1978

Anatomical Studies During Floral Induction, Evocation and Initiation in Eucharis Grandiflora, Planch., The Amazon Lily

Raphael T. Deba

ANATOMICAL STUDIES DURING FLORAL INDUCTION, EVOCATION AND INITIATION IN EUCHARIS GRANDIFLORA, PLANCH., THE AMAZON LILY

BY

RAPHAEL T. DEBA

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Biology, South Dakota
State University

1978

ANATOMICAL STUDIES DURING FLORAL INDUCTION,
EVOCATION AND INITIATION IN EUCHARIS
GRANDIFLORA, PLANCH., THE AMAZON LILY

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

ACKNOWLEDGEMENTS

This investigation would not have been completed without the cooperation of many people.

I would like to express my gratitude to the Faculty of the Botany-Biology Department at South Dakota State University for their friendliness without which my life would have been intolerable, and for their valuable criticism of the methods and photographs.

Dr. Gerald A. Myers, my academic and thesis adviser, deserves special thanks for his remarkable patience and kindness as well as his direction of the thesis.

John Swanson also deserves much credit for the production of the bulk of the photographic prints used in the study.

Finally I would like to thank Mrs. Cathy Haan for her excellent typing of the thesis.

RTD

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

> "Bulbs are in many ways extremely appropriate for fundamental researches on flower formation and extension growth at various temperatures. In the first place, the vegetative offspring of bulbous plants, carefully selected for uniform size, can be used in limited numbers for each experiment. A series of bulbs requires only small space for treatment or storage, ... Moreover, the growing point inside the bulb, protected by the surrounding scales, provided by them with food substances, is able to initiate a flower and to develop under the influence of temperatures without interaction of light-controlled processes. Finally, slightly differing temperatures were found to produce distinctly different effects, either directly or as an after-effect." (Hartsema, 1961)

Wellensiek (1961) emphasized that a better understanding of the theoretical backgrounds of flowering would be highly valuable, since control of flowering would be rational and more certain.

There are five major areas which are appropriate to the comparison of the vegetative shoot apex and the apex in transition to flowering: (1) Zonation anatomy, a description of the topography of the shoot apex; (2) Thermoinduction, achieving floral induction through heat elevation for differing durations; (3) Initiation, growth and development of the lateral (axillary) bud; (4) Photoinduction; and (5) Cytohistology, an indexing of mitotic activity, chondriome, vacuome, RNA content, nucleolar volume, nuclear-cytoplasmic ratio and polysaccharide storage. Cytohistological studies and the study of possible interaction between photo- and thermoinduction were not included within the scope of the present investigation.

Because a variety of definitions have been found in the literature for several terms used in the study, a list of definitions which

best fit the needs of this investigation follow:

FLORAL EVOCATION. Events at the shoot apex following the arrival there of a floral stimulus, but before the differentiation of flowers begins. (Waddington, 1966, cf. Evans, 1969).

FLORAL HORMONE. Graft transmissable substances from photoperiodically (or thermoperiodically) induced plants which evoke flowering in non-induced receptors (Myers, 1976).

FLORAL INDUCTION. Events in a leaf which commit plants to flowering (Moshkov, 1937, cf. Evans, 1969).

FLORAL INITIATION. Morphological manifestations of floral initials (Myers, 1976).

FLORAL MORPHOGENESIS. Events which are subsequent to floral evocation and which give rise directly to flower primordia (Evans, 1969).

FLORAL STIMULUS. Any translocated substance that evokes flowering (Evans, 1969).

FLORIGEN. Immediate products of leaves undergoing photoperiodic (or thermoperiodic) induction which causes evocation (Chailahjan, 1959, cf. Evans, 1969).

GROWTH TABLE. Greenhouse benches upon which plants were placed which exposed them to hours of light and temperatures of no higher than 18° C.

HEAT TABLE. Greenhouse benches upon which plants were placed which exposed them to pot temperatures of 26° C and hours of light (see Figure 2).

LITERATURE REVIEW

Zonation Anatomy: the Vegetative Apex

Popham (1966) reviewed the contributions of several authors on the anatomy of the shoot apex: Hanstein, 1878; Hofmeister, 1851; Lund, 1878; Nageli, 1845; Smith, 1924; and Wolff, 1759. On the basis of these studies, Popham identified seven types of shoot apical organization in vascular cryptogams and higher plants, based on anatomical structure.

Wardlaw (1968) reviewed recent works on shoot apex organization based on functional studies. He reported that Clowes used radioisotopes to determine the zonal rates of cell division in apices. Cutter used cytohistological techniques and experimental morphogenesis in her studies of shoot apices. Wardlaw presented his concept of shoot apical organization in terms of reaction systems and physiological fields. Schuepp analyzed histogenesis and growth mathematically.

Nougarede, *et al.* (1965) grew *Amaranthus retroflexus* under different photoperiodic regimes, recognizing pyroninophilic (cytoplasmic RNA concentration) responses in each zone of the apex.

In a thorough review of shoot apical organization, Gifford and Corson (1971) described many other organizational patterns reported up to that time in the literature: The metrameristem organization of Johnson and Tolbert; the gymnosperm type of Foster, with its surface initials and central mother cell zone and the *meristem d'attente-anneau initial* of Bersillon, which became the accepted pattern in much of the French literature. Other shoot apical organization patterns described

by Gifford and Corson included the dermatogen-subdermatogen-central meristem of Guttenberg, the *mantle-zentral mutterzellen* meristem of Senghas, the *zone apicale-meristem de flanc* of Nougarede, the dermatogen-hypodermis-central zone of Barnard, the protoderm-central zone of Klaus and the quiescent zone of Clowes.

All of the patterns reviewed by Gifford and Corson seemed to be variations that could be identified in the zonation patterns described by Popham (1966).

Zonation Anatomy: The Transitional Apex

In the review of the literature of the shoot apex, Gifford and Corson (1971) described the concept of homology between vegetative and flowering shoots. They also found the '*anneau initial*' functions during vegetative growth in the production of leaves and in the reproductive cycle in the production of sepals, and it may be used up in petal production. The *meristem d'attente* produced carpels and stamens. Literature reviewed also included writings on the vegetative apex and transition to flowering for both dicots and monocots, indicating citations, plant groups studied and general features studied.

Profound changes occur at the cellular and molecular levels in the shoot apical meristem during the transition from the vegetative to the reproductive condition. Studies on the behavior of the vegetative meristem during the change to the reproductive phase and in response to one or several photoinductive cycles have been made by Gifford and Tepper (1961) in *Chenopodium album;* Popham and Chan (1960) in *Chrysanthemum morifolium;* Bernier (1970) in *Sinapis alba;* and Salisbury

(1955) in *Xanthium strumarium*. There were other examples in the literature: Nougarede, *et al.* (1965) studied *Amaranthus retroflexus* and Evans (1969) studied *Lolium temulentum*.

Esau (1965) stated that the zonation of the vegetative apex sometimes might be retained in the reproductive stage. Indeterminate inflorescences of the family Cruciferae are of this type. The zonation of the vegetative apex disappeared in other cases as in the family Compositae which included the sunflower (*Helianthus annuus*) and the bachelor's button (*Gomphrena globosa*). Zonation of the vegetative apex was lost in the reproductive apex, as in the Monocotyledoneae.

Thermoinduction

Hartsema (1961) reviewed the literature relative to the understanding of the effects of temperature on flowering of bulbous plants. This review included the effects of temperature on flower initiation and flowering in a variety of bulbous monocots, including *Tulipa gesneriana, Hyacinthus orientalis, Narcissus pseudonarcissus, Iris tingitana, Iris reticulata, Allium cepa, Allium escalonicum, Allium sativum, Lilium longiflorum, Lilium regale, Galtonia candicans, Hippeastrum hybridum, Amaryllis belladonna, Nerine sarniensis, Zephyranthes rosea,* and some corm and crown producing species, *Gladiolus hybridus, Freesia sp., Convallaria majalis* and *Veratrum viride*. In connection with the work on bulbous monocots, seven different types of flower initiation were distinguished: Flowers were formed (1) during spring or early summer of the year preceding that in which they open; (2) after the end of the previous assimilation period

after the bulbs have been harvested and during the storage period; (3) sometime after replanting at low temperatures of winter or early spring; (4) started during or towards the end of the storage period, but has to be completed after planting; (5) after replanting in spring; (6) began more than a year before flowering; and (7) occurred alternate with leaf formation during the whole assimilation period. Young, developing flower buds were present at the same time with one-year-older, larger flower buds.

Studies of the influence of temperatures on development have allowed scientists to understand the limitation of some plants in special regions and the worldwide distribution of others. Understanding the natural potentials of plants enables us to grow these plants in localities outside their native habitats. Greenhouses and hothouses were built to provide capability of controlled environments which would allow the growth of foreign plants.

Study of the effects of environmental factors on flowering in bulbous plants has been rewarding and productive both for scientific and practical purposes.

In this review, Hartsema recognized that in some bulbous plants, flower formation took place long before flowering while in others, flower formation occurred almost simultaneously with leaf emergence.

The use of other-than-optimal temperatures produced growth and development but at a slower speed. Thus in native conditions, normal development required a whole year, whereas only part of the year would be sufficient if optimal conditions could be supplied.

In general, bulbs must attain a certain size before they can proceed to the formation of flowers and this size is dependent upon and influenced by temperature treatment.

In tulip (*Tulipa gesneriana*) "Pride of Haarlem", the growing point was actively producing leaves and floral parts during the "resting period", after spring flowering and lifting. The new main bud was situated in the axil of the innermost scale and could not be identified before flower formation began. The main bud developed into the main bulb. A number of "lateral" bulbs developed from buds in axils of outer scales.

In general, flower formation of bulbous and tuberous plants was favored by higher temperatures, as demonstrated by the optima recorded for different species: *Hyacinthus*, 23.5°C; *Tulipa*, 17-20°C; *Lilium*, 23°C; and *Amaryllis*, 23°C. Extension growth from flower formation to to flowering of tulips and hyacinths was initiated by rather low temperatures, the optima of which was between 7° and 13°C.

Hartsema further stated that high temperatures will not initiate flowering in plants with low optima.

Wellensiek (1961) has stated that flower formation in most bulbs depends directly and largely on temperature, although the minimum, optimum and maximum differ in different stages of development for the same genus and for different genera.

Hartsema's review (1961) indicated several investigators having reported that flower initiation, especially after photoperiodic induction, did not start until a minimal number of leaves were formed. Though flower formation with bulbous plants was not affected by light, nor by

day-length, with tulips it started after all the leaves had been formed. With onions and irises, the number of leaves before flower formation varied with temperature. Hyacinths could be induced to interrupt leaf formation and flower at any time.

Salisbury (1963) recognized three different flowering response types to temperature: (1) no response; (2) quantitative response in which a specific temperature promoted flowering; and (3) qualitative response in which flowering was dependent upon a specific temperature. It may have been low, high or alternating temperatures.

The results of a study by Lindstrom (1971) involving the regulation of water and heat available to Amazon lily plants in different seasons indicated that plants flowered four times in about a 10-month period. Autumn and spring treatments resulted in earlier and more flowering while winter treatment resulted in later and less flowering.

Adams and Urdahl (1971) studied the influence of temperature on floral initiation in Amazon lily. Plants were held in 18, 21, 24, 27, 29 and 32° C for periods of two, three, four and five weeks.

Significant flowering took place in the 21-24° C treatment providing they were held at that temperature for three weeks. At 27-29° C, only two weeks were necessary.

Treatment temperatures had little effect on the number of days to flowering.

Extending the heat treatment past 2 weeks only extended the time to flowering, suggesting that although floral induction (and possible evocation took place during the elevated temperature treatment, floral initiation did not ensue until removal from heating.

Adams and Urdahl concluded that temperature manipulation was more expedient than drying periods to initiate flowering in Amazon lily.

The Lateral Bud

Gifford (1951) reviewed the literature on the origin and development of lateral branches or axillary buds. He found that lateral buds were cauline, arising in the axil and later than the third or fourth leaf primordium from superficial meristem cells of the apex but not in direct continuity with terminal meristem.

The shell zone developed very early in the axillaries of many species. Its cells have cambial-like orientation and have been postulated to serve as a barrier which keeps the bud from receiving a stimulus to become ground tissue. After bud formation it becomes rib tissue of the new bud (Gifford, 1951; Yeung and Peterson, 1972).

A vegetative axillary bud in *Drimys winteri* var. *chilensis* arose as a detached apical shoot meristem at about the fourth leaf primordium by anticlinal divisions of the inner tunica layer (T_2) and in the peripheral zone of the corpus. The lateral bud, in its early stages was an isolated shoot apex, because of the shell zone and lack of procambial connections. Prophylls arose at right angles to the subtending leaf. Acropetal and basipetal procambium formation in the prophyll base, connected the prophyll with the future branch axis while basipetal procambial development connected the main axis leaf traces which were developing acropetally. It was at the second prophyll stage that *Drimys* overwintered. In the spring, it produced several leaf primordia before stem elongation took place (Gifford, 1951).

In *Cuminum cyminum*, the vegetative axillary bud was differentiated from the peripheral zone of the shoot apex at the second node. It was delimited by a shell zone which helped in changing the apical position of the bud to foliar. The emergence of the bud was effected by the meristematic activity of the tunica and corpus cells. A single prophyll was formed at right angles to the axillant leaf at the fifth or sixth node by periclinal divisions of T_2 and corpus cells and procambial divisions of T_2 and corpus cells and procambial division below that sector (Shah and Unnikrishnan, 1969).

Shah and Patel (1972), in 35 species of dicots, studied the origin, development and probable function of the shell zone, which they defined as an arcuate zone of cambiform cells delimiting the early axillary bud meristem. The shell zone lost its identity at different stages of bud development depending upon the species and ultimately contributed to the ground meristem, procambium and pith cells of the axis.

Yeung and Peterson (1972) studied transition from the vegetative phase to flowering, inflorescence development and particularly axillary bud development in *Hieracium floribundum*. At floral induction, the apex became domed with starch accumulating in the subapical region. Axillary buds developed close to the main apex in the early prefloral stage, a feature commonly shown by many species. A distinct shell zone was evident at this stage. The upper five or six axillaries in *H. floribundum* developed as inflorescences. Buds, formed in the axils of last formed leaves, developed as stolons while buds associated with

the basal rosette remained dormant until after seed set. Although stolons may have developed into inflorescences, rosette axillaries did not.

Shortly after formation of a domed primordium of the axillary bud, two prophylls were formed. When four lateral appendages formed from this rosette leaf axillary, the plant became dormant. This was also reported by Marr and Blaser (cf. Yeung and Peterson, 1972). After seed set, these dormant axillaries became active and formed small rosettes. If the plant was exposed to conditions favorable to flowering, dormant buds in the upper rosette leaves developed into inflorescences.

Accumulation and retention of starch during the reproductive phase indicated its importance in the flowering process. Axillary buds may have been undetermined meristems during initial stages of development with their subsequent development depending on the environment to which they were exposed.

Zabka (as reported by Adams and Urdahl, 1971) found, in *Amaranthus retroflexus*, that the induction cycle was effective only if plants had attained a "critical age" of 30 days. Adams and Urdahl observed in *Eucharis grandiflora* a "critical age" of six weeks before the lateral bud could be effectively induced to flower.

Floral Stages

Salisbury (1971) indicated that in the past, plant scientists have deduced that a profitable way to examine the flowering process was to divide it into a series of component or partial processes,

implying that a number of steps were involved and that one step led to the next until finally the mature flowers were present on the plant. Since some species (e.g., cocklebur) could respond to a single dark period, it was assumed that the series of necessary steps is essentially completed during each light-dark cycle. Plants that required more than a single inductive cycle may have simply required more flowering hormone for the conversion to the reproductive state than can be produced in a single cycle.

