences. Although they may not be related, shortening of the plastochron, change in phyllotaxis, and onset of rapid involucral bract initiation all occur at the time when apparent levels of auxin-like substances A and B increase and when levels of auxin-like substance C begins to decline at a slow rate (Fig. 60). Because exogenous auxin applications sometimes cause changes from decussate to spiral phyllotaxis (Snow and Snow, 1937), it appears that not all of these correlations are fortuitous.

In 'Criterion' the majority of involucral bracts were initiated between the seventh and the 21st short days. That period was one in which precipitous declines in levels of gibberellins appeared to continue. There was a small increase in level of substance C and a continued rapid increase in auxin-like
substance B (Fig. 60). Thus, tentatively, it appears that involucral bracts are initiated during periods of increasing levels of these two hormones in 10-week chrysanthemums.

If, early in capitulum development, short light periods are intercalated during long nights, involucral bract initiation is favored (Okada and Hiraki, 1954). If short cuttings of primordial terminal inflorescences of chrysanthemum are subjected to long days, additional bracts form on the receptacles and each bract subtends a floret (Schwabe, 1959). In the case of some florets, on which corollas have been initiated, the remaining meristematic portion of the floret differentiates into a small capitulum on a short peduncle (Schwabe, 1951).

Likewise, in short day varieties of \textit{Cosmos} (Compositae), several investigators (Biddulph, 1935; Greulach, 1942; Madsen, 1947) found that intercalation of long photoperiods during otherwise photoinductive cycles, resulted in marked deviations from usual capitulum development. Typically, supernumerary involucral bracts formed and internode elongation occurred within the involucre. In some cases, plants in early stages of inflorescence development reverted to a vegetative state.

In many respects, the unlobed, crowded involucral bracts resemble cataphylls of woody plants. Involucral bracts are thinner than foliage leaves and contain no palisade mesophyll. Similar modifications accompany cataphyll ontogeny in woody plants (Romberger, 1963, p 44). Romberger (p 45, citing Dostal, 1952) indicated that exogenous auxin promotes budscale formation in \textit{Aesculus}. Moreover, he reported that the auxin antagonist 2,3,5-trilodobenzoic acid promotes differentiation of foliage leaf primordia in \textit{Aesculus}. In \textit{Acer}, Thomson and Millington (1966) found that cataphylls
are initiated under increasing daylength conditions; foliage leaf primordia develop under decreasing daylengths. Exogenous applications of gibberellic acid promoted cataphyll formation in Acer.

Although the evidence is indirect, there are indications that involucral bract initiation occurs during a period of increasing endogenous auxin biosynthesis in 'Criterion'. At present, of course, there is no unequivocal proof that these circumstances are causally related. It is indeed remarkable that Schwabe (1959), with considerably less data, was able to propose "that in long days higher auxin levels are attained".

Within the involucre, per se, no appreciable internode elongation occurs between bracts, even though pronounced internode elongation, subjacent to the involucre, usually accompanies inflorescence maturation in chrysanthemums. Similarly, the scape of Gerbera (Compositae) rapidly elongates prior to anthesis, but the short involucral internodes do not (Sachs, 1968).

In short day species, Fratianne (1965) and Bhargava (1969) have suggested that inhibitors of flower bud initiation may form in non-inductive photoperiods. If flower initiation inhibitors form during long days in chrysanthemum, as suggested by Weise and Seeley (1964), it would appear likely that synthesis of inhibitors ceases upon onset of photoinduction. Furthermore, it is likely that such inhibitors would have been degraded during the first 15 to 20 short days because floret initiation commences during this period in 'Criterion'. Indeed, Wellensiek (1962) proposed that short day plants may not flower in long days because inhibitors, formed in long days, are not sufficiently inactivated during short nights. Proponents (Weise and Seeley, 1964; Fratianne, 1965; Bhargava, 1969) have not definitively
demonstrated that these proposed inhibitors are different from auxin, however.

Between the 14th and 21st short day, pronounced physiological reorganizations occur in chrysanthemum apices, as indicated by changes in plastochron duration, phyllotaxis, and commencement of rapid floret initiation. Seemingly, these events may be associated with shifts toward increasing levels of auxin biosynthesis. By the Triticum coleoptile straight growth test there is approximately twice as much extractable auxin in 'Indianapolis' chrysanthemum shoot tips on the 21st short day as in vegetative shoots (Leshem and Schwarz, 1968). Endogenous levels of gibberellins may be at their lowest point on the 21st short day (Fig. 60).

The period of rapid floret initiation appears to be one in which there are high endogenous concentrations of auxins and shifts from low to increasing levels of gibberellins (Fig. 60). Cessation of floret initiation appears to occur when endogenous levels of gibberellins may be approaching stability and when there are initial declines from the highest level (28th short day) of endogenous auxin-like substance C (Fig. 60).

As noted earlier, plastochron duration is difficult to ascertain during floret initiation because florets are probably simultaneously initiated within one cycle. Whereas 'Sunbeam' chrysanthemums were reported to have a mean floret initiation plastochron of 24 minutes, Schwabe (1959) did not state that florets can be initiated simultaneously within a cycle. Nevertheless, changes of plastochron, growth substance levels and simultaneous commencement of floret initiation are probably not chance correlations.

After the 28th short day, following a phase of rapid involucral bract growth and interdigititation over the capitulum, apparent auxin concentration
seems to decline in 'Shasta' chrysanthemums. Simultaneously, the concentration of extractable substance C declines precipitously in 'Shasta' chrysanthemums (Fig. 60).

Since leaf primordia synthesize significant quantities of auxin (Wetmore and Pratt, 1949; Wetmore and Wardlaw, 1951), rapidly growing young involucral bracts might do likewise. If so, maturation of involucral bracts would undoubtedly result in declines in amount of extractable auxin, and, thus explain trends apparent on the 28th short day. Unfortunately, because Harada and Nitsch (1959) extracted shoot tips with intact involucral bracts, the source of their substance C is not known.

Between the 28th and 35th short days, when rapid floret elongation occurs on 'Criterion' terminal capitula, differentiation of ray and disc florets occurs. During this phase, endogenous levels of auxin-like substance C apparently decline precipitously and gibberellin levels appear to be approaching a period of stability (Fig. 60).

Among angiosperms, many factors contribute to promotion of stamen and pistil growth or to their inhibition (Heslop-Harrison, 1957b, 1959, 1960; Nitsch, 1952, 1965). Not infrequently, the complex interaction of external environmental factors obscures patterns of development. At present, there is no evident, consistent, set of conditions which culminate in the maturation of stamens or pistils among angiosperms.

Without exogenous applications of growth regulators, sex expression is under strong genetic control in many species (Heslop-Harrison, 1957b, 1959, 1960; Shifriss, 1961a; Galun, 1961; Jain, 1959; Beeskow, 1967; Koopmans, 1959; Martin, 1966; Kooistra, 1967; Yampolsky, 1957). In these species, sex chromosomes or mendelian-inherited genes are primary determinants
of stamen or pistil maturation. Among angiosperms, inorganic salt concentrations (Shifriss and George, 1966), temperature variations (Quagliotti, 1967) and fluctuations in daylength (Jones, 1947; Vergely et al., 1967) play major roles in controlling stamen or pistil maturation or inhibition-abortion. In the gynoecious or monoecious cucurbit species, gibberellin applications frequently promote the maturation of increased numbers of staminate flowers (Peterson and Anhder, 1960). By contrast, auxin applications promote ovulary development in potentially staminate cucumber flower primordia (Galun et al., 1963). In some Ricinus, gibberellins "markedly increase their female tendency" (Shifriss, 1961b). In Xanthium, exogenously applied gibberellins increase the number of pistillate inflorescences (Kirk et al., 1967).

Most commonly, exogenous applications of auxins promote pistil development, or increased femaleness, and inhibit stamen development (Heslop-Harrison, 1957b, 1959, 1960; Smith, 1967). Rarely, exogenously applied cytokinins have been implicated in "increasing femaleness" (Catarino, 1964), or in converting primordia of usually staminate flowers into hermaphroditic flowers which are morphologically bisporangiate (Negi and Olmo, 1966). Kinetin promotes increased staminate flower development in Carex (Smith, 1967).