Salisbury (1955) measured the rate of bud growth in *Xanthium strumarium* based on a series of stages of development of the staminate floral bud, a stage system similar to several other investigators. The average of the plants in a treatment were referred to as the floral stage for that treatment as follows:

0. Vegetative growing point.
1. First clearly visible (swelling) of growing point ... hemispherical shape.
2. Inflorescence primordium at least as high as broad but not yet constricted at the base.
3. Inflorescence primordium constricted at the base but no flower primordia yet visible.
4. First visible flower primordia.
5. Flower primordia later development.

6.7.8. Progressively later development in floral parts.

Salisbury concluded that the floral stage was proportional to time, starting 2.5 days after beginning of induction. The floral

stage, a given number of days after induction, was proportional to the length of the inductive dark period.

Hartsema (1961) described successive stages in the dissected apex of *Tulipa gesneriana*, which consisted of seven stages:

I. Scales and foliage leaves splitting off; apex low and flat.
II. Apex dome-shaped.
III. (P1) 3 outer tepals separate primordia.
IV. (P2) 3 inner tepals separate primordia.
V. (A1) 3 primordia of first whorl of stamens distinguished.
VI. (A2) 3 primordia of second whorl of stamens distinguished.
VII. (G) 3 carpel-primordia visible.

Other Effects of Induction

Manifestations of cytohistological effects of floral stimuli in induced plants at subsequent stages in transition included effects on mitotic activity, DNA synthesis, elongation of cells in the pith rib meristem, nucleolar size (Jacqmard, *et al.* 1976), and changes in width and height of the apex (Nougarede *et al.* 1965). Jacqmard, *et al.* found cytohistological zonation absent in evoked meristems of *Xanthium strumarium*. These meristems were divided arbitrarily into three sections: the central sector (central zone) and two lateral sectors (peripheral zone).

OBJECTIVES OF THE STUDY

Several questions were posed at this point which led to the hypotheses to be tested in this investigation. The experimental design was divided into 3 phases. For a detailed description of the design, see Appendix A.

Phase I of this study involved a comparative study of the anatomy of the apices of plants of *Eucharis grandiflora*, the Amazon lily, exposed to heat (27-29° C) for one, two, three, four or five weeks, in an effort to see if manifestations of floral evocation and/or initiation are in evidence when compared to the control.

Since the time from the end of heat to flower was essentially the same at all temperatures (Adams and Urdahl, 1971), a difference in the velocity of evocation (and/or initiation) must occur. Another problem to be addressed in this phase of the study will be whether differences in the velocity of apical change is the same whether heated for one, two, three, four or five weeks. If there is no apparent changes taking place, then how long after heating has stopped must one wait before visible changes in the apical meristem will occur?

Phase II will be concerned with floral morphogenesis of three-week heated plants. The question to be answered here will be: What was the developmental sequence of the thermoinduced apex through the life cycle when bulbs are heated for three weeks at 29° C and then returned to 18° C? Phase II will also compare developmental stages of two-, three- and five-week heated plants.

Phase III will correlate Phase II development with the develop-

ment of the first lateral, which will become the next floral axis (the next bulb). This will be collected through the twenty-third week (160 days) or up until the bulb is ready to start the next reproductive cycle. These questions present themselves for consideration. In the developmental sequence of the new terminal growing point: When is the new apex visible? When does it begin to function? When does it initiate new leaf primordia? What is the developmental condition six weeks after the last flower has died on the scape? Is it structurally ready to be induced to flower?

MATERIALS AND METHODS

Eucharis grandiflora, Planch. (*Eucharis amazonica*, Lind.), is commonly called the Amazon lily, although it is from the Andes of Colombia and Peru.

A team headed by Goodspeed (1961) collected 100 bulbs of *Eucharis* at about Puente Durand, a small settlement on the bank of the river Rio Chinchao, near the city of Tingo Maria (lat. S. 09.1°, long. W. 75.5°, elevation 1,000-1,000 m.) in the Montana Region of Peru. This was their second expedition in 1938-1939. The bulbs were loaded in a truck that left Lima, Peru on the first lap of their journey to Berkeley, California; from there, they were distributed to different areas of the United States and the rest of the world. It is very probable that this is the original source of the bulbs used in this study.

Eucharis, from the Greek meaning very graceful, is a bulbous, very fragrant member of the family Amaryllidaceae and is a common warmhouse flowering plant. It produces a few large leaves (See Figure 1A) from a bulb (Figure 1B) and a scape up to 60 cm tall (Figure 1C) bearing four to eight narcissus-like, waxy-white flowers up to seven cm across (Figure 1D). *Eucharis* seems to have no specific blooming period in the U.S., but plants are most apt to flower in the spring and fall (Schulz, 1954; Bailey, 1925).

Norman Evers, SDSU Horticulture Department, indicated that sporadic flowering of Amazon lilies took place in March and October in Brookings in untreated greenhouse grown plants. Profuse flowering occurred in October when plants were grown outdoors in a shaded, north exposure location, indicating that photoperiod may play a role in induction in Amazon lilies.

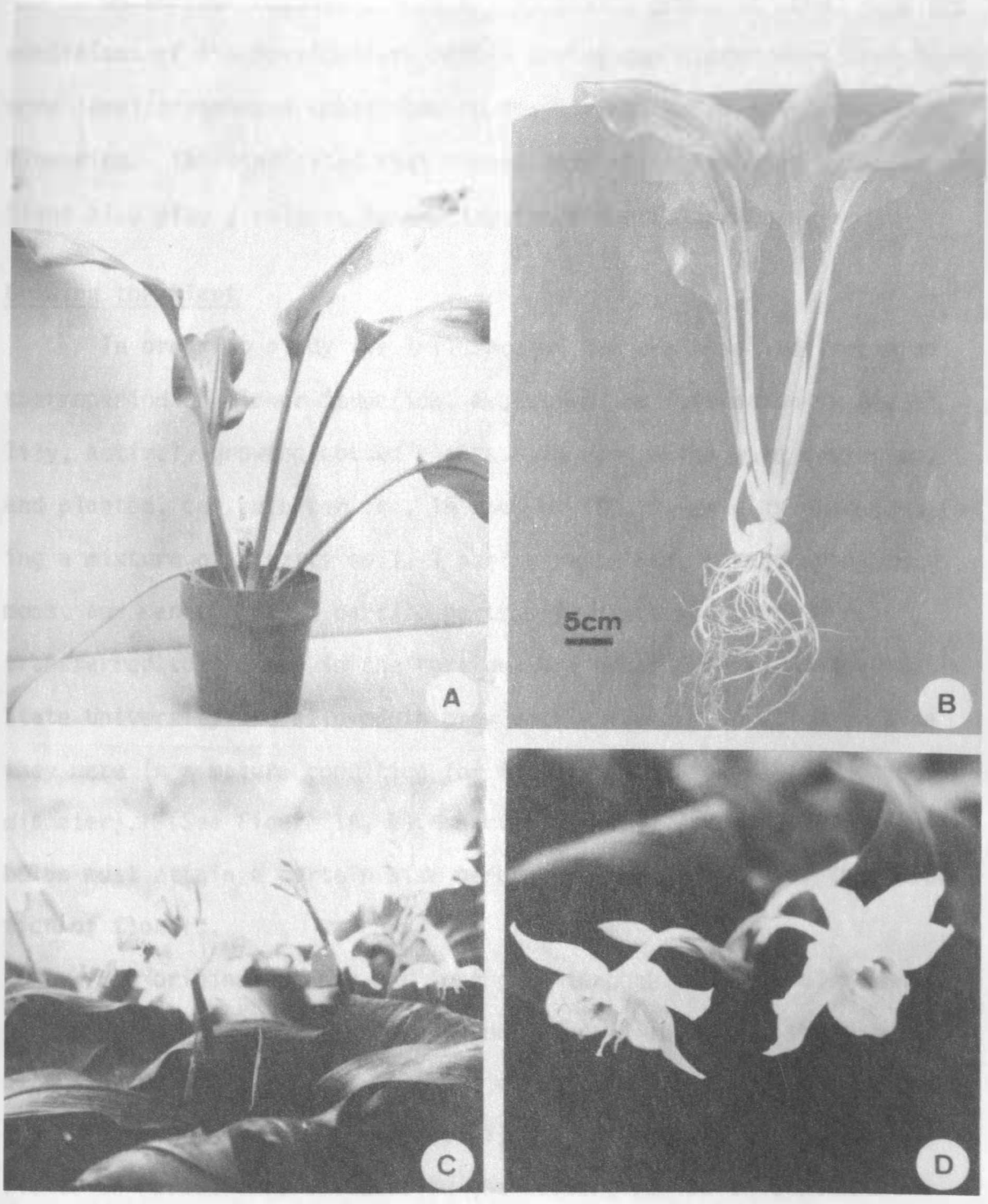
Figure 1. *Eucharis grandiflora*, Planch. the Amazon lily.

A. Potted plant ready for heat table/growth table experiment.

B. Mature plant ready for floral induction.

C. Amazon lilies on the growth table in their mature stage bearing an umbel of 4-6 waxy-white flowers on a terete scape up to 60 cm tall.

D. Closeup of 2 of the 4-6 waxy-white flowers.


A
5cm
B
C
D

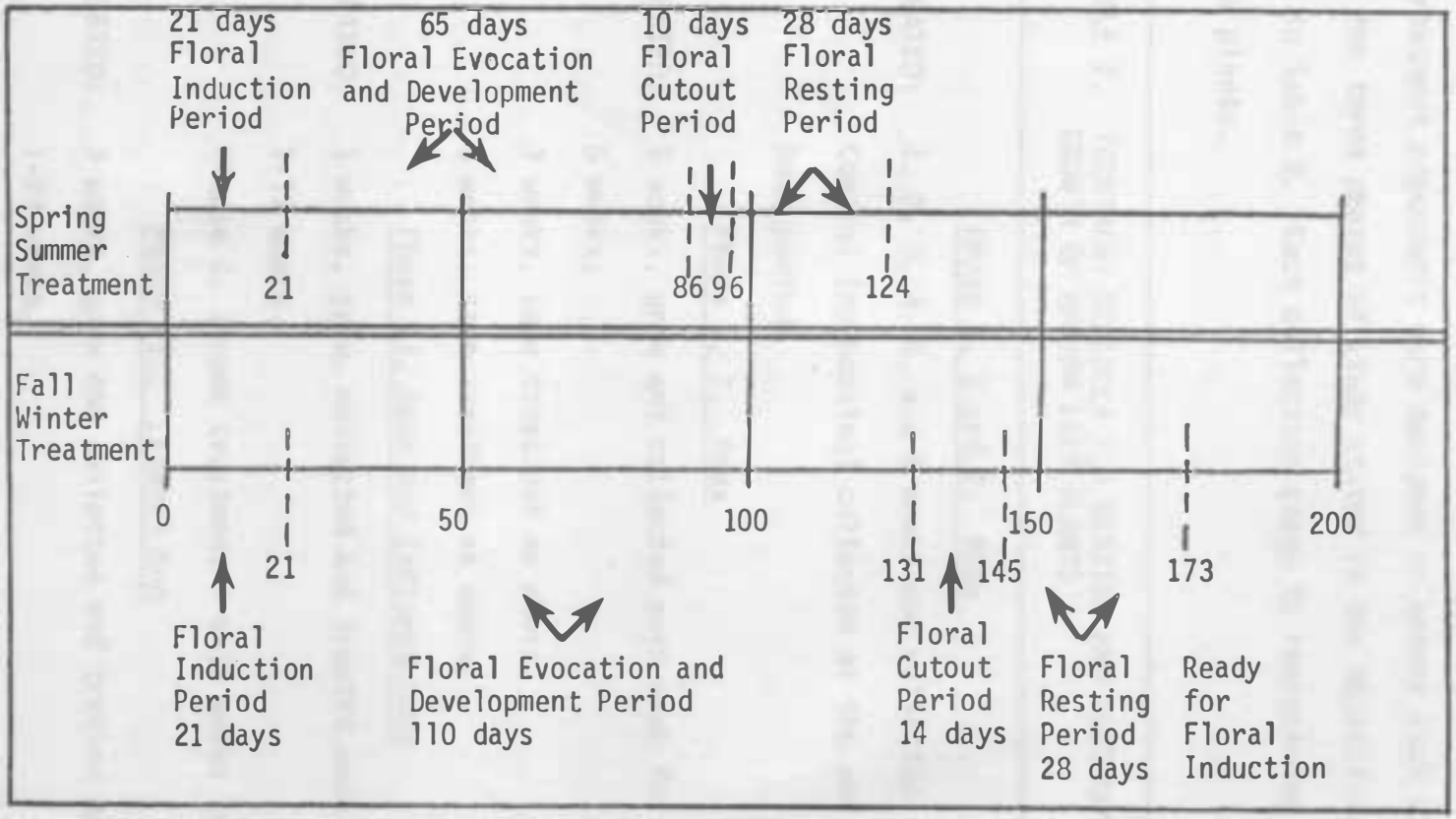
On another occasion, Amazon lily plants grown in warm, very dry conditions of the horticutlure office during one winter were transferred to more ideal greenhouse conditions in the spring, which triggered profuse flowering. This indicated that stress conditions followed by ideal conditions also play a role in triggering the flowering process.

Growing the Plant

In order to study the influence of temperatures and length of thermoperiod on flower induction, evocation and initiation in Amazon lily, actively growing potted plants with many bulbs were broken up and planted, one bulb per pot, in each of 200, 15-cm clay pots containing a mixture of 3 parts soil, 1 part vermiculite, 1 part sphagnum moss, and sand (about 1 part/10 parts soil mixture). They were transferred to a bench in the horticulture greenhouse at South Dakota State University and allowed to grow until December 1, at which time they were in a mature condition for further study (bulbs at least 5 cm diameter). (See Figure 1A, B). Hartsema (1961) found that in general, bulbs must attain a certain size before they can proceed to the formation of flowers.

The original plants had just gone through the natural flowering period so no previous flowering stimulus would influence the results of this study. They were collected during the floral resting period of the spring-summer cycle (See Table 1). If they had been induced to flower in April, they would be in the resting period in September. Transplanting at that time for use in the December study would assure vegetative conditions. It also allowed enough time for the lateral bud to reach the "critical age" for the next induction.

TABLE 1. SCHEDULE OF THE LIFE CYCLE OF AMAZON LILY.



* Spring-Summer Treatment in this table was constructed from data in Adams and Urdahl (1971).

Thermoinduction

Treatment procedures were designed to answer each of the questions in the three phases of study stated in the objectives and are outlined in Table 2. Each collection stage is represented by three replicate plants.

TABLE 2. TREATMENT SCHEDULE FOR HEATING AND SUBSEQUENT GROWTH OF AMAZON LILY PLANTS.

	Phase I, 1 and 2. Apex
HEATED:	1, 2, 3, 4, 5, and 6 weeks and collected.
	Control (no heating) collected at the end of each heat period.
	Phase I, 3. Apex
HEATED:	2 weeks, grew and collected each week for 1 through 5 weeks.
	3 weeks, same treatment as above.
	5 weeks, same treatment as above.
	Phase II, Apex and Inflorescence
HEATED:	3 weeks, grew, collected and treated each week for 1-19 weeks.
	(Phase I, 3 week treatment + 6-19 weeks collection)
	Phase III. Lateral Bud
HEATED:	3 weeks, grew and collected and treated each week for 1-23 weeks.
	(Phase II + 20-23 weeks collection)

Mature plants were labelled for proper transferral to the growth table and placed in the hot water bath using the technique of Adams and Urdahl (1971). (See Figure 2A-D).

Three plants for each time and temperature combination were transferred to 15 cm plastic pots with sealed bottoms and plunged to within three cm of the pot rim in water baths. Following temperature treatments, plants were returned to normal pots and held at 17° C (maximum) on the growth table until the end of the flowering period.