Among species of Compositae, sex expression and modification are equally inconsistent. Jones (1947) found that short day conditions are conducive to pistillate flower development, but are detrimental to staminate flower maturation in ragweed (Ambrosia spp.). Auxin inhibits stamen dehiscence in ragweed (Smith et al., 1946). In Xanthium, gibberellins increase the number of pistillate inflorescences. After initial photoinduction,
long days increase the number of staminate inflorescences (Kirk et al., 1967).

In numerous species there are higher concentrations of endogenous auxins and gibberellins in long days than in short days (Chailakyan, 1967). During capitulum development in a short day cosmos, intercalation of long days into the series of photoinductive cycles disrupts usual pollen development (Madsen, 1947). The auxin, 2,4-D, inhibits disc floret opening in sunflower (Greenshields and Putt, 1958).

In chrysanthemum, increased numbers of disc florets form under longer nights than are necessary to ray floret development (Okada and Hiraki, 1954). From the data of Cooke (1954), it is conceivable that auxin synthesis may continue declining under increasingly longer nights.

Under continuous night temperatures of 15.6°C, some chrysanthemums have no disc florets; under certain regimes of fluctuating night temperatures, typical disc florets form (Cathey, 1955). Tsukamoto (1957, Table 4), in experiments with 'December King' chrysanthemums, found that auxin applications generally decreased the total number of florets formed per capitulum. At 100 ppm, auxin application resulted in the formation of 0.23 mean (range = 0 to 2) disc florets per capitulum. Control plants had, on the average, 8.62 (range = 0 to 22) disc florets per head.

At weekly intervals in the first 49 inductive days, Harada and Nitsch (1959) methanol-extracted several endogenous auxin and/or gibberellin-like compounds from shoot tips of the 10 week chrysanthemum 'Shasta' (Fig. 60). Although endogenous inhibitors severely confounded their results, they found clear, weekly fluctuations in concentrations of auxin and/or gibberellin-like substances. Most notably, their compound C decreased during the first
three weeks of photoinduction, then significantly increased to an all-time high concentration on the 28th short day. Subsequently, precipitous declines in compound C occurred at times when compounds A and B increased in concentration over the next weeks. If compound C was auxin, there is significant additional evidence that auxins can inhibit disc floret development at a critical time and, thus, promote maturation of the male-sterile ray florets in chrysanthemum. Tsukamoto's (1957) data fully corroborate this inference.

Moreover, continued developmental events in the chrysanthemum capitulum ontogeny are not inconsistent with an hypothesis that declining auxin levels are conducive to disc floret maturation. It seems likely that auxin (Substance C, Fig. 60) biosynthesis by the apex may decline as the capitulum becomes floret covered. The involucral bracts, themselves a likely source of auxins at earlier stages, have either slowed or ceased growth by the 28th to the 35th short day, at which time disc-ray differentiation is expressed morphologically.

Because at least four auxin and/or gibberellin-like compounds and one major inhibitor (Harada and Nitsch, 1959) are extractable from chrysanthemum shoot tips during capitulum ontogeny, it is evident that no complete explanation of ray-disc maturation is yet at hand.

After the disc and ray florets are morphologically distinct, their rapid continued growth may be related to increased gibberellin synthesis as interpreted from data of Cathey and Stuart (1958) (Fig. 60). They noted that exogenous gibberellin application promoted significant stem elongation (ca. 13 cm) on the sixth photoinductive week, but equivalent applications, seven days later, induced less than two cm elongation.
In the Compositae, final peduncle elongation appears to be a result of gibberellins, auxins and other, unknown, substances synthesized in the capitulum. In Gerbera, for example, exogenous applications of gibberellins and auxins can essentially replace the usual effects of florets in promoting peduncle elongation (Sachs, 1968). The receptacle, per se, and/or involucral bracts also appear to have a measurable role in final peduncle elongation (Sachs, 1968).

Chrysanthemums, or the Compositae in general, are not unique in their distribution and formation of two kinds of flowers in one inflorescence. In at least some species or varieties of Hydrangea and Viburnum, strikingly similar variations occur within one inflorescence. In some hydrangeas and in Viburnum tomentosum Thunb., the peripheral flowers are male-sterile, have large corollas and sometimes are zygomorphic; central flowers are morphologically perfect, have small corollas, and are usually actinomorphic. In the Japanese snowball (Viburnum tomentosum var. sterile Koch) the majority of flowers are male-sterile with large petals. At the tip of an inflorescence branch, however, one morphologically perfect flower, with a small corolla, usually forms. Resemblance to chrysanthemum is so striking that a similar ontogenetic physiology is possible.

In several angiosperms, correlative growth of flower parts has been reported (Heslop-Harrison, 1959; Nitsch, 1965; Plack, 1958; Greyson and Tepfer, 1967). Typically, exogenously applied substances promote development of either corolla and androecium or calyx and gynoecium; correlative effects of inhibitors are often consistent. Plack (1958) found that early stamen emasculation resulted in retarded corolla enlargement. Exogenously applied gibberellic acid replaced the usual effects of intact
stamens, thus promoting usual corolla growth in Glechoma (Labiatae).

Auxins, proline, or 2,3, 5-triiodobenzoic acid did not replace the effects of the androecium. In emasculated Nigella flowers, gibberellins have similar effects (Greyson and Tepfer, 1967) to those noted in Glechoma.

In chrysanthemum, however, correlative growth is remarkably different. Sepals are absent, except, perhaps, for the possibly homologous small protruberances which form at the ovulary summit as epigyny occurs. If, in reality, these extensions are sepal homologues, then the usual initiation of floret parts is not strictly acropetal. Just as Lythrum, Primula, and other species (Cheung and Sattler, 1967), chrysanthemum would be an exception to the general pattern of initiation of flower parts.

If the possible formation of a calyx-homologue is ignored in chrysanthemum florets, it appears that promoters of corolla elongation are inhibitors to stamen elongation and maturation. Alternatively, promoters of stamen maturation may be inhibitors to continued corolla growth. Intermediate florets, half disc and half ray, are consistent with these hypotheses. In them, stamens mature only in the actinomorphic disc portion; stamens fail to mature in the elongated corolla portion which is ray-floret-like in respect to its sympetaly, length, and coloration in 'Criterion'. In these intermediate florets, the disc-floret-like portion typically is oriented toward an original growth-center summit; the ray-floret-like portion is usually oriented toward the receptacle base, or toward an original microsurgical wound site. Thus, there is some evidence for an interaction, within the receptacle, which culminates in the differentiation of ray and disc florets. Perhaps one or more materials, from the inflorescence apex summit or from the incipient disc florets, may move basipetally in the receptacle and, subsequently, inhibit
the corolla development at the nearest point in each of the incipient ray florets. Such a phenomenon could explain the flat, open, bilaterally symmetrical form of ray florets. That each ray floret elongates most extensively at its outermost face strongly suggests that corolla growth promoters may move acropetally from the receptacle base. Thus, chrysanthemums are excellent subjects for experimental approaches toward characterizing the physiological causes of zygomorphy, actinomorphy, stamen and corolla growth, as well as patterns of sex reversal. Pursuit of such investigations could, perhaps, explain why stamens usually mature in only the last formed flowers in species of Alismataceae, Typhaceae, Araceae, and Sparganiaceae.

The physiological mechanisms of reproductive morphogenesis are so great between species that no one biochemical pathway of floral ontogeny is yet evident. Not only is the developmental physiology of a single stamen extremely complex (Sparrow et al., 1961; Porath and Galun, 1967), but also, the essential inorganic salts and growth regulators of complete floral development remain to be ascertained for a single species. Undoubtedly, there are myriads of nutritional and hormonal changes which occur during the development of one flower, as the data of Cooke (1954), Harada and Nitsch (1959) and Bilderback et al. (1968) have ably demonstrated. Moreover, the multitude of contradictory data among plants militates against accepting a single physiological pathway in angiosperm reproductive morphogenesis, (Carr, 1967; Wellensiek, 1962).
B. Effects of Microsurgery and Gamma-Radiation on
Floral Morphogenesis

Among all the results of microsurgical and gamma-irradiation studies, very few are interpretable from a firm biochemical-physiological base of established facts. Recently, for example, Smith et al., (1969) demonstrated that ionizing irradiation treatments resemble plant ageing in that both result in the formation of increased kinds of separable isozymes. Nevertheless, data from ionizing radiation and microsurgical treatments allow some insight into chrysanthemum reproductive morphogenesis.