Following the treatment schedule of Table 2, plants were removed from the heat table and either collected for microscopic sectioning or transferred to the growth table. They were then removed from the growth table at specified times and prepared for microscopic study (See Table 3).

A detailed schedule for collection purposes showing the heating and growth periods for each treatment set was designed (Table 4). Those asterisked were used for Phase II and III of the study while treatments 1-63 were used for Phase I only.

If the length of time on the heat table (one to five weeks) makes no difference in the anatomical structure of the apex or in the time of flowering, then the specimens heated two, three, four and five weeks should compare anatomically after one week on the growth table, two weeks on the growth table, etc. Table 5 was developed to show which treatments should correspond anatomically.

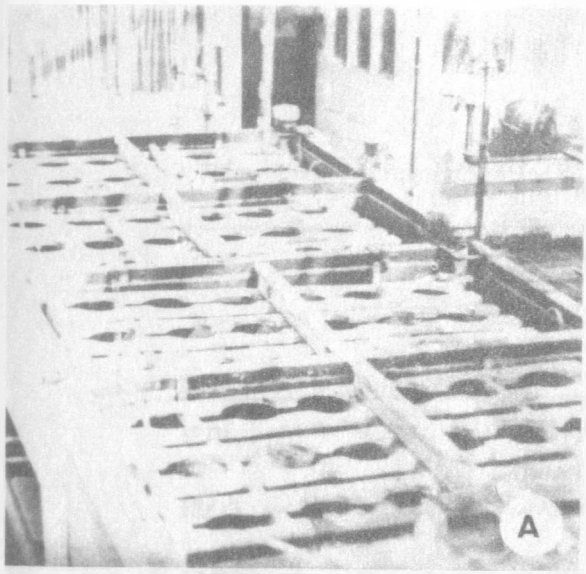
Figure 2. Heating table without plants and with plants in place.

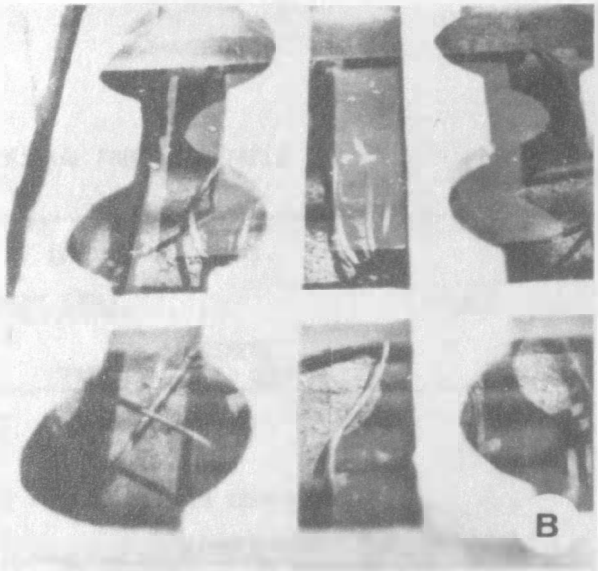
A. Heat table in horticulture greenhouse prior to placement of Amazon lily plants.

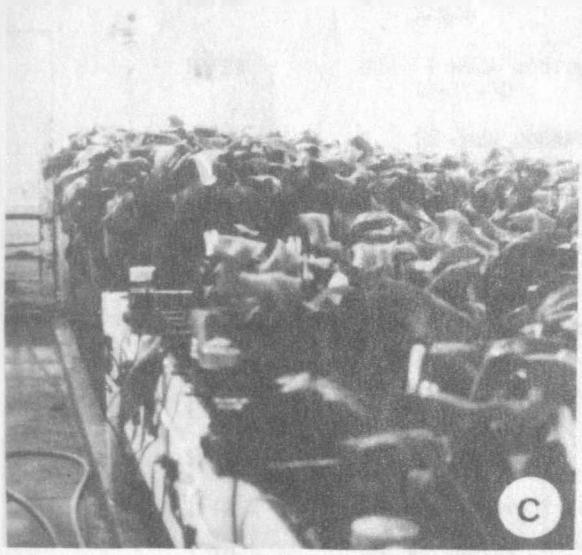
B. Close-up of heat table.

C. Heat table with Amazon lily plants in place for heat treatment.

D. Close-up of loaded heat table.


A


B


C

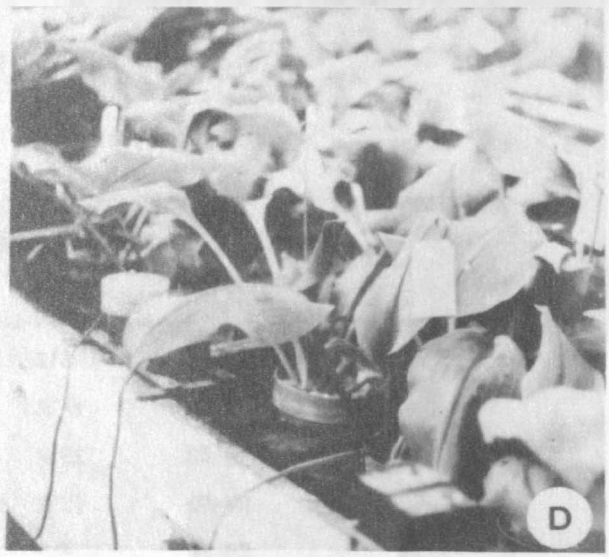

D

TABLE 3. SCHEDULE FOR REMOVAL FROM HEAT TABLE AND GROWTH TABLE

Placed on heat table 12/1 (except controls).

TAKEN OFF HEAT TABLE

DATE	SPECIMENS KILLED/FIXED	SPECIMENS TRANSFERRED TO GROWTH TABLE
12/8	A. (1 week control) 1-2-3	-----
12/15	B. (2 week control) 4-5-6	16-17-18, 19 through 30
12/22	C. (3 week control) 7-8-9	31 through 42, 64 through 108
12/29	D. (4 week control) 10-11-12	43-44-45, 46-47-48
1/5	E. (5 week control) 13-14-15	49 through 63

TAKEN OFF GROWTH TABLE

DATE	SPECIMENS KILLED/FIXED	DATE	SPECIMENS KILLED/FIXED
12/22	16-18	3/8	79-81
12/29	19-21, 31-33	3/15	82-84
1/5	22-24, 33-36	3/22	85-87
1/12	25-27, 37-39, 49-51	3/29	88-90
1/19	28-30, 40-42, 52-54	4/5	91-93
1/26	43-45, 55-57	4/12	94-96
2/2	46-48, 58-60, 64-66	4/19	97-99
2/9	61-63, 67-69	4/26	100-102
2/16	70-72	5/3	103-105
2/23	73-75	5/10	106-108
3/1	76-78		

TABLE 4. DETAILED SCHEDULE SHOWING HEAT AND GROWTH PERIODS OF AMAZON LILY

REP. NO.	TREATMENT
A-E	Heated 0 weeks. Collected after 1, 2, 3, 4, 5 weeks on growth table.
*1-3	Heated 1 week. Collected.
*4-6	2
*7-9	3
*10-12	4
*13-15	5
16-18	Heated 2 weeks. Collected after 1 week on growth table.
19-21	2 2
22-24	2 3
25-27	2 4
28-30	2 5
*31-33	Heated 3 weeks. Collected after 1 week on growth table.
*34-36	3 2
*37-39	3 3
*40-42	3 4
*43-45	3 5
46-48	Heated 4 weeks. Collected after 5 weeks on growth table.
49-51	Heated 5 weeks. Collected after 1 week on growth table.
52-54	5 2
55-57	5 3
58-60	5 4
61-63	5 5
*64-66	Heated 3 weeks. Collected after 6 weeks on growth table.
*67-69	3 7
*70-72	3 8
*73-75	3 9
*76-78	3 10
*79-81	3 11
*82-84	3 12
*85-87	3 13
*88-90	3 14
*91-93	3 15
*94-96	3 16
*97-99	3 17
*100-102	3 18
*103-105	3 19
*106-108	3 20

*Used for Phase II and III of the study.

TABLE 5. SPECIMEN IDENTIFICATION AND HEAT/ GROWTH TABLE TREATMENTS OF AMAZON LILY

TOTAL WEEKS GROWN	0 HEAT	1 WK HEAT	2 WK HEAT	3 WK HEAT	4 WK HEAT	5 WK HEAT
1	A	1-3 (1)				
2	B		4-6 (2)			
3	C		16-18 (2+1)	7-9 (3)		
4	D		19-21 (2+2)	31-33 (3+1)	10-12 (4)	
5	E		22-24 (2+3)	34-36 (3+2)		13-15 (5)
6			25-27 (2+4)	37-39 (3+3)		49-51 (5+1)
7			28-30 (2+5)	40-42 (3+4)		52-54 (5+2)
8				43-45 (3+5)		55-57 (5+3)
9				64-66 (3+6)		58-60 (5+4)
10				67-69 (3+7)		61-63 (5+5)
11				70-72 (3+8)		

Zonation Anatomy:

Microtechnique

At specified times (see Table 3) plants were removed from the growth table and the bulb was dissected to remove the growing point for fixation as illustrated in Figure 3 A-F. Details of this technique appear in the Appendix.

In the event of the possibility of diurnal fluctuations in the synthesis of certain substances (as found by Teltscherova and Pleskotova, 1973) which might be reflected in the collected data, all specimens were dissected, killed and fixed at the same period of the day, between one PM and three PM.

After all the fleshy leaf bases were removed from the crown stem, the tip of the youngest visible leaf was exposed (Figure 4A), and enlarged for clarity in Figure 4B. The leaf base was removed exposing the young leaf (Figure 4C). Figure 4D illustrated the crown stem with the base of the young leaf and the approximate position of the vegetative shoot apex (induced). Figure 4E showed the tip of the youngest visible leaf in the microscopic sections. It was this level of dissection of the apex that was prepared for killing and fixing prior to sectioning.

Three vegetative apices were collected, killed, fixed and embedded using the technique of Johnshoy, 1978 for study on the scanning electron microscope (SEM). Specimens were photographed on the SEM at the University of Minnesota, Veterinary Science Department.

Each of the dissected vegetative apex treatments numbered 1-72 were killed and fixed in Formalin-Acetic Acid-Alcohol (FAA) according

to Sass (1964). Specimens 73-108 were preserved in 10% formalin for future observations if necessary.

Crafs V (Sass, 1964) was also used in earliest sectioning procedures to determine which killing agent yielded best results.

Dehydration of the specimens in preparation for embedding for light microscope studies was carried out using the tertiary butyl alcohol schedule of Sass, 1964.

Tissue was then embedded in 22 x 22 x 6 cm Tissue-Tek Base Molds (Lab-Tek Products) and placed in ice water for fast cooling to prevent fracturing of the paraffin during sectioning.

All apices were sectioned longitudinally, 8 um thick on a Spencer rotary microtome, mounted on adhesive treated microscope slides, floated in a 2% formalin solution and placed on a Chicago Surgical and Electrical Co. warming plate set at 9° C to flatten paraffin ribbons prior to staining. The Triarch quadruple staining technique was used (Sass, 1964). The completed slides were cured in an oven for 24 hours and were then ready for observation.

Microscope slides were photographed using a Bausch and Lomb microscope and a Polaroid M-P4 camera unit.

Floral Stages

Floral stages in Phase II of this study were classified using a modified form of the stage system of Hartsema, 1961. Whereas both Salisbury and Hartsema used *in toto* dissected apices, this study based stages on sectioned material. Three plants were used to represent each treatment. That specimen within the replicates showing the most well-developed stage was photographed and studied. Hartsema's stages were

Figure 3. Procedure for dissecting bulbs to obtain the growing point

A. Amazon lily plant as it was taken off the growth table prior to dissection.

B. Amazon lily plant removed from pot to expose bulb.

C. Close-up of Amazon lily plant to demonstrate 1/2 phyllotaxy allowing ease of dissection to get apex in proper plane for sectioning.

D.E.F. Steps in removal of fleshy leaf bases of the bulb.

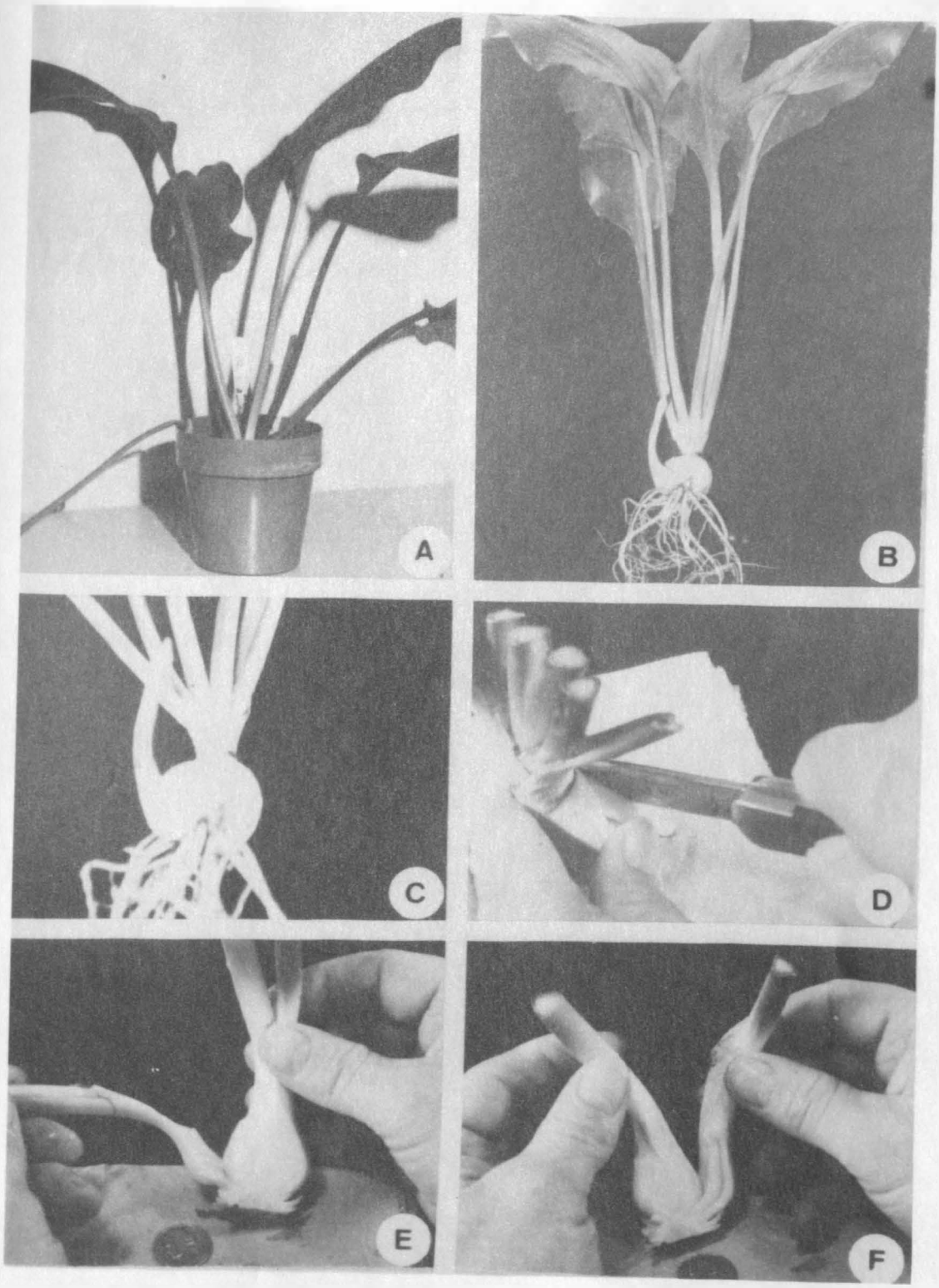

A
B
C
D
E
F

Figure 4. Locating the Youngest Foliar Leaf to Determine the Position of the Growing Point