Irradiation treatments did not irreparably damage qualitative photoperiodic receptivity of leaves and/or apices with respect to early inflorescence development. Indeed, there was no evidence that phytochrome-mediated phenomena, associated with floral initiation, were damaged at all. That is, first floret initiation occurred at essentially the same time among control plants and plants irradiated after zero or seven short days. Because last floret initiation, in these plants, was delayed by approximately one week over controls, it appears that such delay was more a result of mitotic inhibition than a result of inhibition of the initial phytochrome reactions.

When Xanthium plants were irradiated at various times in respect to photoinduction, apparently contradictory results have been reported among investigators. For example, Chorney and Rakosnik (1965) found that "an X-ray exposure of 3.0 kr [kiloroentgens] within the first few hours of the light period following the inductive dark period suppressed flowering". Moreover, they added, "exposures between 250 r and 5.0 kr prior to the inductive night stimulated the production of flower primordia". Brown and
Taylor (1967) reported that floral production generally was suppressed by irradiation treatments (mean absorbed dose = 1927 rad) given during the first five light periods or during the first five dark periods which commenced the photoinduction. Discrepancies may have resulted from time-dose differences between these two investigations. Gamma-ray doses up to 30 kr did not prevent photoperiodic receptivity of exposed leaves of a short day Perilla (Savin, 1968). Because results vary greatly among species studied, no broad generalizations can yet be drawn to relate phytochrome radiosensitivity and floral initiation.

Results of irradiation treatments at weekly intervals during inflorescence growth coincidentally disclosed patterns of leaf maturation in association with inflorescence initiation and development. The highly significant linear relationship between time of irradiation and mean percentage of gamma-ray-damaged foliage leaves and bracts (Fig. 12) suggests that foliar primordia mature at an essentially linear rate. Therefore, at a certain age each foliar primordium must pass a developmental threshold beyond which it is no longer susceptible to observable radiation damage. Even though several shifts in physiological processes occurred in terminal apices and capitula between the first and 35th short day, it appears that foliage leaf and bract maturation rates were not affected in a similar saltatory manner. That is, no concomitant speed-up or delay in foliage maturation was evident.

Significant increases in the number of leaves initiated up to the 30th short day occurred in Xanthium plants which were irradiated either during the light or dark periods of the first five photoperiodic days (Brown and Taylor, 1967). Brown and Taylor did not describe whether or not the leaves originated from terminal or axillary apices. Conceivably, the additional
leaves could have developed from axillary meristems after loss of apical dominance occurred in terminal buds. No similar increase in the number of leaves was found in 'Criterion'.

Among endogenous hormones, there is little evidence that high-energy irradiation affects cytokinins and gibberellins \textit{in vivo} (Brown and Taylor, 1967). Lockhart (1961) found that none of his observed effects of X-rays upon bean stem growth could be attributed to disruption of the gibberellin system. In some cases, there even may be hints that ionizing irradiation may enhance gibberellin biosynthesis. Many of the "abnormalities" of "straighthead" - diseased rice plants (Nunes, 1964) appear to be identical to those which Shimizu et al. (1966) induced in non-diseased rice plants by gamma-irradiation and/or exogenous applications of gibberellins. Moreover, Shimizu et al. noted that gibberellins, applied after irradiation, greatly enhanced irradiation-induced deviations in rice spikelets. Results with rice may be difficult to interpret in view of the fact that Magdon (1964) found that 10 to 75 micrograms/ml of gibberellic acid reduced the radiosensitivity of \textit{Vicia faba} roots. Magdon attributed this effect to "an increased production of catalase, induced by gibberellic acid, that removed the harmful oxidizing products of radiation" (Romani, 1966).

High doses of gamma-radiation may influence endogenous levels of gibberellins, however. In aqueous solutions of $10^{-6}$M gibberellic acid, molecules are inactivated by the formation of the hydroxyl free radical. The inactivation dose ($D_{37}$) of gibberellic acid is approximately 15 kilorads (Sideris and Nilan, 1969). Biological activity (measured by ability to induce alpha-amylose activity) of buffered gibberellic acid solutions is almost completely destroyed by \textit{in vitro} gamma irradiation at 32 kr (Sideris et al.,
Kutacek et al. (1966) found that the in vitro treatment of standard samples of gibberellic acid with doses up to 1000 kr gamma-irradiation "did not lead to marked radiochemical degradation" of gibberellic acid. In vivo, treatment of barley grains (at 5.9% water content) with 5 kr, or higher, doses of gamma-rays caused a significant reduction in endogenous levels of gibberellins (Kutacek et al., 1966). Post-irradiation additions of zinc (as Zn SO$_4$), prior to germination, restored subsequent levels of endogenous gibberellins to control values if original irradiation doses did not exceed 30 kr (Kutacek et al., 1966).

Recently, it became apparent that auxins, cytokinins, and gibberellins are involved in inhibition of bud growth (Jacobs and Case, 1965; Davies et al., 1966; Helgeson, 1968; Stowe and Yamaki, 1959). Since loss of apical dominance is a rather common consequence of ionizing irradiation treatments (Sax and Schairer, 1963), one might infer that biosynthesis of gibberellins and cytokinins may be generally less affected by high-energy radiation than is auxin synthesis.

Because of differences in major synthesis sites of plant hormones, it is not unexpected that gibberellin and cytokinin biosynthesis appear to be more radioresistant than auxin synthesis mechanisms. It is increasingly evident that significant quantities of cytokinins are synthesized in roots from whence they move to aerial portions of plants (Letham, 1967; Skene and Kerridge, 1967; Smith, 1969). Similarly, significant quantities of gibberellins are formed in roots from which they move into shoots (Skene, 1967). Thus, aerial irradiation of intact plants, potted in soil, would not be expected to reveal symptoms of declines in gibberellins and cytokinins even if the two synthesis pathways were equally radiosensitive to that of auxins. Even though
physiological interactions among cytokinins, auxins and gibberellins are well known (Helgeson, 1968; van Overbeek, 1966), it would be rational to attribute irradiation effects more to declining auxin levels than to increased gibberellin and cytokinin levels. Thus, there is little reason to suspect that endogenous levels of gibberellins and cytokinins were significantly affected by the 2500 r dose employed in this chrysanthemum study.

The evidence that ionizing irradiation retards auxin synthesis is so extensive as to be considered a well-known fact. Most of this evidence is derived from studies of irradiation effects upon well known auxin-mediated phenomena as noted below.

Rooting of cuttings is inhibited or greatly delayed following unshielded irradiation of African violet (Sparrow et al., 1960), poplar (Scandalios, 1964), coleus (Gordon et al., 1965) and chrysanthemum (William Duffett, personal communication) cuttings. Gamma-irradiation inhibits geotropic phenomena in herbs (Holmsen et al., 1964) and woody species (Sax and Schairer, 1963). Apical dominance loss often results from ionizing irradiation treatment. This is sometimes followed by such enhanced lateral shoot growth that radiation-induced growth enhancement is claimed (Meiselman et al., 1961b; Sax, 1963; Sax and Schairer, 1963; Savin, 1968).