A. Amazon lily shoot with youngest visible leaf emerging through ostiole of the larger leaf petiole.

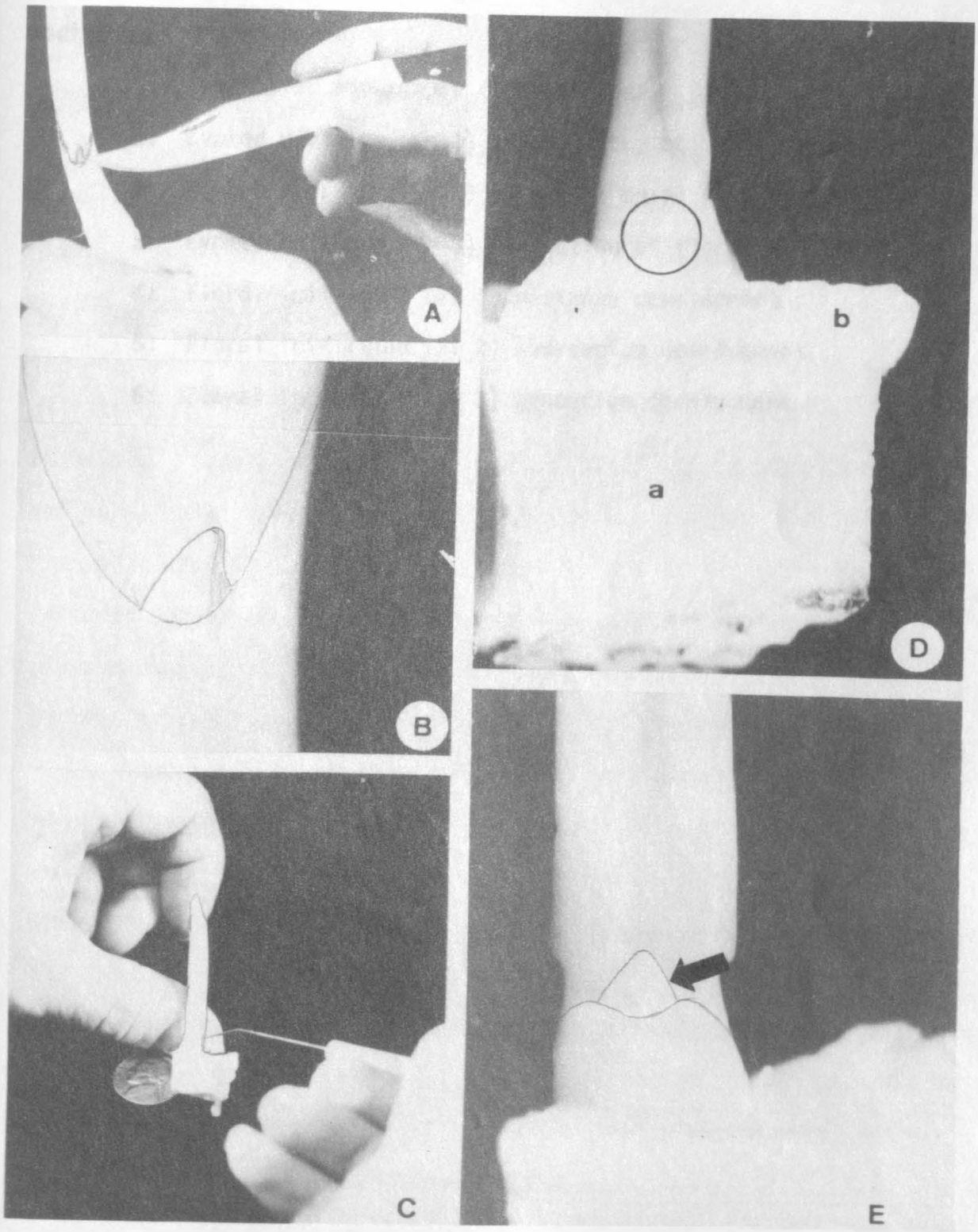
B. Close-up of A (10x)

C. Removal of petiole and leaf base shown in B showing youngest visible leaf in its entirety.

D. Crown stem with all leaf bases removed and young leaf showing position of shoot apex in circle (magnified 10x over C).

E. 90° rotation of D showing the largest leaf to be seen in sectioned material at arrow (magnified 10x over C).

*Photos A, B, C and E were retouched for clarity of youngest expanded leaf.


A
B
C
D
a
b
E

modified as listed below:

0: Vegetative shoot apex (induced apex)

1: Evoked meristem (EM 1) change in cytohistology (evoked apex)

2: Evoked meristem (EM 2) change in shape (initiated apex)

3: Evoked meristem (EM 3) elongation of floral axis

4: Floral initiation (FI 1) perianth development

5: Floral initiation (FI 2) androecium development

6: Floral initiation (FI 3) gynoecium development

RESULTS

Zonation Anatomy

Popham's Type VII: Usual Angiosperm Type (Popham, 1966) was accepted as the shoot apical organization in Amazon lily (Figure 5A). The diagram in Figure 5B illustrated Zone 1, the Mantle, which consisted of two tunica layers, in which most divisions were anticlinical. Zone 2, the self-perpetuating Subapical Initials (SA) showed division walls in all planes. This zone produced all tissue of the shoot except the epidermis. Zone 3, the Central Meristem (CM), was produced from SA and itself. Transverse divisions produced tiers which differentiated into pith tissue. Zone 4, the Peripheral Meristem (P) formed a cylinder around the CM. It formed from T_2 and SA and within itself. It was somewhat stratified and from it was initiated foliar appendages, cortex, procambium and lateral buds, which originated subhypodermally.

Scanning Electron Microscope (SEM) studies (see Figure 6) revealed the Maximum-area (6A), intermediate (6B) and minimum-area (6C) phases of the vegetative growing point of Amazon lily. Although the growing points in the photographs were about the same diameter in A (75x), and C (90x), the magnification of B was 161x. This indicated a larger apex in A before leaf primordium elevation. As the leaf primordium separated from the apex through differentiation and elevation through growth, the apex became reduced in size until the next leaf primordium began its initiation in the next maximum-area phase.

Thermoinduction: Phase I

The first question (Phase I) addressed in this section was: Do

Figure 5.

A. Photograph of a median longitudinal section of Amazon lily, vegetative shoot apex, 8 um thick, magnification 80x, from which zonation determinations were made.

B. Diagram illustrating zonation (according to Popham, 1966).

Zone 1. Mantle

(T_1) outer tunica

(T_2) inner tunica

Zone 2. Subapical initials (SA)

Zone 3. Central meristem (CM)

Zone 4. Peripheral zone (P)

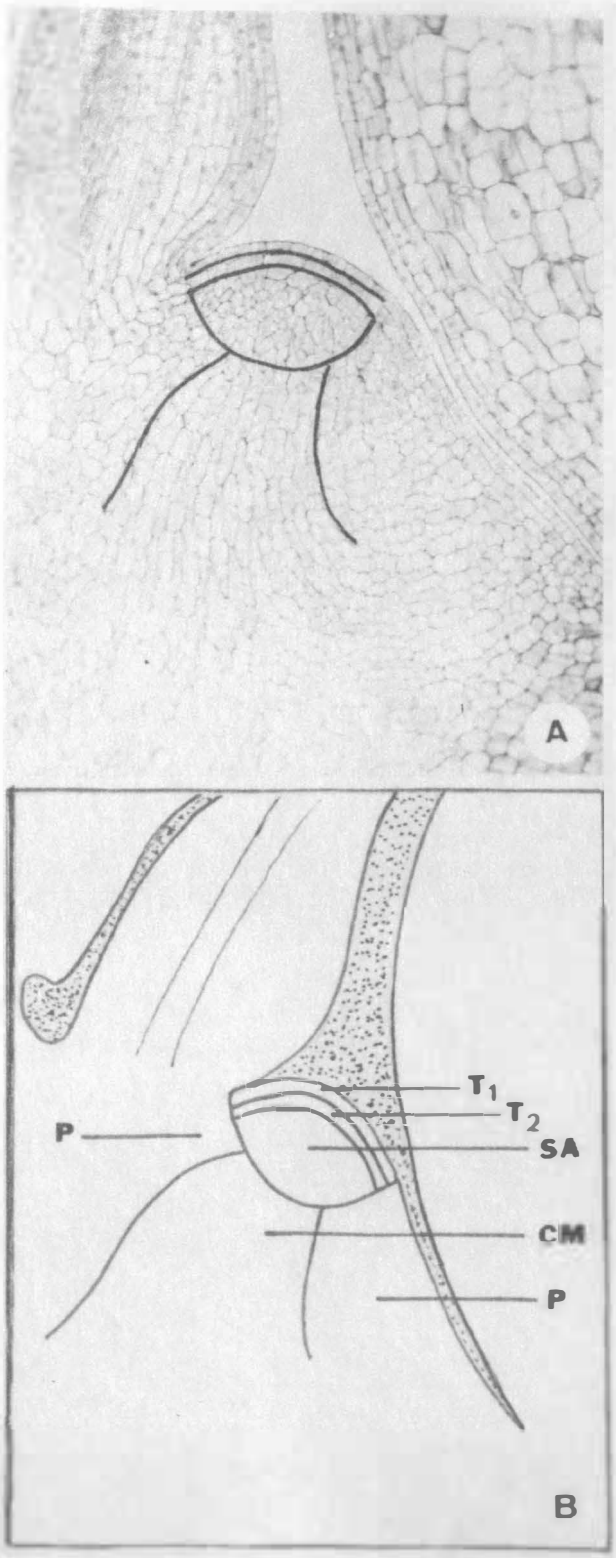
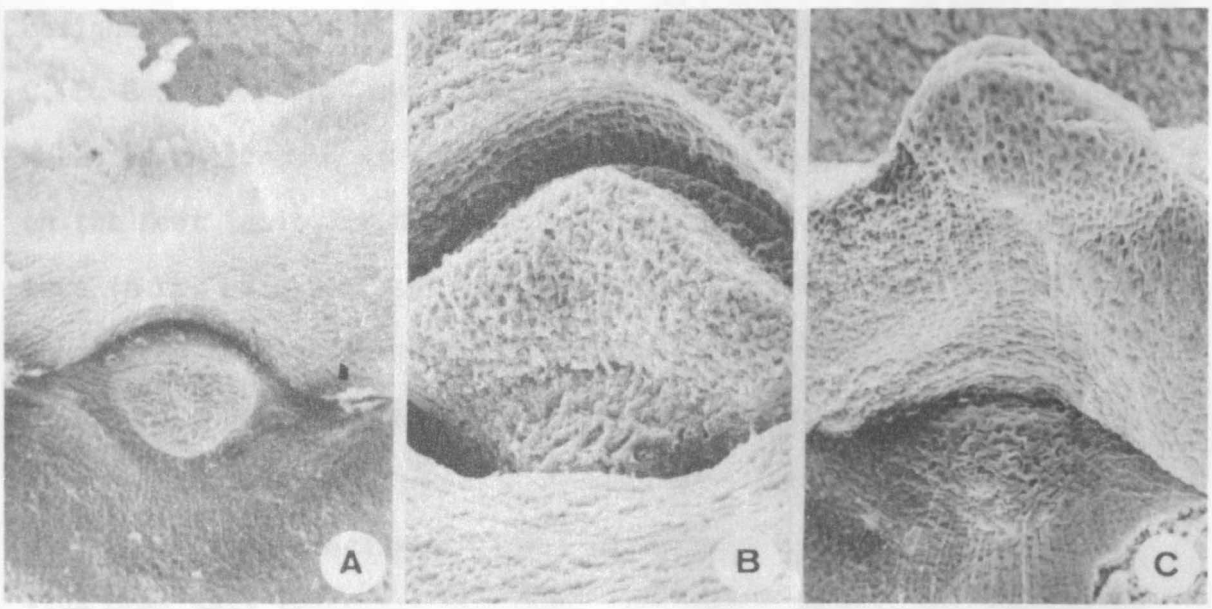

A
T1
T2
P
SA
CM
P
B

Figure 6. Scanning electron micrographs depicting maximum-, intermediate- and minimum-area phases in the Amazon lily vegetative apex.

A. Maximum-area phase, before the elevation of the newly forming leaf primordium (75x).

B. Intermediate-area phase, during the departure from the apex and elevation of the last formed leaf primordium (161x).

C. Minimum-area phase after the elevation of the last leaf (90x).


A
B
C

anatomical changes take place in apical meristems during the heating period? Specimens that were observed to lend supporting evidence were selected as most representative of the replicates collected within each treatment group. The most well-developed stage within each replication group was considered most representative. For the control group (no heat, one through five weeks) the specimens designated by A, B, C, D and E in Table 5 were used. For the treatment group, specimens designated 2, 4, 9, 11 and 15 were used (see also Table 5). Figure 7A, C, E, G and I represented control plant apices grown for one to five weeks on the growth table. B, D, F, H and J represented those grown on the heat table for the same intervals. There was no apparent difference in the nature of the apex between the control and induced apices.

The next question considered in Phase I was: Is the velocity of apical change the same whether heated for three or five weeks? Figure 8 compares three and five weeks heat treatments illustrating the differences in velocity of floral development. Both three week and five week heat treatment specimens were selected from the replicates (see Table 5) and illustrated in Figure 8 as follows:

Plants depicted in Figures 8A, C, G, H and I were heated for three weeks and collected: immediately (no. 8) A; after one week (no. 32) C; two weeks (no. 34) E; three weeks (no. 39) G; four weeks (no. 41) H; and five weeks (no. 44) I.

Plants depicted in figures 8B, D, F and J were heated for five weeks and collected: immediately (no. 13), B; after one week (no. 50), D; two weeks (no. 52), F; and five weeks (no. 61), J.

Figure 8B (five weeks heating table) and Figure 8E (three weeks heating, two weeks growth table) have both grown the same length of time after initial thermal induction. Floral initiation was indicated in 8E while 8B remained vegetative. Refer to Figure 8D. After one week growth the apex was still vegetative (five weeks heating, one week growth), while Figure 8G (three weeks heat, three weeks growth), although in the same growth interval, perianth initiation and lateral bud formation was observed.

Refer to Figure 8I (three weeks heat, five weeks growth). This apex appeared to be in the same stage of floral development as Figure 8J (five weeks heat, five weeks growth).

Figure 9 depicted a comparison of apices, all of which had been grown for a period of six weeks under different heat regimes, from zero to five weeks heat. Figure 9A, zero heat and six weeks growth (Table 5 specimen numbers are listed in parentheses) was still in the vegetative condition. Figure 9B, one week heat and five weeks growth (not in Table 5) was also vegetative. Figure 9C, two weeks heat and four weeks growth (27) and Figure 9D, three weeks heat and three weeks growth (39) showed considerable floral development. Figures 9E (11) and 9F (not in Table 5) showed no floral development although they were thermally induced to flower.