In addition to gamma- and X-ray-induced auxin declines there is increasing evidence that ultraviolet light (ca. 254 nm) also causes declines in auxin biosynthesis (Klein, 1967). There are scores of other cases of irradiation-induced inhibition of auxin synthesis (e.g., Gunckel and Sparrow, 1961; Moore and Hough, 1962; Stein and Sparrow, 1966; Spang, 1966). Auxin molecules can be destroyed by in vitro irradiation (Skoog, 1935).
The elegant, thorough, researches of Gordon (1957) have formed a classical body of proofs that auxin biosynthesis mechanisms are extremely radiosensitive -- perhaps the most sensitive of enzyme systems in higher plants. He found that elongation of internodes and coleoptiles and rooting of leafed poplar cuttings was markedly inhibited by ionizing irradiation. Although apical dominance was lost subjacent to irradiated sunflower stem tips, exogenous applications of auxin were necessary for only two weeks to prevent lateral bud growth. Thereafter, all irradiated shoot tips had resumed usual levels of auxin biosynthesis and no lateral shoots grew. In addition, Gordon found that indoleacetaldehyde conversion into indoleacetic acid was inhibited by X-ray doses as low as 10 r. Auxin degradation mechanisms were apparently unaffected by any X-ray doses employed by him. Applied irradiation levels were directly correlated with amount of time necessary to the formation of usual endogenous concentrations of auxin, with little or no recovery above the dose range of 1000 to 2000 r in mung beans (Gordon, 1957).

In stating his generalizations on recovery time among irradiated plants, Gordon (1957) related auxin synthesis recovery times to amount of time necessary to the resumption of shoot growth. If these suggested correlations are correct, then it might be inferred that auxin synthesis and apex growth recovery times might vary among species beyond the absolute upper limits of 2000 r which Gordon found for mung beans. Moore and Hough (1962) suggested that auxin biosynthesis mechanisms might be more radioresistant in strawberry than Gordon noted for mung bean. These authors reported a seven day recovery time for auxin synthesis in irradiated strawberry plants. Exogenous applications of zinc, tryptophan, and/or indoleacetic acid can lead
to restitution of usual auxin levels in irradiated grains of barley (Kutacek et al., 1966) and wheat (Abrol et al., 1969).

Wounding, both by irradiation and other means, may have other pronounced subsequent effects upon auxin levels in angiosperms. In his second literature review on wound healing, Bloch (1952) noted that wounding frequently had an accelerating effect upon growth, sometimes by the occurrence of numerous mitoses parallel to wounds. Occasionally, the auxin precursor, indoleacetaldehyde, and auxin-like substances are found near wound sites. More recently, Leopold and Plummer (1961) reported that three phenols can be complexed independently with indoleacetic acid if polyphenolase is added to the reaction mixture.

Gordon and Paleg (1961) also investigated the formation of auxin through the action of polyphenolase. They discovered a reaction between phenols and tryptophan which resulted in auxin biosynthesis. They concluded that the "tryptophan-phenolase-phenol reaction could be considered as a latent, potentially operative, mechanism for auxin synthesis" in angiosperms. The phenolase-promoted transformation of tryptophan to auxin was not impaired by in vivo irradiation doses of 5000 r. Gordon and Paleg suggested that this auxin biosynthesis "probably is activated by the disruptions in cellular integrity accompanying chronic high-energy irradiation". Moreover, they noted that "consequent hyperauxiny could well be the basis for the hyperplasia and neoplasms that are commonly observed in such irradiated plants". Unfortunately, Gordon and Paleg did not suggest a general time relationship between wounding and in vivo maximum auxin synthesis by the phenolase system. No quantitative or time relationships were suggested between auxin
declines following irradiation, and the phenolase-mediated promotion of auxin synthesis.

There can be little doubt, then, that irradiation of intact plants probably results in immediate inhibition of the usual auxin biosynthesis mechanisms. Subsequently, however, the latent phenolase pathway appears to be activated and may account for higher than usual auxin levels.

Although many investigators have reported pronounced differential radiosensitivity among zones or regions in vegetative apices (e.g., Miksche et al., 1962; Pratt et al., 1959; Crockett, 1957, 1968; Kuehnert, 1962; Pratt, 1967, 1959; Sagawa and Mehlquist, 1957), no comparable zonal susceptibilities to gamma-ray-damage occurred in 'Criterion' irradiated at zero or seven short days. Commonly, other workers have noted that the most extensive damage occurs in the second or deeper mantle layers (e.g., Pratt, 1967; Pratt et al., 1959; Kuehnert, 1962; Crockett, 1957, 1968).

In some respects, similar phenomena were observed in 'Criterion' but necrosis of any one entire mantle layer was never observed. Most commonly, scattered cells in various mantle and sub-mantle layers (Figs. 37, 41, 44, 46) died and disintegrated. At other times, necrotic lesions included the protoderm (Figs. 36, 41). Thus, the relationships between highest radio-sensitivity and location of cells in chrysanthemums remain obscure. The lack of agreement between this study and those of others provides additional evidence that reproductive apices are significantly different, physiologically, than vegetative apices. Indeed, there is a wealth of literature which details physiological and morphological variations between vegetative and reproductive apices (Searle, 1965; Cutter, 1965; Allsopp, 1964). Nevertheless it
would be worthwhile to investigate spatial differential radiosensitivities in chrysanthemums growing under strictly non-inductive conditions.

Although there was a measurable, pronounced nuclear shrinkage, particularly in the apex summit, induced by early irradiation treatments, it appears that the majority of affected cells survived. That is, no massive necrotic lesions subsequently developed at the summit of apices irradiated at zero or seven short days. The survival and apparent recovery of cells in which nuclei shrank measurably, does suggest that a careful reconsideration of some histochemical data and chromosomal volume calculation methods are in order.

Since interphase nuclear volume varied greatly among apex positions, the quantity of stain in a nucleus must be considered with extreme reservation when the physiology of apices is studied. In assaying histochemical staining variations in shoot apices, intensity of nuclear staining must be related to specific nuclear volume. Otherwise, one cannot draw realistic inferences concerning differentiation within apices. On the other hand, qualitative staining variations of nuclei undoubtedly reflect physiological differences among nuclei.

As Sparrow (1962) has pointed out, mean interphase nuclear volume has an unquestioned relationship to differential radiosensitivity. Typically, mean chromosomal volumes are derived by dividing mean interphase nuclear volume by the chromosomal number (Sparrow et al., 1968a), implying that nuclei consist merely of chromosomes. If post-irradiation nuclear shrinkage occurs, chromosomal and/or non-chromosomal nuclear material undoubtedly move out of affected nuclei. Perhaps, some of the "nucleoplasm" (Whaley et al., 1960) moved out of nuclei after early gamma-ray treatment.
Whereas mean chromosomal volume, within certain limits, frequently correlates directly and well with lethal or inhibitory irradiation doses, it appears that mean chromosomal volumes, as calculated above, are not fortuitous. Thus, it might be inferred that each chromosome of a nucleus is endowed with a definite amount of nucleoplasm in direct proportion to chromosome volume.

If the overall mean interphase nuclear volume (ca. 309 cubic micra) in 'Criterion' vegetative apices were considered, irrespective of chromosome number, then approximately 200 r daily (chronic) exposure to gamma-rays should produce "severe growth inhibition". Mean interphase nuclear volume of vegetative 'Criterion' apices, irrespective of chromosome number, should place 'Criterion' intermediate among Allium cepa, Helianthus annuus, Vicia faba, and Pisum sativum, with respect to doses necessary to promote "severe growth inhibition" (Sparrow and Miksche, 1961). Whereas greenhouse and garden chrysanthemums are usually hexaploid (2n = ca. 54 = 6X) (Dowrick, 1953; Sampson et al., 1958), 'Criterion' may be more radio-resistant than diploid Pisum, Helianthus, Vicia and Allium. Unfortunately, there are no published data for radiosensitivity of vegetative 'Criterion' plants growing under chronic gamma radiation.

In many apices, especially from early irradiation treatment dates (0,7 short days) radiation-induced damage to cells at the apex summit was not evident until 14 or more days had elapsed (Figs. 37, 38, 39). In general, it often appeared that such delayed necrosis was not expressed in apex summits, or floret primordia (Fig. 41) until they were involved either in floret initiation or initiation of floret parts.

Superficially, such results could be interpreted to mean that the summit
portion of early inflorescence apices is in a more quiescent state than the apical flanks. Although it is by no means universal, there is evidence in several species that cells at the summit of shoot apices divide less frequently than other apex cells (Clowes, 1961, Ch. 6).