Figure 7. Apices of Control (non-heated) and heated plants of Amazon lily (magnification 80x)

A. No heat, 1 week growth table and collected

B. Heat, 1 week and collected

C. No heat, 2 weeks growth table and collected

D. Heat, 2 weeks and collected

E. No heat, 3 weeks growth table and collected

F. Heat, 3 weeks and collected

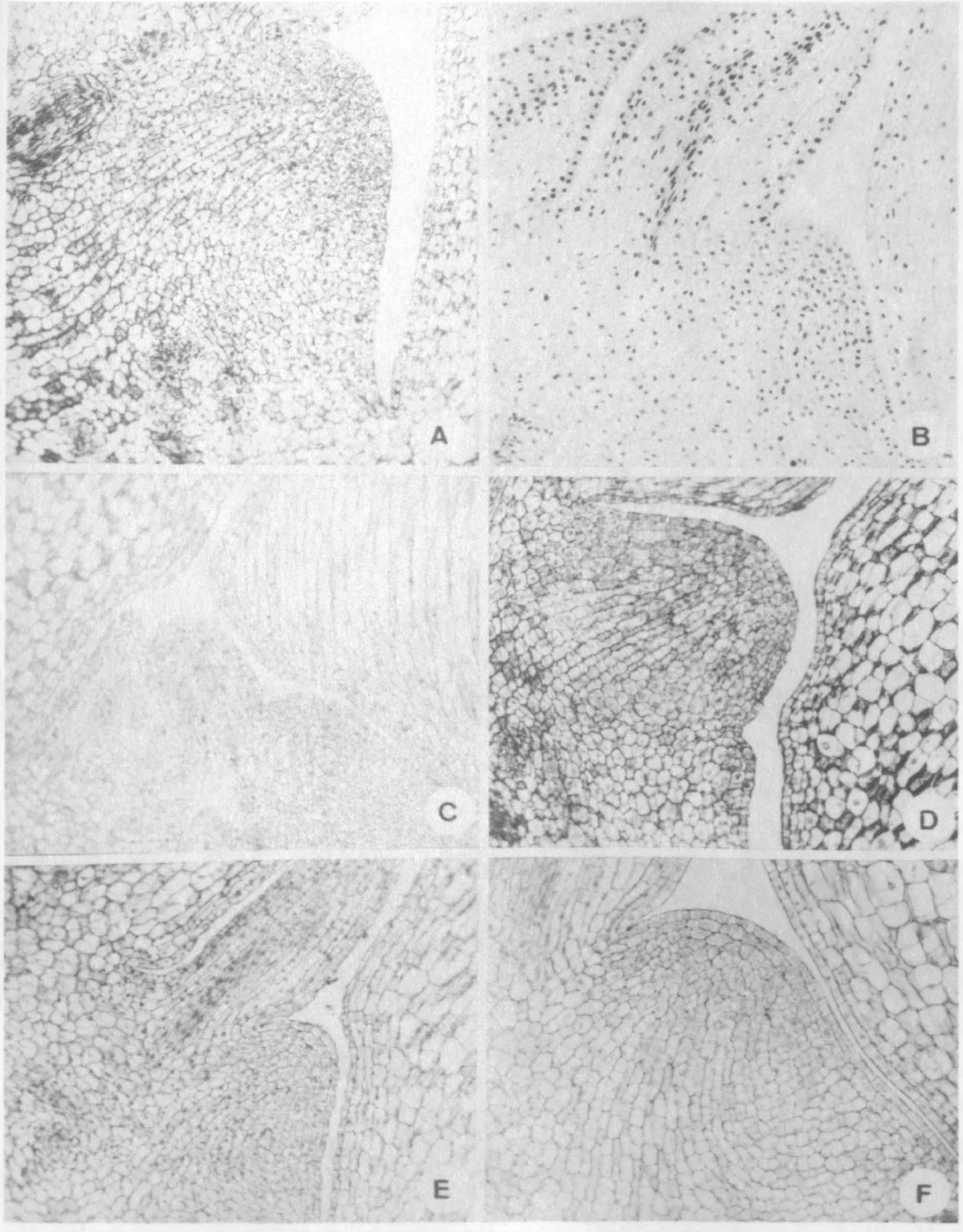

A
B
C
D
E
F

Figure 7. (continued)

G. No heat, 4 weeks growth table and collected

H. Heat, 4 weeks and collected

I. No heat, 5 weeks growth table and collected

J. Heat, 5 weeks and collected

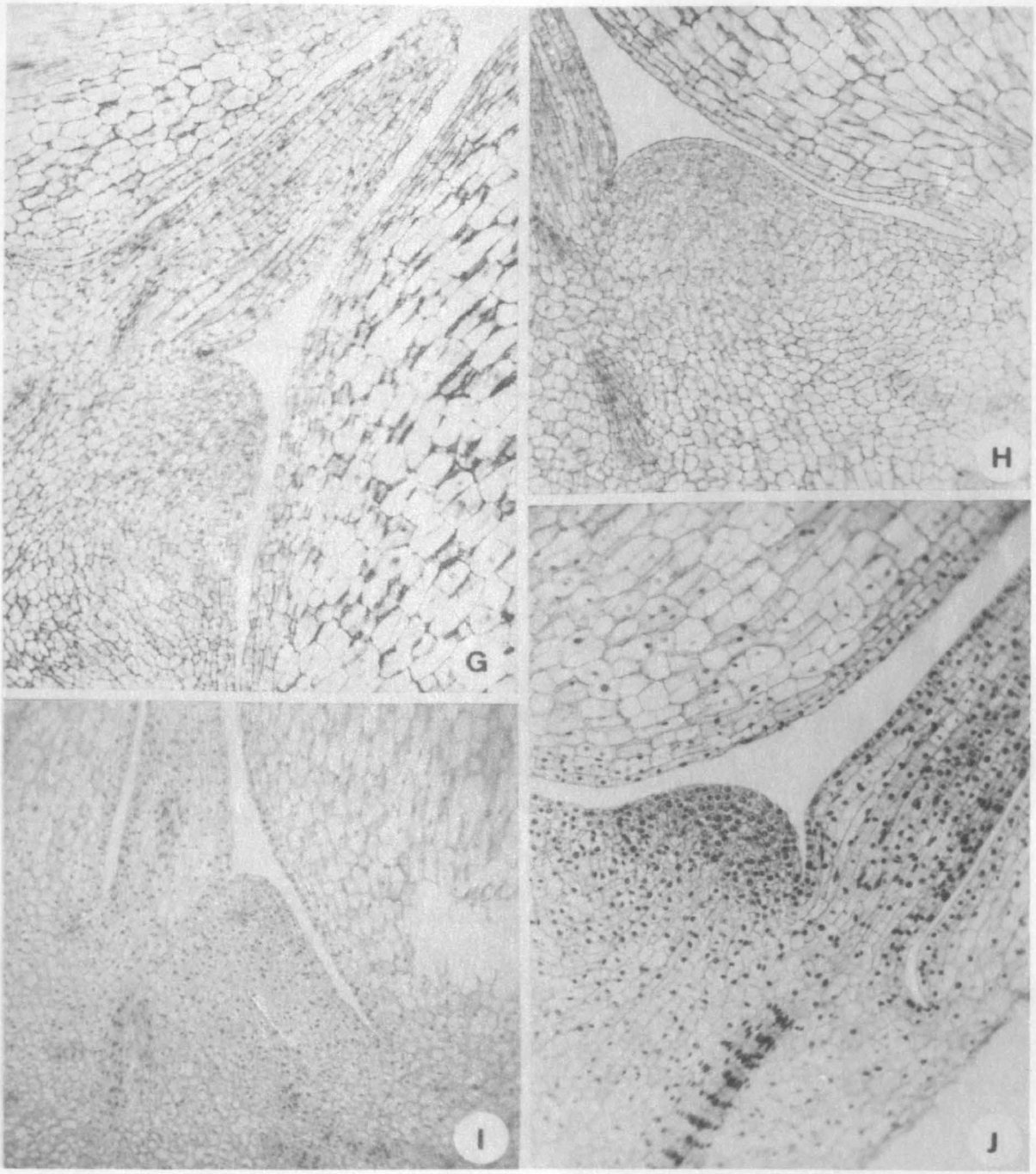

G
H
I
J

Figure 8. Comparisons of differences of floral development of specimens of Amazon lily subjected to three and five week heat treatments.

A. 3 weeks heat, collected immediately (80x).

B. 5 weeks heat, collected immediately (80x).

C. 3 weeks heat, collected after 1 week (55x).

D. 5 weeks heat, collected after 1 week (80x).

E. 3 weeks heat, collected after 2 weeks (55x).

F. 5 weeks heat, collected after 2 weeks (65x).

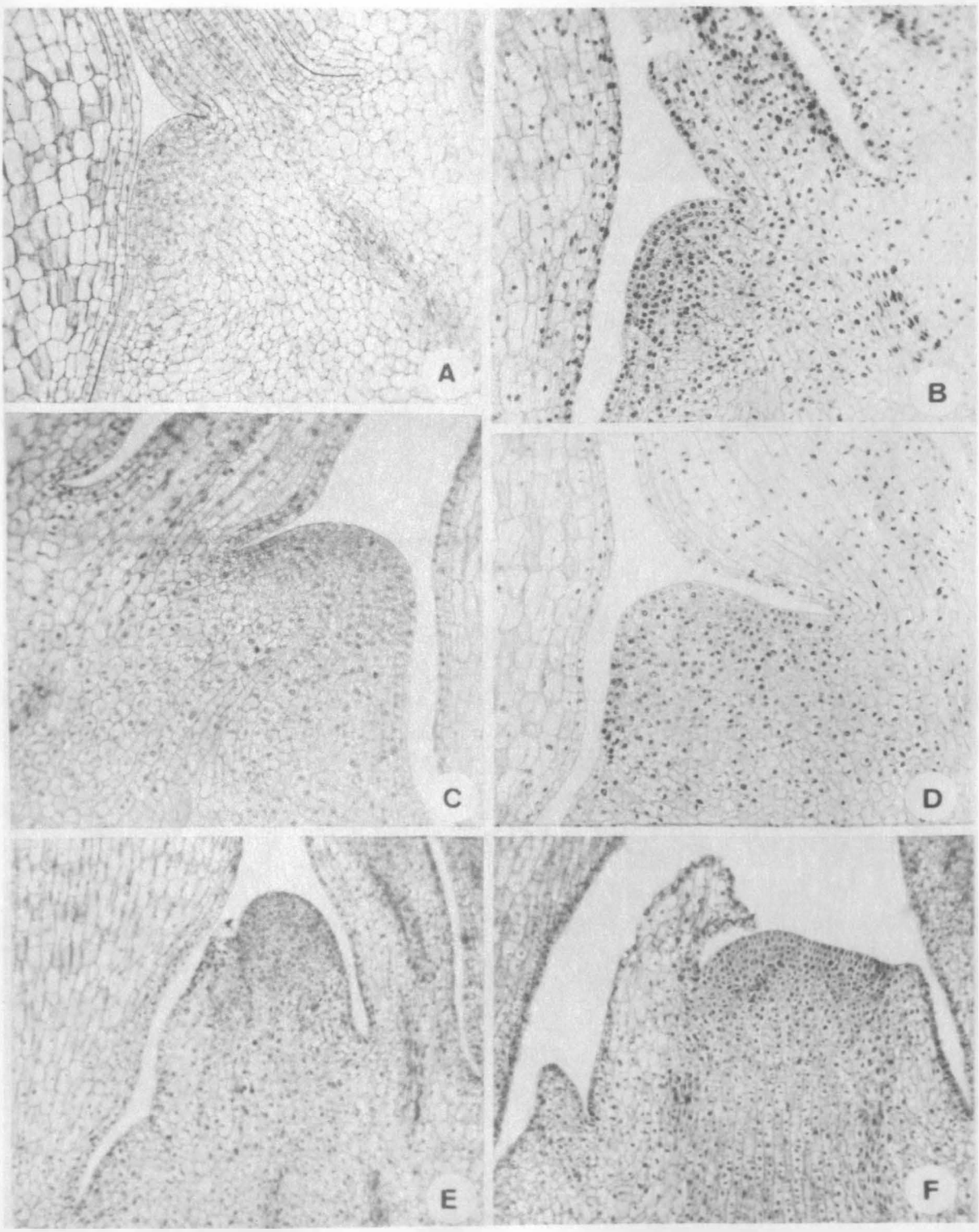

A
B
C
D
E
F

Figure 8. (continued)

G. 3 weeks heat, collected after 3 weeks (60x).

H. 3 weeks heat, collected after 4 weeks (80x).

I. 3 weeks heat, collected after 5 weeks (45x).

J. 5 weeks heat, collected after 5 weeks (55x).

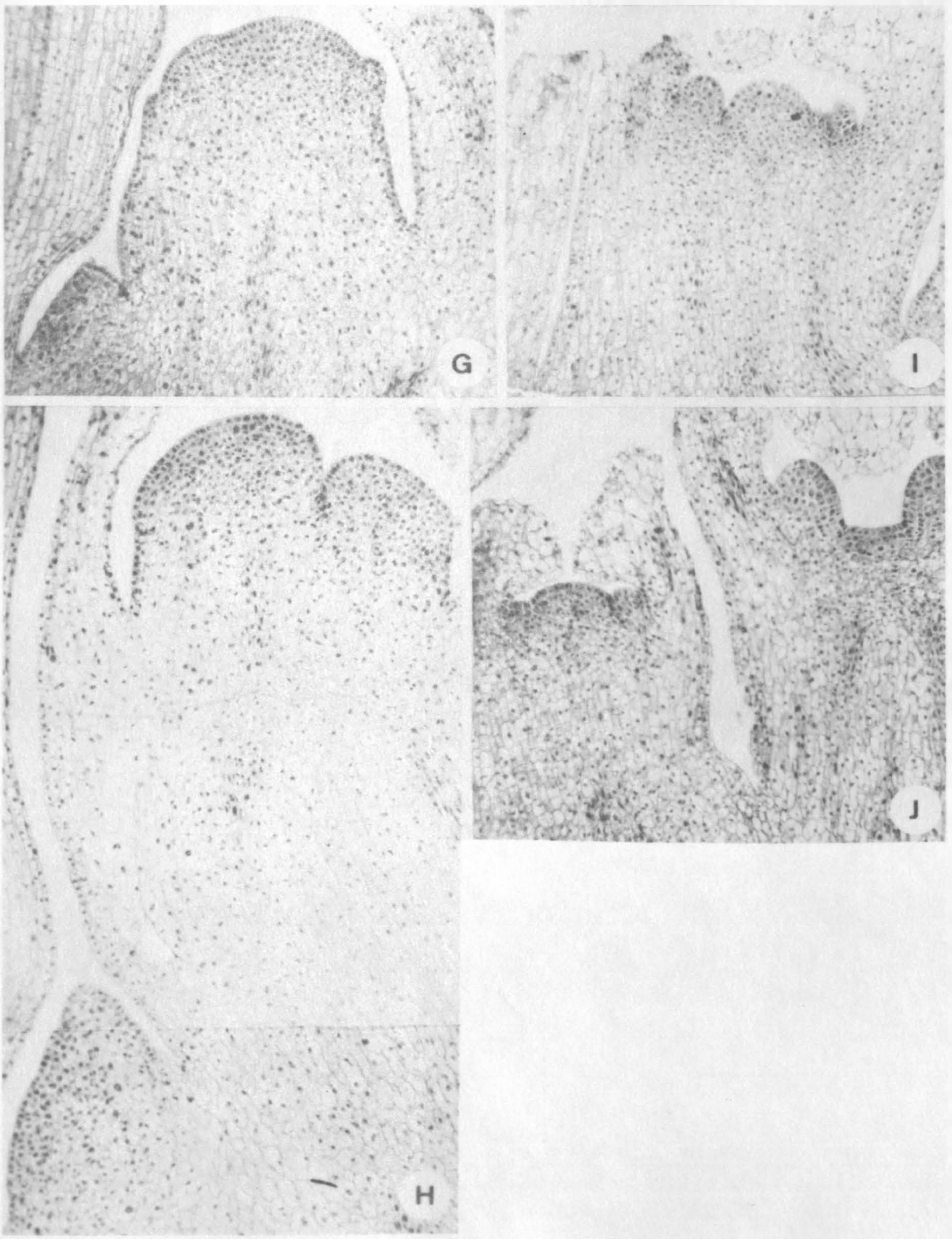

G
I
J
H

Figure 9. Comparison of apices of Amazon lily plants grown for six weeks under different heat regimes.