Vegetative shoot apices of Helianthus annuus are comprised of a central zone, in which nuclei are large and stain faintly in the Feulgen DNA reaction, and a peripheral zone in which nuclei are smaller and stain much darker in the Feulgen reaction (Steeves et al., 1969). There is no significant incorporation of tritiated thymidine in the central zone but there is extensive thymidine incorporation in the peripheral zone of in vitro Helianthus apices (Steeves et al., 1969). With the onset of inflorescence ontogeny, tritiated thymidine is incorporated uniformly throughout Helianthus apices (Steeves et al., 1969). Steeves and associates inferred that DNA synthesis and mitosis were "arrested" in the central zone of vegetative apices of sunflower.

In vegetative Chrysanthemum morifolium plants, cells in the "flank zones" divide with an average frequency of 70 to 73 hr (Berg, 1969). In the "apical zone", mantle cells divide with a mean frequency of 135 hr; cells of the "central zone" (the corpus) divide with a mean frequency of 102 hr (Berg, 1969).

The delayed necrosis of cells at the apex summit, especially following irradiation at zero or seven short days, may be another clue that cells of the apex summit are relatively quiescent in vegetative and early reproductive stages in chrysanthemums. Nevertheless, significant numbers of mitoses have been observed at the summit of vegetative apices of several angiosperms (Soma and Ball, 1963; Ball, 1961; Popham, 1958). The summit of vegetative apices is physiologically active in synthesis of insoluble
carbohydrates, proteins, and RNA (West and Gunckel, 1968a, 1968b). The distal portion of shoot apices in many species has a lower rate of DNA synthesis than other apex portions (West and Gunckel, 1968b). Considerably more data are necessary to evaluate the physiological and morphological dynamics in vegetative and reproductive apices of chrysanthemums between zero and 28 short days. Only then can the relative importance of the somewhat quiescent cells in the summit of *Chrysanthemum* apices be evaluated.

Unfortunately, some highly pertinent information was not obtained from 'Criterion' terminal capitula at maturity when multiple floret 'mutant' color sectors were measured. It would be extremely valuable to ascertain the precise acropetal-basipetal extent of 'mutant' ray florets in respect to each irradiation treatment date between zero and 21 short days, after which multiple floret 'mutant' sectors were no longer inducible (Table 5). Suppose, for example, that irradiation at 14 short days resulted in the formation of one multiple floret 'mutant' sector which included all rays from the most acropetal down to those adjacent to the involucre. Then, presuming that all 'mutant' cells of one sector originate from one original 'mutant' cell, it could be inferred that one cell, on the apex flank, was the forerunner of all of the 'mutant' epidermal cells of one multiple floret sector. Since apices are large on the 14th short day, it might be considered unusual that one cell could be the forerunner of the epidermis of all ray florets of one sector in plants irradiated on the 14th short day. If one apex flank cell were such a forerunner, then one might be able to suggest that some cells of the apex flank contributed significantly more cells to ray florets than others, and that some cells of the apex might, consequently, be relatively quiescent. Because
exact acropetal-basipetal distribution of 'mutant' cells was not ascertained, however, these eventualities are, at present, mere conjecture.

The delayed necrosis of some apical cells and the apparent relative quiescence of some *Chrysanthemum* apex cells would lead to the inference that many more data are necessary to elucidate and evaluate apex dynamics during reproductive growth in chrysanthemums. At a minimum, data concerning histochemistry, mitotic frequency, mitotic cycle time, and acropetal-basipetal distribution of induced 'mutant' florets are necessary for the period zero through 21 short days in order to clarify the dynamics of growth and the relative cellular contributions of each apex cell in 'Criterion'. Only then can the existence of relatively quiescent areas be defined or rejected.

Another explanation of delayed necrosis might involve lethal gene action. Conceivably, certain late-acting, but essential, genes could have been damaged by early (zero through seven short days) irradiation treatments without immediate effects. Then, as inflorescence ontogeny progressed, it is conceivable that cell necrosis might occur at a time when affected essential, late-acting genes usually function. Such a mechanism has been proposed to explain sequential lethal action of genes during animal embryology (Gluecksohn-Waelsch, 1963).

The extensive cell necrosis which followed irradiation at 14 short days provided evidence that terminal apices of chrysanthemums were most radiosensitive at that time and also fully confirmed that early stages of inflorescence ontogeny are extremely radiosensitive as several other investigators have indicated in other species (Gunckel, 1957; Yamakawa and Sparrow, 1966; Sagawa and Mehlquist, 1957; Gupta and Samata, 1967; Brown
and Taylor, 1967). At 14 short days, apices had extremely rapid mitosis as indicated by changing apex dimensions and cell counts. Since extensive cell necrosis and most extreme fasciation resulted from treatment at 14 short days, it may be inferred that apices in stages of rapid mitosis are most radiosensitive. Rapidly growing apices of woody species, with large interphase nuclear volume, are more radiosensitive than dormant apices with smaller interphase nuclear volume (Sparrow et al., 1968b). Mikaelson (1969) reported that barley cells are most susceptible to gamma-ray damage when they are in the G₂ phase, immediately after DNA synthesis. Evans (1965) found that meristematic cells were highly radiosensitive, particularly in the G₂ phase, late in interphase. Contrary to these results, Haber and Rothstein (1969) reported equal radiosensitivities among meristematic and non-dividing leaf cells. Nevertheless, there are numerous data which indicate that apical meristems are extremely radiosensitive (Pratt, 1959, 1960; Pratt et al., 1959; Miksche et al., 1962; Sagawa and Mehlquist, 1957).

Massive necrosis of the apex summit appeared to be the primary cause of fasciation observed among irradiated apices. Microsurgical experiments, particularly those performed before 21 short days, caused similar bifurcations of apices. Thus, one of the main causes of chrysanthemum fasciation appears to be the isolation of growth centers in meristematic apices after massive wounding.

Wound healing, by cambium-like growth, observed in many apices after irradiation, was probably associated with increased auxin biosynthesis. Gordon and Paleg (1961) suggested that a usual result of irradiation-induced lesions might be hyperauxiny by the phenolase pathway of auxin biosynthesis. Gorter (1965) reported that three auxin-like substances were present in
higher concentrations in the *fasciata* type of *Pisum sativum* than in non-fasciated types. Moreover, she found no evidence that variations in endogenous gibberellin levels were associated with fasciation. Elsewhere, unusually high or low concentrations of growth substances have been assumed to cause fasciation (Bloch, 1953).

That supernumerary involucral bracts frequently formed adjacent to wound sites, whether induced by irradiation or whether induced by early microsurgery, suggests that involucral bracts differentiate under increasing auxin levels. In *Tradescantia*, additional bracts form following irradiation of primordial inflorescences (Gunckel et al., 1953). Although no micro-surgically bisected chrysanthemum apices reverted to the vegetative state, Wardlaw (1961b, 1963) found that such reversions were common after apex bisection in *Petasites* (Compositae). Neither Gunckel et al. (1953) nor Wardlaw (1961b, 1963) presented any possible physiological explanations of these results.

Since irradiated 'Criterion' apex recovery and subsequent bifurcation (Figs. 40, 47) strikingly resemble recovery from apex bisection (Figs. of Soetiarto and Ball, 1969; Soma, 1958; Ball, 1950a), one can conclude that post-irradiation apex bifurcation is a result of extensive cell necrosis and subsequent wound healing. There is little reason to assume that apex fasciation in chrysanthemums is a consequence of any direct radiation-induced effects other than the causing of cell necrosis. It appears that enhanced auxin synthesis, by the phenolase pathway, may be a usual result of apex wounding and a concomitant occurrence with wound healing.

Genotypically different color sectors on ray florets were extremely common results of all gamma-ray treatment times. Ionizing irradiation is
an effective method of inducing visible and cryptic chimeras at frequencies higher than spontaneous levels under natural background irradiation. Moreover, if only the multiple floret sectors in terminal heads (Table 5) are considered, the greatest number and largest size of sectors resulted at the time of renewed or most rapid mitosis in terminal apices (seventh and 14th short day, Table 5). Precise relationships between mitotic frequency and induced 'mutation' frequency are difficult to evaluate, however. The difficulty results because (a) color-'mutants' possibly represent only a small fraction of actual induced genotype changes, and (b) precise genetic mechanisms of color changes cannot be defined in these chrysanthemums.

Occasionally, induced mutant cells have different mitotic frequencies than non-mutant cells, in which case there may be differential survival of living mutant and non-mutant cells ("diplontic selection", Gaul, 1964). Diplontic selection is a common consequence of irradiation treatment (Gaul, 1964). Thus, actual mutation frequency cannot be assessed because there often is differential survival of cells in irradiated multi-celled apices.

Other approaches to study the relationship of mitotic frequency to efficiency of induced mutations in plants must be made. One new approach that would yield valuable data has been described (Broertjes et al., 1968; Broertjes, 1969). Broertjes and associates evaluate mutation induction in plant species in which individual adventitious plantlets originate from single cells. Thus, most of the difficulties of diplontic selection in chimeral plants are circumvented.

Theoretically, irradiation of the small, vegetative apices should result in the induction of largest ray floret variant color sectors, merely because the ultimate possible cell lineage contribution of any one cell in an apex
should be greatest at zero short days. Such was not the case. In terminal heads, the most frequent and the largest multiple floret 'mutant' sectors appeared in plants irradiated at 14 short days, at which time inflorescence apices were large (compare Figs. 2, 4, 5). It appears likely that sectors were smaller, on the average, in plants irradiated at zero short days than in plants irradiated at 14 short days because diplontic selection occurred before florets matured. Theoretically, diplontic selection would have occurred over a longer period (70 days) in plants irradiated at zero short days than in plants irradiated at 14 short days (56 days of diplontic selection). Such a mechanism of diplontic selection would explain the smaller, less frequent sectors found in plants irradiated at zero short days, versus the larger, more frequent sector found in plants irradiated at 14 short days if all 'mutant' cells mitosed less frequently than the original 'Criterion' cells. Conversely, and irrespective of sector size, the higher number (29 sectors, Table 5) of multiple-floret sectors in plants irradiated at 14 short days versus the 13 sectors (Table 5) observed in plants irradiated at zero short days, may indicate that there is a direct relationship between mitotic frequency and number of 'mutants' induced. At present, there are not sufficient facts to resolve these apparent incongruities.

Because greenhouse and garden chrysanthemums are hexaploids (2n = ca. 54) (Dowrick, 1953; Sampson et al., 1958) they undoubtedly harbor considerable genetic redundancy. If only one of six possible alleles of a trait were destroyed or damaged by ionizing irradiation, then there should be five other genes of the trait which could function without inevitable inhibition of growth or death of the 'mutant' cell. Theoretically, then,
diplontic selection should occur less frequently, in respect to essential genes, in a polyploid than in a haploid or diploid plant.

'Mutant' sector induction in 'Criterion' could be a result of one or more genetic phenomena exclusive of diplontic selection. In an extensive survey of many chrysanthemum clones, Dowrick (1951, 1953) found that chromosome number variations frequently were associated with changes in leaf form and flower color in numerous 'families' ('mutant' series). Specific causal relationships could not be claimed with certainty because chrysanthemum chromosomes are small and lack inter-chromosomal morphological distinctness (Dowrick, 1953). Thus, there could be a simultaneous loss of one specific chromosome and a gain of another specific chromosome without any visible effect upon karyotype but with a change in cell genotype. With similar thoroughness, Sampson et al., (1958), from root tip squashes, reported that gain or loss of chromosomes accompanied flower color changes in 26.3% of varieties investigated.

Dowrick and El-Bayoumi (1966) have suggested that most mutant chrysanthemums are periclinal chimeras. This is not surprising because 30% of commercially cultured chrysanthemums have originated by mutation (Wasscher, 1956).

In addition to possibly causal chromosome number changes, many 'mutations' are attributable to "breaking" of periclinal chimeras (see Sagawa and Mehlquist, 1957; Dowrick and El-Bayoumi, 1966; Weaver, 1963; Bowen et al., 1962). Sagawa and Mehlquist (1957) have ably illustrated that radiation-killing of L-1 apex cells may be followed by reconstitution of a shoot apex from surviving sub-protodermal cells. This will alter cell and plant phenotype, in L-1 periclinal chimeras, because petal pigments in
angiosperms are usually limited to epidermal cells which, in turn, are
generally derived from surface mantle cells. Several workers have alluded
to the "breaking" of periclinal chimeras to explain flower color changes in
chrysanthemums (Weaver, 1963; Bowen et al., 1962).

Although there is a remote possibility that some bronze, white or
yellow sectors in 'Criterion' were attributable to the "breaking" of a chimera,
several facts indicate that 'Criterion' is not a periclinal chimera with
respect to ray floret color. Chimeral plants appear to have higher rates
of flower color change than non-chimeral ones (Weaver, 1963; Bowen et al.,
1962; Perea-Leroy, 1969). 'Criterion' has a very low rate of spontaneous
flower color change (William Duffett, personal communication). 'Criterion',
which was a seedling selection which did not originate by mutation in an
established clone (William Duffett, personal communication), has only
yielded one commercial mutant, 'Whiterion'.

Commonly, pink chrysanthemums have the greatest spectrum of
'spontaneous' flower color mutations or irradiation-induced flower color
changes (Nybom, 1960; Jank, 1957; Broertjes, 1966). Since 'Criterion' has
pink-, bronze-, and white-flowered immediate ancestors (William Duffett,
personal communication), genetic heterozygosity may be great. Thus, there
are few reasons to believe that 'Criterion' is a chimeral clone with respect
to ray floret color.

With equal facility, variations in frequency of bronze, white or yellow
'mutant' sectors could be attributed to many genetic phenomena, no one of
which could be ascertained easily. The fact that bronze, yellow, and white
sectors occurred with unequal frequencies (Table 5) may be due to unequal
radiosensitivities of genes which control ray floret color. Different
frequencies of bronze, white, and yellow ray floret 'mutant' sectors may result because the number of controlling genes varies. Theoretically, there could be fewer white sectors than bronze sectors (Table 5) because there are more genotype changes necessary to the formation of white sectors than to the formation of bronze ray floret sectors.

Unequal frequencies of bronze, white, and yellow sectors in terminal heads could also result because the various genes which control flower color may be located at varying distances from chromosome centromeres. Chromosome breaks are a common result of ionizing radiation treatment (Sparrow, 1962). Given a constant dose of irradiation, it is more likely that a chromosome break will occur in a long chromosome than in a short chromosome. Conceivably, the loci which control ray floret color in chrysanthemums may be located at various distances from chromosome centromeres. Thus, unequal frequencies of variant color sectors may be a result of varying frequencies of "lost" genes controlling flower color.

Indeed, there is no absolute proof that ionizing irradiation did induce specific point changes (=gene mutations) in 'Criterion' chromosomes. It is easily conceivable that gene mutation, in the narrow sense, did not occur in any cases of flower color change. Apparent 'mutations' may have resulted from nuclear genotype changes by mere gain or loss of one or more chromosomes or chromosome parts.

Whereas bronze in chrysanthemums is probably due to a mixture of red and yellow pigments (William Duffett, personal communication), it is possible that yellow sectors, which were observed only in lateral heads of irradiated plants, were not due to any genotype change. Lateral heads matured after most terminal heads. Because lateral heads of all intact irradiated and
control inflorescences matured in late June, under increasing temperatures and light intensity, mechanisms elucidated by Rutland (1968) might be important. He found that potentially pink chrysanthemum ray florets are pale or colorless under certain cultural regimes of high temperatures or intense light. Thus, it is highly probable that the superficially yellow cells were genetically identical to those of phenotypically bronze ray florets. Hypothetically, yellowness would result if the red-pink component of a bronze sector were absent.

In view of the polyploidy, heterozygosity, and the lack of unique interchromosomal morphology, it is hopeless to pursue specific genetic and cytogenetic causes of ray floret color changes in 'Criterion'. Indeed, as Nilan (1967) observed, "except for some detailed information from a few well-studied loci in maize, our knowledge about the nature of both spontaneous as well as induced mutations in higher plants is scanty and highly speculative".