A. Zero weeks heat, six weeks growth (80x).

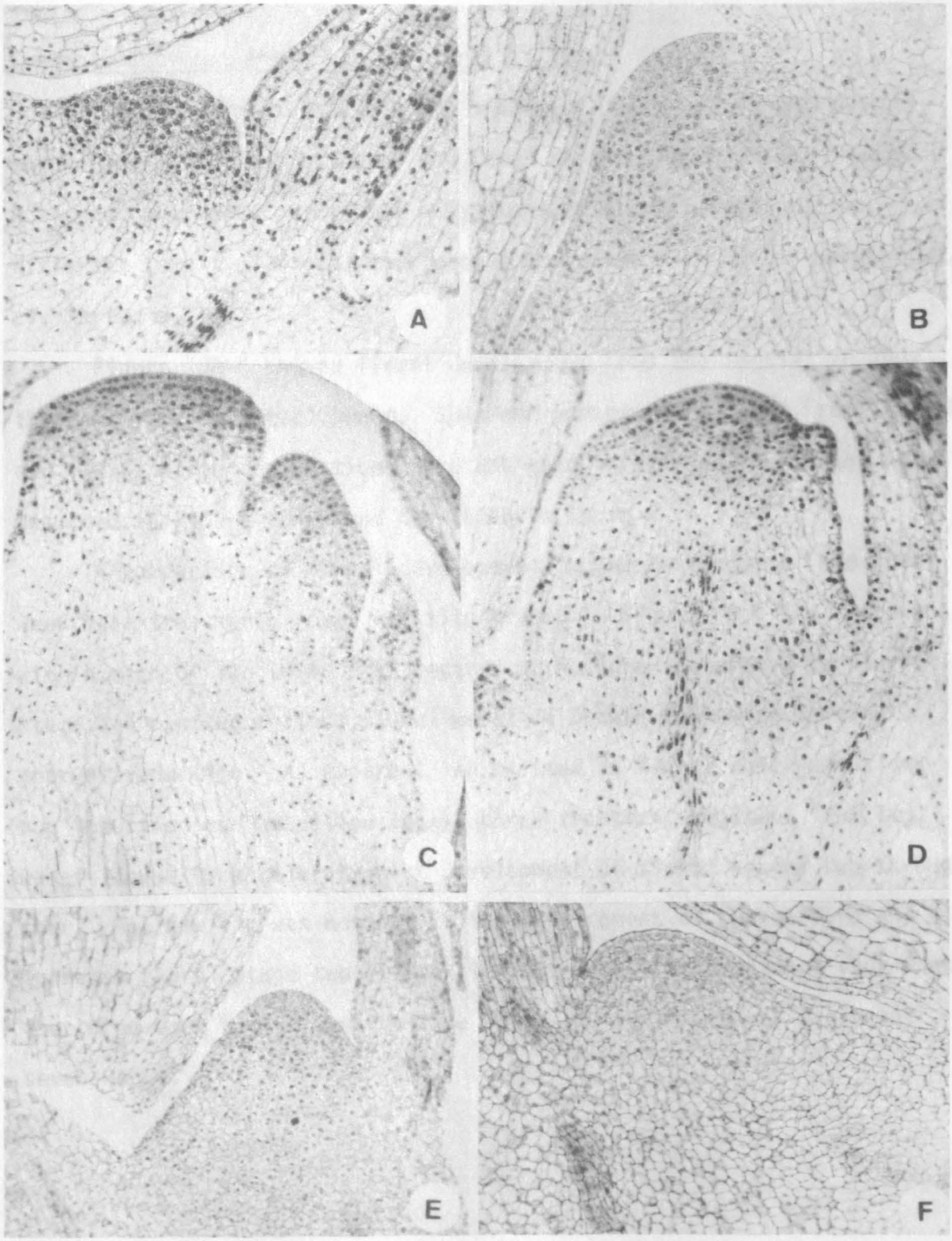
B. One week heat, five weeks growth (80x).

C. Two weeks heat, four weeks growth (45x).

D. Three weeks heat, three weeks growth (60x).

E. Four weeks heat, two weeks growth (60x).

F. Five weeks heat, one week growth (80x).


A
B
C
D
E
F

Thermoinduction: Phase II and Floral Stages.

The next phase of this study addressed Phase II, concerned with morphogenesis following floral induction of three-week heated plants. A second consideration was the assignment of floral stages to the different levels of floral development (modified from Mulder and Leyten, cf. Hartsema, 1961).

Figure 10A-K showed floral development from the vegetative apex through gynoecium development. Specimen numbers were taken from Table 5 and floral stage designations were assigned to each developmental stage depicted in Figure 10A-K and tabulated in Table 6.

A comparison of floral development in the two-, three- and five-week heat treatment groups was illustrated in Figure 11. The specimens within each of the three heat regimes were scored according to floral stage and plotted against floral weeks of growth following initial thermal induction. An apparent lag existed in floral development during induction and evocation in all three treatment regimes. The lag was followed by acceleration of development of floral stages two through four. Another lag was noticed in the development of phases five and six. To reach floral stage two it took the two-week heat treatment four weeks, the three-week heat treatment five weeks and the five-week treatment seven weeks.

Figure 10. Floral development in three-week heated Amazon lily plants showing development from induced apex to gynoecium formation. All collections A-K were heated three weeks.)

A. Collected immediately (80x).

B. Collected after one week growth (80x).

C. Collected after two weeks growth (45x).

D. Collected after three weeks growth (45x).

E. Collected after four weeks growth (60x).

F. Collected after five weeks growth (60x).

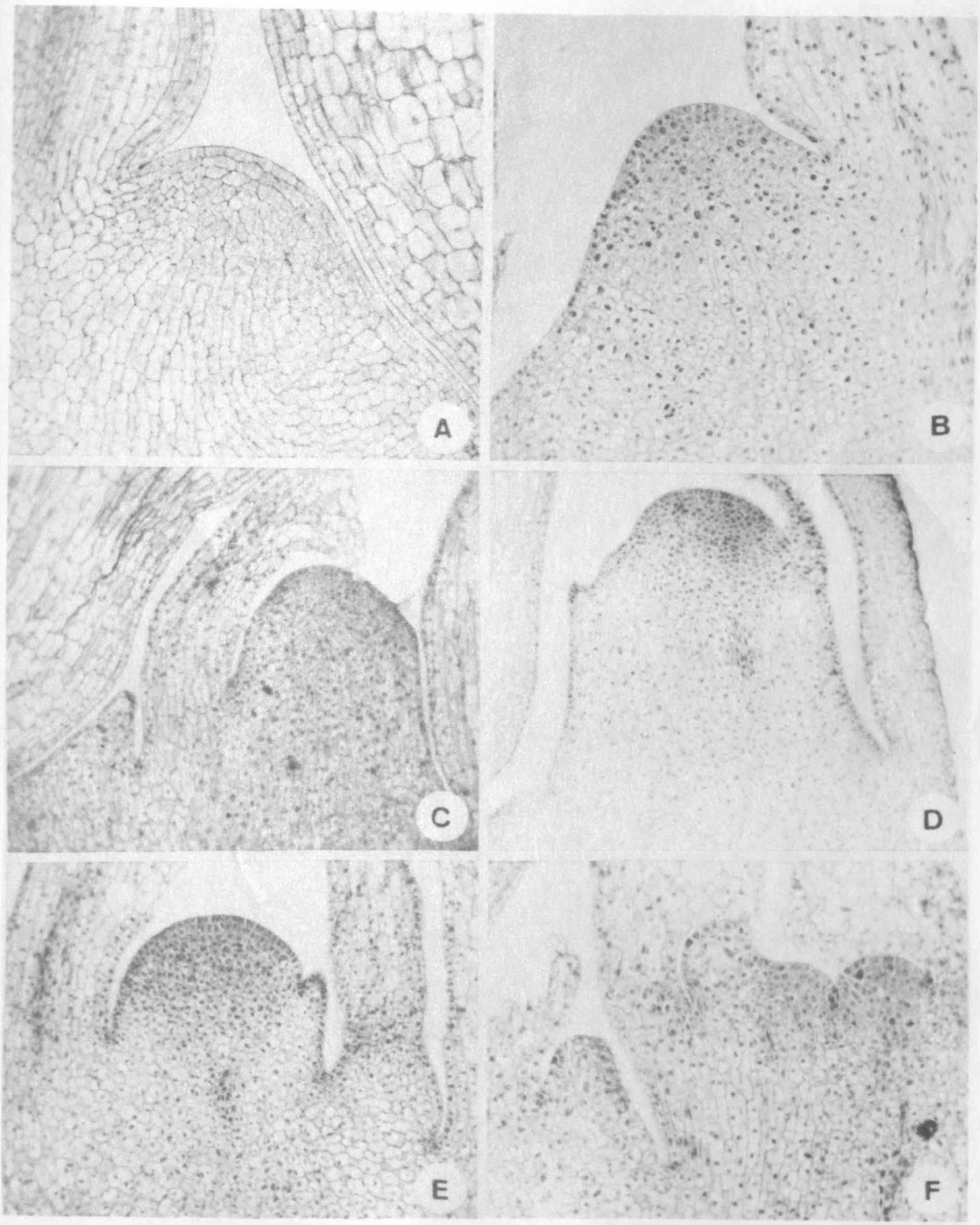

A
B
C
D
E
F

Figure 10 (continued)

G. Collected after 7 weeks growth (65x).

H. Collected after 7 weeks growth (65x).

I. Collected after 7 weeks growth (50x).

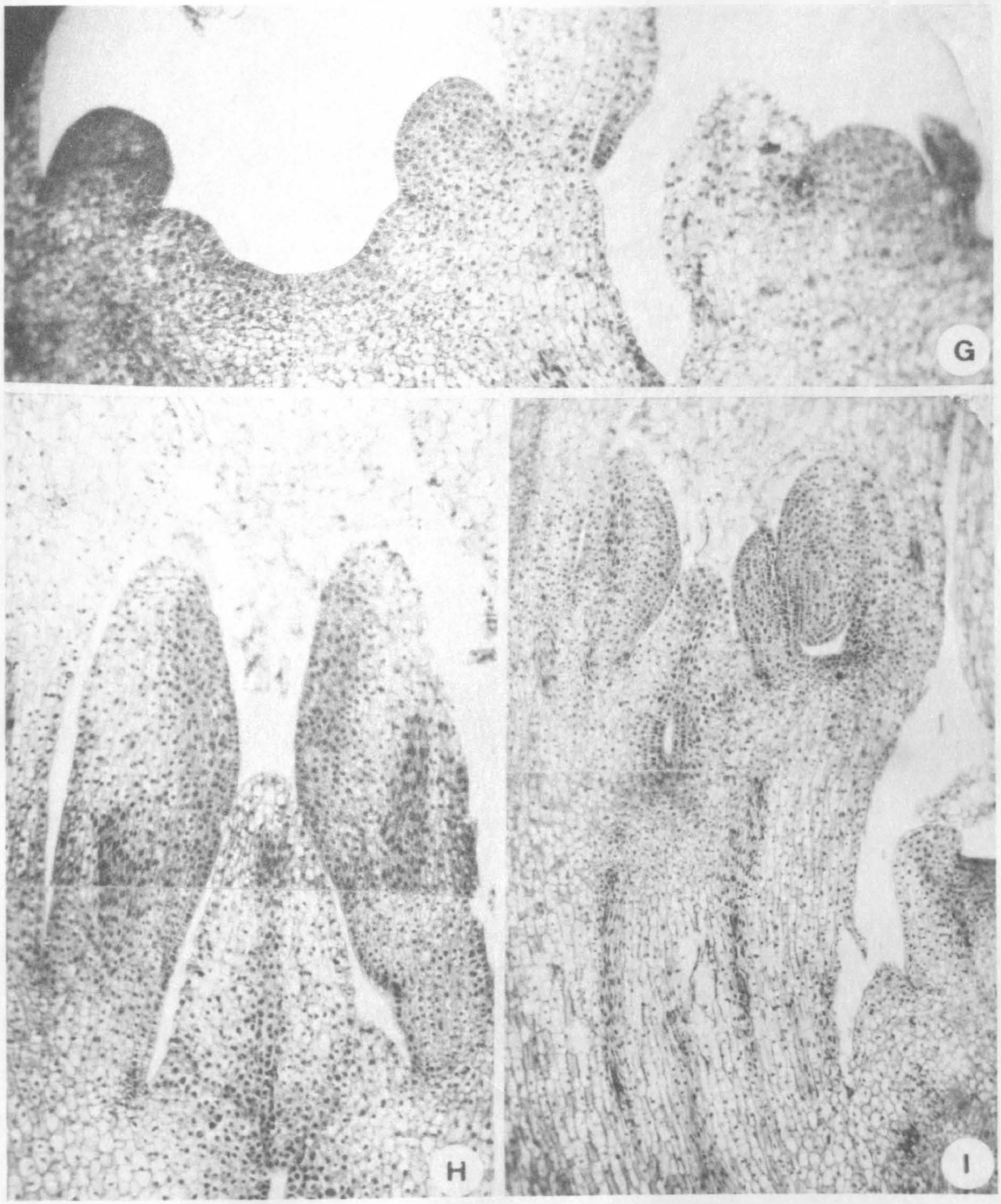

G
H
I

Figure 10 (continued)

J. Collected after 8 weeks growth (65x).

a. Lateral bud at stage shown in J (100x).

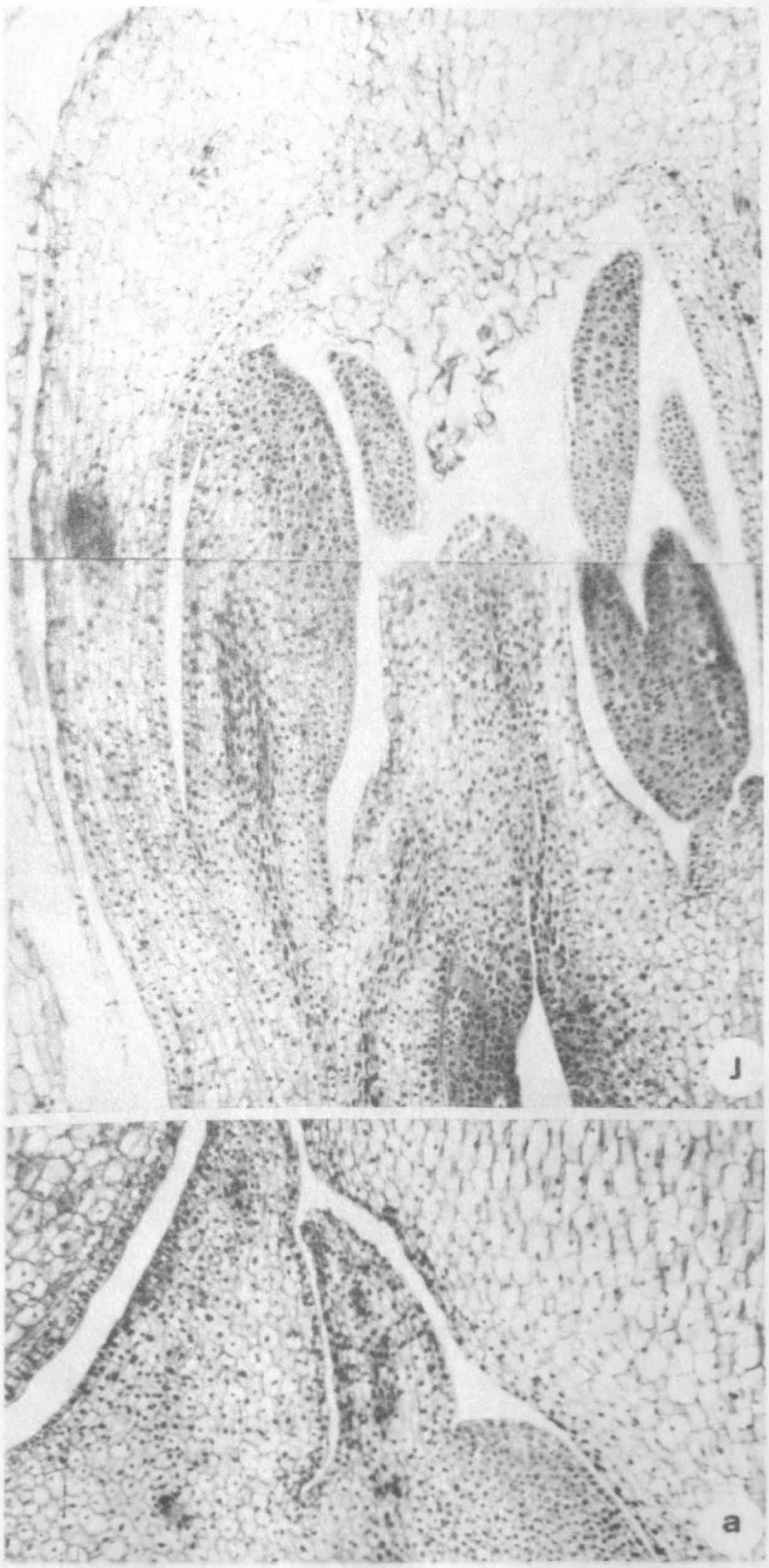

J
a

Figure 10 (continued)

K. Collected after eight weeks growth (45x).

a. Lateral bud at stage shown in K (100x).

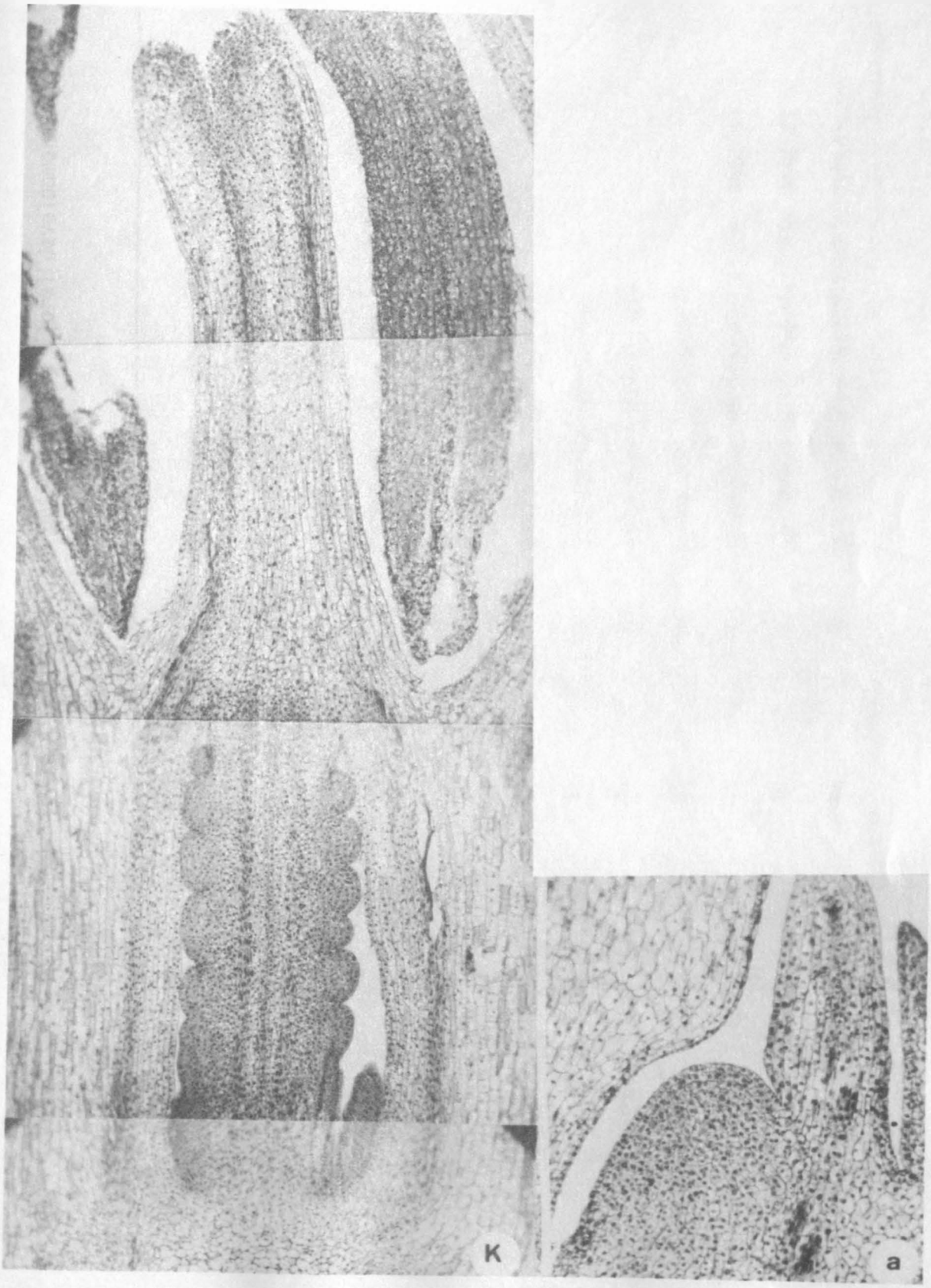

K
a

Table 6. Assignment of specimen numbers from Table 5 and designation of floral developmental stages to each specimen illustrated in Figure 10A-K.

FIGURE 10	TREATMENT (Heated 3 weeks)	TABLE 5 SPECIMEN NO.	FLORAL STAGE	DESCRIPTION (MODIF. HARTSEMA, 1961)
A	Collected immediately	9	0:	floral apex induced.
B	After 1 week growth	31	1:	floral apex evoked.
C	After 2 weeks growth	36	2:	change in shape. 3: some elongation of floral axis.
D	After 3 weeks growth	38	3:	elongation of floral axis. 4: perianth initiation.
E	After 4 weeks growth	40	4:	perianth development
F	After 5 weeks growth	45	5:	androecium initiation
G	After 7 weeks growth	67	5:	androecium development
H	After 7 weeks growth	68	6:	gynoecium development
I	After 7 weeks growth	69	6:	gynoecium development (later than H)
J	After 8 weeks growth	72	6:	gynoecium development (later than H)
K	After 8 weeks growth	70	6:	gynoecium development (ovule formation)

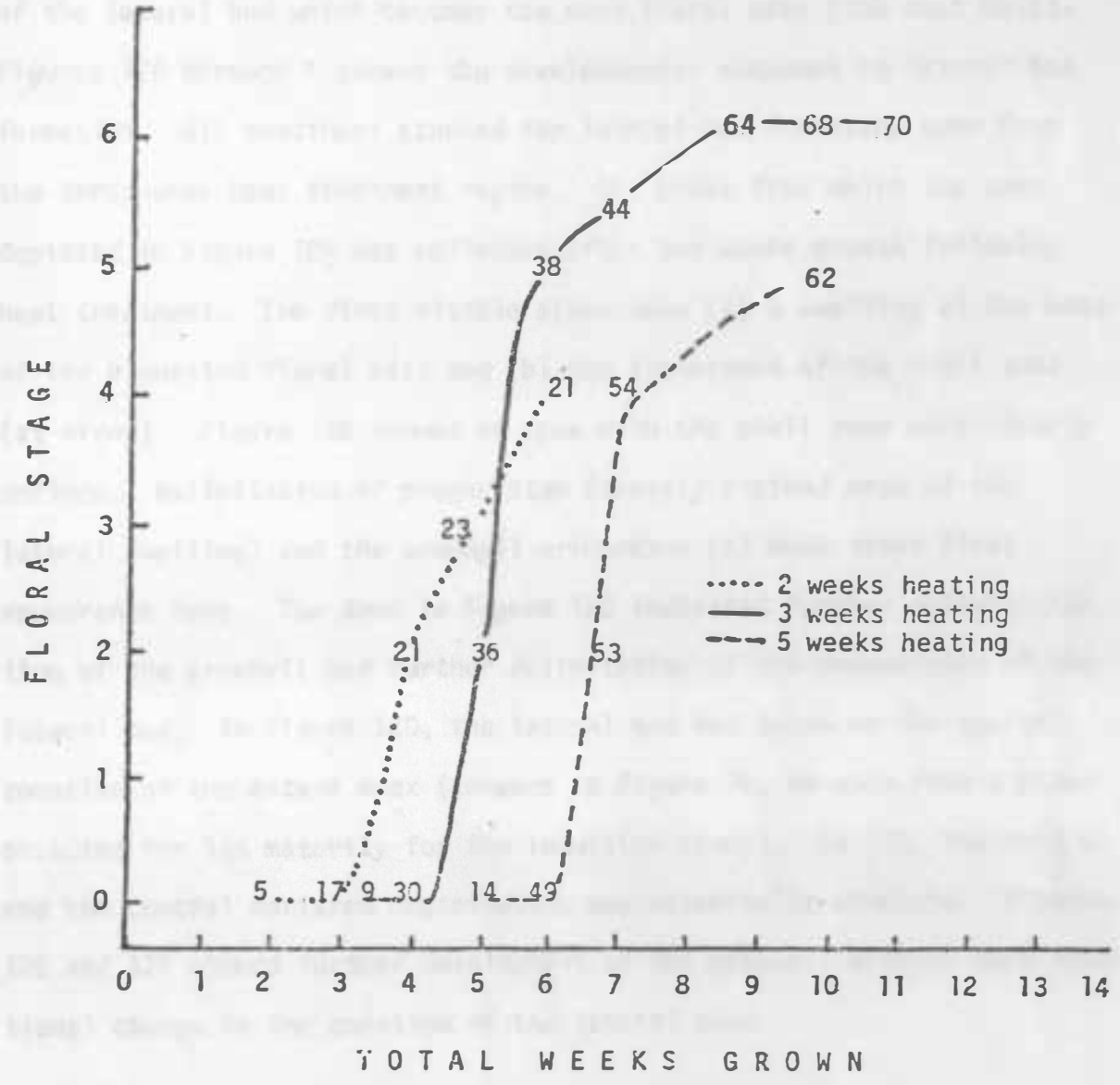


Figure 11. A comparison of floral development by stages in each of three heat treatment regimes over a period of 11 weeks following initial thermal induction. (Numbers of data points represent specimen numbers taken from Table 5.)

Thermoinduction: Phase III.

The third and final phase of the study involved the development of the lateral bud which becomes the next floral axis (the next bulb). Figures 12A through F showed the developmental sequence in lateral bud formation. All specimens studied for lateral bud formation came from the three-week heat treatment regime. The plant from which the apex depicted in Figure 12A was collected after two weeks growth following heat treatment. The first visible signs were (a) a swelling at the base of the elongated floral axis and (b) the appearance of the shell zone (at arrow). Figure 12B showed an apex with the shell zone more clearly defined. Delimitation of promeristem (densely stained area of the lateral swelling) and the prophyll primordium (a) made their first appearance here. The apex in Figure 12C indicated further differentiation of the prophyll and further delimitation of the promeristem of the lateral bud. In Figure 12D, the lateral bud had taken on the typical zonation of the mature apex (compare to Figure 7E, an apex from a plant selected for its maturity for the induction study). In 12D, the mantle and the central meristem organization was essentially complete. Figures 12E and 12F showed further development of the prophyll without much additional change in the zonation of the lateral apex.

Figure 12. Lateral bud development in three-week heat treatment regime of Amazon lily.

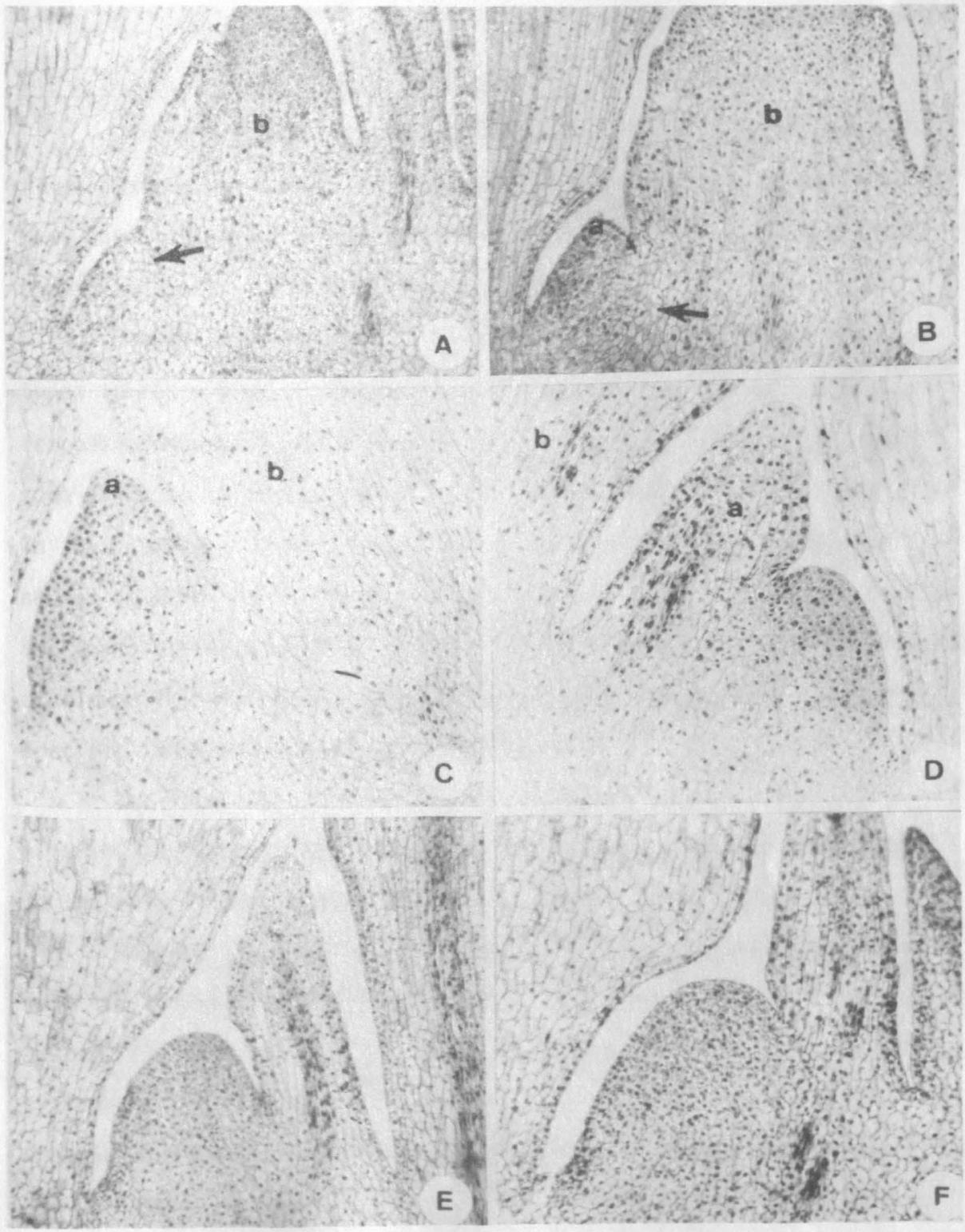
A. Floral apex (b) with lateral bud with delimiting shell zone at arrow (55x).

B. Further lateral bud development. Shell zone more pronounced and prophyll making its appearance (60x).

C. Lateral lateral bud development. Shell zone no longer visible. Prophyll more obvious and promeristem becoming delimited (80x).