On a more positive note, histological facts and the distribution patterns of variant color sectors suggest that 'Criterion' ray floret corollas develop as those of most angiosperms. Esau (1965, p 575) reported that perianth parts usually have initial apical activity followed by intercalary growth. Extensive cell necrosis appeared to occur more frequently in highly meristematic cells than in slowly dividing cells. Since tips of many primordial ray floret corollas were killed by gamma-ray treatment on the 35th short day, results suggested that affected corolla tips were highly meristematic at the treatment time. At later irradiation times, where no detectable cell necrosis occurred, variant-color flecking developed over most of the adaxial corolla surface. Thus, it may be inferred that early
corolla growth is mainly apical, but subsequent corolla development occurs by intercalary mitosis over most of the 'Criterion' ray floret surface.

Apical corolla necrosis played a measurable role in causing some of the common ray floret incision or laceration when plants were irradiated after 28 or 35 short days. Disintegrating cells were observed at corolla tips subsequent to treatments. Because early irradiation treatments, before floret initiation, also induced corolla tip laceration, other mechanisms probably accounted for some of the laceration, or incision, of corolla tips. Perhaps living cells, in which mitotic inhibition was induced by gamma-ray treatment, were lifted to critical sites by growth of adjacent cells. In certain critical positions, inhibition of mitosis and cellular enlargement could account for ray floret tip incision or laceration.

Laceration of ray florets could result from mitotic inhibition or from a gamma-ray-induced modification of gene expression in chrysanthemums. Among chrysanthemum varieties, typical ray florets are highly variable, and corolla tips may be tubular, entire and flat, spoon shaped, incurved or feathered. In the "anemone" types, there often is a gradual transition from typical peripheral ray florets to typical central disc florets. Furthermore, the 'Whitetop' chrysanthemum has open ray florets but some of its mutants have completely tubular rays (e.g., 'Tip Top') or deeply serrated ray floret tips (e.g., 'Feathertop'). Thus, types of ray florets may be controlled by complex genetic mechanisms. Environmental conditions also can play significant roles in determining ray floret types. Under bright, warm days, 'Indianapolis' varieties usually have open ray corollas; under lower temperatures and/or lower light intensities, ray florets are often closed and tubular ('quilled') (William Skou, personal communication).
Irradiation and microsurgical experiments each yielded results which would suggest that disc floret differentiation: (a) occurs under low auxin levels, and (b) may be decisively influenced by auxin formed in involucral bracts. Gamma-ray treatment given at 21 short days, two weeks before the time of usual morphological differentiation of disc florets, markedly enhanced the number of disc florets which differentiated (Table 2; compare Figs. 16, 19, 21).

After early irradiation treatments (0 to 7 short days), terminal heads generally had two alternating sets of ray and disc florets (compare Figs. 10, 42, 20, 22). Capitula which had these alternating cycles of ray and disc florets had two separate periods of physiological conditions which were conducive to ray floret differentiation, since disc florets differentiate acropetally. Moreover, receptacular necrotic lesions and supernumerary involucral bracts formed in terminal capitula after irradiation treatments at zero, seven, or fourteen short days.

In the microsurgical experiments, supernumerary involucral bracts frequently differentiated proximal to sites of apex-capitulum wounding—but only if wounding occurred between zero and 15 short days. Furthermore, disc floret regions did not form adjacent to those wound sites (Figs. 52, 54, 60). That is, early irradiation (0-21 short days), early wounding (0-22 short days), and/or the formation of supernumerary involucral bracts (0-15 short days) caused decisive modification of the typical pattern of disc floret differentiation. Furthermore, longitudinal microsurgical slitting, either as two or three cuts, modified disc floret differentiation patterns if those incisions were made before the 23rd short day was complete. The reader is
reminded that extensions of the disc floret regions formed after these incisions were made.

The following hypothetical model is suggested for future tests. Early (0-15 short days) wounding of apices probably results in promoted auxin biosynthesis by the phenolase pathway (Gordon and Paleg, 1961). Hyperauxiny and subsequent wound repair are, then, associated with differentiation of involucral bracts adjacent to wounds. Later, higher than usual auxin levels, from the wound sites and/or supernumerary involucral bracts, prevent disc floret differentiation adjacent to wounds and supernumerary involucral bracts, but do not prevent ray floret differentiation there.

Early irradiation (0-7 short days) depresses auxin biosynthesis in 'Criterion'. Under sub-optimum auxin levels, disc florets differentiate low on the receptacle flanks in the usual position of ray florets. As recovery of auxin biosynthesis occurs, possibly by the usual pathway, and also by the phenolase pathway (Gordon and Paleg, 1961), levels become sufficiently high that a second increment of ray florets differentiate acropetal to the unusually located extra disc florets. Subsequently, auxin biosynthesis declines and the usual increment of disc florets differentiates at the receptacle summit. Supernumerary involucral bracts do not form, and auxin concentrations decline precipitously if irradiation occurs at a critical later time (e.g., at 21 short days). Then, a large-diameter disc floret region differentiates merely because complete auxin biosynthesis recovery does not occur until after all florets are differentiated as either disc or ray.

Because leaf primordia form auxin (Wetmore and Pratt, 1949; Wetmore and Wardlaw, 1951), it is likely that involucral bracts are significant sites of auxin synthesis.
In the usual sequence of events, disc florets only differentiate at sites distant from involucral bracts. If, however, irradiation treatment occurs at a critical time during rapid involucral bract growth (e.g., at 21 short days), or if the receptacle flanks, including part of the involucre are removed by two or three longitudinal incisions (e.g., at 21, 22 short days), the disc floret region is theoretically modified from the usual merely because the involucral bracts' contribution of growth regulators is disrupted.

Although the evidence is scanty, there is some evidence that substance C (Fig. 60) might be extremely important to ray and disc floret differentiation. Its concentration declines precipitously as the involucral bract growth rate abates at the critical period just before disc florets are differentiated from ray florets (Fig. 60). It is postulated that substance C (Harada and Nitsch, 1959): (a) is synthesized in foliage leaf and bract primordia as well as in involucral bracts, (b) is an auxin, (c) is a substance, the synthesis of which is highly radiosensitive, and (d) moves acropetally in the receptacle where it promotes ray floret differentiation at concentrations which inhibit disc floret differentiation. Substance C may inhibit stamen growth at concentrations which promote ray floret differentiation.

There are numerous, simple experimental procedures which can resolve all of these hypotheses. Initially, a most expedient procedure should involve (a) the careful microsurgical removal of all involucral bracts between the 21st and 28th short day, (b) chromatography of cold methanol extracts of the isolated involucral bracts, (c) Avena coleoptile growth tests of involucral bract extracts by methods identical to those of Harada and Nitsch (1959), and (d) allowing the involucre-deficient capitula to mature at a high relative humidity to prevent drying of the remainder of the capitulum.
It is conceivable that ray or disc differentiation might be elicited by changing ratios of endogenous auxins and gibberellins but this possibility is unlikely. Among numerous papers reporting effects of exogenously applied gibberellins and anti-gibberellins in chrysanthemums, there are no suggestions that ray and disc floret ratios are distorted from the usual. Conceivably, ray and disc floret differentiation also might be affected by cytokinin ratios to gibberellins and auxins. At present, there are no data concerning the effects of varying levels of cytokinins on inflorescence ontogeny in chrysanthemums. In Carex, however, Smith (1969) has presented evidence that cytokinins formed in roots, influence inflorescence form. In spite of conceivable roles of gibberellins and cytokinins on sex reversal and ray and disc floret differentiation in chrysanthemums, the majority of facts leads to the inference that variations in auxin levels are the primary determinants of ray and disc floret differentiation.

A consideration of terminal head diameters at maturity (Table 4, Fig. 31) is necessary. From Sachs' (1968) data, it is evident that rapidly growing floret primordia are sources of gibberellins and auxins in Gerbera (Compositae). Moreover, Harada and Nitsch (1959) found that the extractable, auxin and/or gibberellin-like substances A and B markedly increase from previously stable levels following the 35th short day in chrysanthemum 'Shasta'. Since this time period coincides with one of rapid floret elongation in 'Criterion', it seems probable that: (a) substances A and B are associated with rapid floret growth, and (b) florets form at least part of the extractable quantities of substances A and/or B. Furthermore, it appears that the biosynthesis of these substances might be susceptible to gamma-ray inhibition. If so, causes of significant inhibition of terminal head growth in
diameter, induced by irradiation at 35, 42, and 49 short days (Fig. 31), are resolved. The tentative hypothesis is that higher concentrations of substance C (Harada and Nitsch, 1959) promote corolla development, inhibit stamen development and cause differentiation of florets into ray florets. Lower concentrations of substance C are hypothetically causal to disc floret differentiation. Substances A and B (Harada and Nitsch, 1959) appear to be associated with continued rapid elongation of some or all florets.

Among mature plants of various irradiation treatment times, there are few explanations of patterns of lateral head development (Fig. 33, Table 6). Data are perplexing in that early irradiation treatments (0, 7 and also, perhaps 14 short days) should, theoretically, promote precocious auxin declines in addition to those usual auxin declines of the onset of photoinduction. Therefore it might be theorized that early irradiation treatment should enhance the number of lateral heads which developed instead of significantly inhibiting lateral inflorescence development (Fig. 33).

Because capitular lesions and supernumerary involucral bracts frequently developed on plants irradiated at zero and seven short days, these modifications may have been responsible for depressed lateral head development. It is conceivable that irradiation-caused lesions could be sites of auxin synthesis by the phenolase pathway (Gordon and Paleg, 1961). Thus, the resultant enhancement of auxin synthesis might cause greater inhibition of lateral bud growth than occurred in controls.

As expected, late gamma-ray treatments (28 through 49 short days) were ineffective in altering lateral head numbers. Since rapid lateral bud growth was occurring on the 21st short day (Fig. 6), there is little reason
to suspect that later disruptions of apical dominance mechanisms would appreciably alter the number of lateral heads which flowered.

On any one irradiated plant, numerous morphological variations occurred among lateral inflorescences. Although histological ontogeny of lateral capitula was not pursued in this study, it is virtually certain that one could predict histological stages and acropetal-basipetal sequences of lateral head ontogeny in a simple manner. Since kinds of variations in lateral heads were identical to those of terminal heads, one could use terminal head histological and gross morphological data to project the histological state of each lateral head on any one irradiation treatment date. Thus ionizing irradiation can be a valuable tool for studying normal morphogenesis, thereby minimizing the necessity of making a complete histological examination to ascertain sequences of development of numerous plant structures. Several students of plant morphogenesis (e.g., Mericle and Mericle, 1969; Ichikawa and Sparrow, 1968; Cuany et al., 1957; Kaukis and Reitz, 1955; Gupta and Samata, 1967; Buiatti and Ragazzini, 1965) have observed the distribution, sizes, and shapes of induced mutant sectors to ascertain patterns of ontogeny of leaves, stems, and flowers.
SUMMARY

*Chrysanthemum morifolium* Ramat. 'Criterion' plants were investigated to ascertain effects of single 2500 r total acute gamma-ray treatments, given over 20 hours, at zero, seven, 14, 21, 28, 35, 42, or 49 days after continuous daily photoinduction cycles commenced. Ontogenetic events, ascertained from weekly histological study collections of all previously irradiated plants, were correlated with gross morphological variations observed among mature plants.

In non-irradiated 'Criterion' plants there was little anatomical change in apices during the first week of photoinductive nights. By the 14th short day, rapid growth in width and height occurred in all apices. During subsequent rapid acropetal initiation of involucral bracts, mean involucral bract plastochron duration decreased to 0.4 day from the approximate 2.1 days of the foliage leaf plastochron. After approximately 38 involucral bracts were initiated, acropetal floret initiation commenced on the enlarging inflorescence apex between the 15th and 21st short day. Acropetal floret initiation was rapid and occurred in cycles of 22 to 40 florets each. Mean floret-cycle plastochron was approximately 23 hours. By the 28th short day, the majority of florets were initiated on most terminal capitula. By the 35th short day, involucral bracts, which enveloped the receptacle and florets, decreased in growth rate. Concomitantly, florets commenced rapid growth and had differentiated into either ray or disc florets by the 35th short day. About 350 ray florets and 17 disc florets formed in typical terminal heads. By the 42nd
short day, the incipient disc florets were longer than the adjacent, slightly older, ray florets. Meiosis had commenced in some ray floret ovules and disc floret anthers by the 49th short day. Ray florets were strictly pistillate but disc florets were perfect. Phasic changes in growth rate and kinds of parts which differentiated were, in part, attributed to phasic changes in endogenous levels of gibberellins and auxins.

Irradiation at zero or seven short days did not markedly delay first floret initiation. Irradiation after the 14th short day did, however, delay first floret initiation by seven to 14 days. Inhibition of last floret initiation was delayed in all plants irradiated after zero, seven, 14 and 21 short days. Delayed floret initiation appeared to result from mitotic inhibition. There was no evidence that phytochrome mediated phenomena were affected by treatments.

Radiosensitivity varied greatly among the weekly treatment groups as well as among criteria considered. Maximum inhibition of stem elongation and terminal head growth in diameter and maximum promotion of fasciation occurred in plants irradiated just after the 14th short day. Irradiation at zero short days enhanced terminal head growth in diameter but greatly inhibited lateral inflorescence development. The greatest number of gamma-ray-damaged foliage leaves occurred following irradiation at zero short days.

Alterations in terminal head symmetry and distribution of ray and disc florets could be induced by irradiation treatments only at zero through 21 short days. Gamma-ray treatment at 21 short days was most effective in inducing differentiation of a maximum number of disc florets. Gamma-ray treatment promoted the differentiation of perfect disc florets from floret
primordia which otherwise were in locations typical of male-sterile ray florets.

Necrotic lesions on the apex-receptacle occurred after all irradiation treatments given between zero and 14 short days. Largest lesions occurred in plants irradiated after 14 short days. Subsequently, and during wound healing, supernumerary involucral bracts differentiated adjacent to radiation-induced lesions. Induced supernumerary involucral bract initiation occurred in comparable but unirradiated plants following microsurgical wounding.

Median microsurgical bisections or punctures of apices made in early stages (after zero through 14 short days) caused many of the same effects of ionizing irradiation treatments. Microsurgery induced fasciation, supernumerary bract formation as well as modification of disc and ray floret differentiation patterns and ratios. Fasciation, therefore, appeared to result from isolation of living growth centers by cell necrosis caused by irradiation or by microsurgical treatment.

Typically, involucral bracts appear to differentiate during periods of increasing auxin concentrations. Similarly, there is evidence that supernumerary bract formation occurs during wound healing accompanying hyperauxiny.

There is considerable evidence that disc florets differentiate during stable or decreasing auxin levels, whereas ray florets differentiate during increasing or high auxin concentrations. Irradiation-caused decline and recovery in auxin biosynthesis appear to be the chief causes of modification in ray and disc floret distribution and ratios.

In non-irradiated, as well as irradiated and microsurgically treated plants, involucral bracts appear to be likely sources of growth substances
which play a role in disc floret differentiation. Microsurgical removal of
some involucral bracts and receptacle flanks enhanced localized development
of disc florets. Disc florets usually differentiated in control plants after
involucral bract growth slowed or ceased. With the exception of variant
color sectors, most of the effects of gamma irradiation were interpreted to be
the result of cell death, wound healing and disruption of usual auxin levels.
There was no evidence that gibberellin and cytokinin biosynthesis was
affected by irradiation treatment.

Multiple-floret variant color sectors in terminal heads were induced by
irradiation after zero, seven, 14 or 21 short days. Largest and most
frequent terminal head multiple floret sectors were found in plants
irradiated after 14 short days. Among lateral heads, multiple floret variant
color sectors were inducible by treatments through 28 short days. In them,
largest sectors appeared in plants irradiated after seven short days. Highest
mean frequency of multiple floret sectors in lateral heads occurred in plants
irradiated after 14 short days. Since there is little evidence that 'Criterion'
is a periclinal chimera, all variant color sectors were attributed to
irradiation-caused genotype changes.

The distribution and frequency of laceration and 'mutant' spots, streaks,
or sectors among ray florets confirmed that initial ray floret corolla growth
is apical; subsequently, there is intercalary growth over the entire ray floret
corolla.
### Statistical Data

Analysis of Variance and Covariance (Factorial Design)

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LITERATURE CITED