D. Mid-development of the prophyll and lateral bud apex. zonation apparent (80x).

E.F. Lateral bud fully matured. Note elongating prophyll and further differentiation of the prophyll procambium (80x) (100x).


b
A
b
B
a
b
C
b
a
D
E
F

DISCUSSION

Thermoinduction: Initial treatments

Hartsema (1961) distinguished among seven different types of flower initiation in bulbous monocots. The Amazon lily seemed to fit best into category 7 in which flowering occurred alternate with leaf formation during the assimilation period.

Salisbury (1963) recognized three different flowering response types to temperature. Amazon lily appeared to have a qualitative response dependent upon a specific temperature. However, there was some evidence that photoinduction may have played a role in flowering in that sporadic flowering took place in constant temperature greenhouses in Brookings in March and mid-October. For plants grown outside and therefore subjected to cold periods of mid-October, a high percentage of flowering resulted, indicating a quantitative response in which a specific temperature promoted flowering.

The fact that daylength in Brookings in March is 11:50.06 to 12:56.06 and in October is 10:30.87 to 11:39.87, gave further support to the role of photoinduction in flowering in Amazon lily.

Popham's Type VII (Usual Angiosperm Type) (Popham, 1966) was selected as the most appropriate type of shoot apex organization for the Amazon lily (See Figure 5). Popham's was a structural classification system and since the study was anatomically based, the functional organization patterns of other authors were not deemed appropriate.

Nougarède, *et al.* (1965), in their cytohistological studies found the shoot in maximal-area phase at the time of leaf buttress

formation and in minimal-area phase after the elevation of the first two leaves.

The scanning electron microscope photographs, Figure 6, showed three stages of leaf development and changes in maximal-, intermediate-, and minimal-area phases of the apex of Amazon lily. This plate was useful to explain the differences in the topography (shape) of the apex and the developmental stage of the last-formed leaf. An example of sectioned apices corresponding to area phases, Figure 7B showed that apex in minimal-area phase, since the last leaf was well-developed but still an obvious extension of the apex. The stage of the apex in Figure 7D was in the maximal area phase, since the apex was well delimited from the developed leaf primordium and the next leaf buttress was forming at the basal flank of the apex. The series of SEM photographs in Figure 6 illustrated a reason for the diversity of apical shapes prior to floral initiation.

Figure 7 compared the plant apices of non-induced (controls) and induced (experimental) plants grown for one to five weeks. There was no difference between the plant apices of the controls (A, C, E, G and I) and the experimental plants (B, D, F, H and J). This lends supporting anatomical evidence to Adams' and Urdahl's (1971) statement that little development takes place until after plants are removed from heating.

As expected, the three- and five-week thermoinduced plants showed no signs of floral initiation either after immediate collection (Figures 8A and 8B) or when collected after one week following induction (Figures 8C and 8D). The earliest sign of floral initiation in this study appeared

in plants after two weeks growth following three weeks of thermoinduction, a total of five weeks after the beginning of induction (Figure 8E). Note that Figure 7J, five weeks heating, showed no floral development. Figures 8F and 8G were in similar stages of development, both of which are in the perianth initiation stage. However, 8F had been heated two weeks longer and was one week older from the initial induction. Figures 8I and 8J indicated initiation of stamen primordia. Both were in very similar stages of development. However, 8J had grown two more weeks in the induction phase and was consequently two weeks older than 8I, post initial induction. This further supported the arrest of development during the induction phase past three weeks.

Adams and Urdahl (1971) found that the appearance of the first flower in the five-week heated plants was 105 days after the end of the induction period, while the three week heated plant's first flower appeared after 110 days. Since floral initiation and therefore development was arrested during the thermoinduction period, there must have been a difference in the velocity of floral development after the induction cycle ended. In fact, since 8I (8 weeks after initial thermoinduction) and 8J (10 weeks after initial thermoinduction) were in essentially the same stage of floral development, any changes in velocity toward first flower production must have come after 10 weeks following initial thermoinduction (between 70 and 105-110 days from beginning of the heat treatment).

Figure 9A and 9B indicated that plants exposed to zero and one week heating, and allowed to grow for a total of six weeks, were not induced. Figure 9D, three weeks of heating, was in a later

developmental stage than Figure 9C. Since they were both grown for six weeks after initial thermoinduction, it appeared that the three-week heating may have stimulated more rapid floral development than two-week heating. Figure 9E and 9F showed no floral development post-induction.

The data from the apices in Figure 9 seemed to support an hypothesis that one week was not adequate for the synthesis and/or transport of florigen from the young leaves to the apex. Further, it appeared that more than four weeks may tend to arrest the synthesis and/or transport of florigen. Two or three weeks seemed optimal for the synthesis and/or transport of florigen to the apex, thus stimulating it to undergo floral evocation.

In studying Phase II and the development of three-week heated plants, floral initiation and subsequent formation of floral appendages were recorded (Figure 10A-K) and assigned stages according to Hartsema, 1961. Salisbury, 1955, studied *in toto* apices and assigned stages based upon the average stage within each treatment. Hartsema's study on tulips assigned stages based on floral parts which resembled Amazon lily development very closely. This study utilized sectioned apices and selected as representative of the treatment that specimen which was most well-developed. It omitted only those stages of Mulder and Leyten, 1928 (cf. Hartsema, 1961) which were duplicate whorls of appendages.

The anatomical study of floral development was terminated with the 72-day collection because the buds were too large to study effectively microscopically and no more fruitful results could be obtained with

older flowers. After the production of a floral branch, the sequence of floral appendage initiation was outer tepals, inner tepals, androecium and gynoecium.

The graph shown in Figure 11, indicated that after an initial lag, the velocity of floral development increased sharply but at different rates, for all three temperature regimes through the floral stages. This supported Adams' and Urdahl's results (1971) that the rate of floral development for the two-, three- and five-week heated plants differed from one another.

Before the second floral stage and before the fifth week of growth, very little difference existed between the velocity of floral development for the two-week and three-week treatments. From the beginning of heating, floral development for two-week heated plants reached the second floral stage in the fourth week and the three-week heated plants reached the second floral stage in the fifth week. The rate of change of floral development was highest for the five-week heated plants. The fact that specimens 53 and 54 (five-week heating and two-week growth) were replicates of the same treatment showing a difference of two stages of floral development lends further support to the rapid rate at which floral development was taking place in the five-week treatment plants.

From the second floral stage on, the rate of change in the velocity of floral development of two-week heated plants slowed down more than that of the three-week heated plants, which slowed much less and therefore developed faster than that of the two-week treatment.

Consequently, floral development of the two-week treatments reached the fourth floral stage during the sixth week after initial heating, while the three-week heating reached the fifth floral stage in the same duration. This supported our suggestion in Figure 9 that although two- and three-week heating was optimal for floral initiation, three-week treatment showed more rapid development than two-week treatment.

The rate of change in velocity of floral development of the five-week heated plants was most rapid, reaching the fourth floral stage within the sixth week after initial heating. Floral development of the two-week treatment after the onset of stage two progressed more slowly than the three- and five-week treatments.

Lateral bud development in Amazon lily followed a pattern similar to most bulbous plants. Further, a shell zone was recognized in an early stage of lateral bud ontogeny (Figures 12A and B). Its disappearance by the seventh week from initial induction (Figure 12C) corresponded to a stage of bud development where the promeristem and prophyll was clearly delineated. This indicated that no reversion of the bud primordium to pith rib meristem was likely to occur. This would support the statement of Shah and Patel, 1972, that one of the functions of the shell zone was to control differentiation of the lateral bud primordium, keeping it from undergoing parenchymatization.

By comparing Figure 12D (lateral bud from plant heated three weeks plus four weeks on the growth table) to 7E, a thermoinduced, mature bud, there was essentially no difference anatomically. Therefore, by the fourth week following the end of the induction period, the lateral had reached a structurally mature state. Any lag between four weeks (28 days)

post-induction and six weeks after the end of flowering (108 days till the end of flowering plus 42 days or 150 days total) was probably due to an immature physiological state. The "critical period" (Adams and Urdahl, 1971) may be the result of the prophyll requiring more time to produce the florigen necessary to evoke the apex to flower.

SUMMARY AND RECOMMENDATIONS

Summary

1. This study on Amazon lily strongly supported the concept that vegetative and floral buds are homologous and that the ontogeny of both began subhypodermally.
2. Apical zonation was categorized as Popham's (1966) Type VII: Usual Angiosperm Type.
3. Variability of apex topography was due to whether the apex was in minimum-, intermediate-, or maximum-area phase of development.
4. No anatomical differences were seen in non-heated (control) plants and those thermoinduced, regardless of the length of the induction period.
5. Floral initiation made its first appearance in the three-week heated plants five weeks after the beginning of the thermoinduction period. No floral initiation was observed in five-week heated plants indicating arrested development during the induction cycle.
6. There appeared to be an increased velocity of floral development in the five-week heated plants over the three-week heated plants, but that accelerated development must have been expressed after ten weeks following initial heat treatment.
7. The highest probability for flowering came during the two-week and three-week thermoinduction cycles.
8. The Amazon lily floral development fit Hartsema's (1961) floral stages described for *Tulipa gesneriana*.

9. A shell zone was initiated very early in the lateral bud primordium and disappeared by the time the promeristem and prophyll made their appearance.
10. The lateral bud (the bulb of the next flowering cycle) was anatomically fully developed seven weeks (140 days) after initial thermoinduction of the three-week heated plants. This was about 132 days before the necessary "critical period" of six weeks after the end of first flowering (Adams & Urdahl, 1971).

Recommendations

1. There seemed to be a weak photoperiodic reaction in Amazon lily and the thermoperiodic induction caused a qualitative response promoting flowering. Studies on the interrelations between photo- and thermo-period responses would be important.
2. Cytohistological studies should be made during the evocation cycle following thermoinduction, especially studies on mitotic index, polysaccharide storage and nucleolar volume changes.
3. Apical zonation studies, coupled with cytohistological studies, should be made using a functional classification scheme to categorize the apex of Amazon lily.
4. Clarification of causal factors relative to differing velocities of floral inititation after different heat treatment regimes would be of value.
5. Hormonal studies relative to when the lateral bud completes the "critical period" and becomes functionally ready to be thermally induced could shed light on the long lag between anatomical maturity and physiological maturity.

LITERATURE CITED

Adams, D. G. and Urdahl, W. A. (1971). Influence of bulb temperature on floral initiation in the Amazon lily, *Eucharis grandiflora* Planch. Hort. Science 6(3), 205.

Bailey, L. H. (1925). Manual of cultivated plants. The MacMillan Co. N. Y. p. 184.

Bernier, G. (1970). Cellular and molecular aspects of floral induction. London: Longman Group Ltd. 492 p.

Esau, K. (1965). Plant anatomy, John Wiley and Sons, Inc. New York. 767 p.

Evans, L. T. (1969). Induction of flowering: Some case histories. Cornell U. Press, Ithaca, N. Y. 488 p.

Gifford, E. M. Jr. (1951). Ontogeny of the vegetative axillary bud in *Drimys winteri* var. *chilensis*. Amer. J. Bot. 38(3), 234-243.

Gifford, E. M. Jr. and Corson, G. E. Jr. (1971). The shoot apex in seed plants. Bot. Rev. 37(2), 143-229.

Gifford, E. M. Jr. and Tepper, H. B. (1961). Ontogeny of the inflorescence in *Chenopodium album*. Amer. J. Bot. 48, 657-667.

Goodspeed, T. H. (1961). Plant hunters in the Andes. Univ. of Calif. Press, Berkeley.

Hartsema, A. M. (1961). Influence of temperatures on flower formation and flowering of bulbous and tuberous plants. Handbuch der Pflanzen Physiologie. Springer-Verlag, Berlin, 123-167.

Jacqmard, A., Raju, M. V. S., Kinet, J. M. and Bernier, G. (1976). The early action of the floral stimulus on mitotic activity and DNA synthesis in the apical meristem of *Xanthium strumarium*. Amer. J. Bot. 63(2), 166-174.

Johnshoy, M. W. (1978). Rotaviral enteritis in swine: a scanning electron microscopic study. M. S. thesis. South Dakota State University.

Lindstrom, R. S. (1971). Amazon lily -- year around production. Florists Review 149 (3864), 20.

Myers, G. A. (1976). Advanced plant anatomy. Class notes.

Nougarede, A., Gifford, E. M. Jr. and Rondet, P. (1965). Cytohistological studies of the apical meristem of *Amaranthus retroflexus* under various photoperiodic regimes. Bot. Gaz. 126(4), 248-298.

Popham, R. (1966). Principal types of vegetative shoot apex organization in vascular plants. Laboratory Manual of Plant Anatomy C. V. Mosby Co, St. Louis, 228 p.

Popham, R. and Chan, A. P. (1950). Zonation in the vegetative stem apex of *Chrysanthemum morifolium*. Amer. J. Bot. 37(6), 476-484.

Salisbury, F. B. (1955). The dual role of auxin in flowering. Plant Physiology 30, 327-334.

Salisbury, F. B. (1963). The flowering process. Pergamon Press. N. Y. and London.

Salisbury, F. B. (1971). Time Measurement. (Chapter 5). The biology of flowering. The Natural History Press, Garden City, N. Y.

Sass, J. E. (1964). Botanical Microtechnique. The Iowa State College Press. Ames, Ia. 223 p.

Schulz, P. (1954). Amaryllis - and how to grow them. M. Barrows and Co. Inc., N. Y., 106.

Shah, J. J. and Patel, J. D. (1972). The shell zone: its differentiation and probable function in some dicotyledons. Amer. J. Bot. 59(7), 683-690.

Shah, J. J. and Unnikrishnan, K. (1969). The shoot apex and the ontogeny of axillary buds in *Cuminum cyminum* L. Aust. J. Bot. 17, 241-253.

Teltscherova, L. and Pleskotova, D. (1973). Nucleic acid synthesis in *Chenopodium rubrum* L. during photoperiodic induction and its relation to endogenous rhythmicity. Biol. Plant. (Praha) 15(6), 419-426.

Wardlaw, C. W. (1968). Morphogenesis in plants. Methuen and Co., Ltd. London. 451 p.

Wellensiek, S. J.(1961). Theoretical backgrounds of flowering: The role of temperature. Amer. Soc. for Hort. Sci. 66, 397-402.

Yeung, E. C. and Peterson, R. L. (1972). Studies on the rosette plant of *Hieracium floribundum*. I. Observations related to flowering and axillary bud development. Can. J. Bot. 50(1), 73-78.

APPENDIX

INITIAL QUESTIONS POSED, DETAILED CODING, TREATMENT AND COLLECTING SCHEDULES AND MICROTECHNIQUE DETAILS USED IN THE FLORAL INDUCTION STUDIES OF AMAZON LILY.

PHASE I

1. Do anatomical changes occur in the apical meristem during the three weeks heating period at 26° C?
2. Do anatomical changes occur in the apical meristem if heated longer than three weeks?
3. If there is no apparent change taking place, then how long after heating has stopped must one wait before visible changes in the apical meristem occur?

   Is the velocity of apical change the same whether heated for 1, 2, 3, 4, 5 weeks?

PHASE II

1. What is the developmental sequence of the floral apex when bulbs are heated for three weeks at 26° C and then returned to 18° C? Weekly sampling through flowering occurrence until pollen is shed (19 weeks).

PHASE III

In the developmental sequence of the new terminal growing point:

1. When is it first visible?
2. When does the new terminal growing point begin to function? Make new leaf primordia? Record rate of new leaf production by

measuring comparing to developmental and chronological scale of floral development.

3. What is the final developmental situation six weeks after the last flower has died on the scape?

   Standard heating period -- three weeks at 26° C.

PHASE I

TREATMENT AND COLLECTING SEQUENCING

IA0 I = PHASE I. A refers to questions 1 and 2

0 = no heating
underlined = heating

IA1	IA1	IA1	IA1	IA1
IA2	IA2	IA2	IA2	IA2
IA3	IDb3	IA3	IA3	IA3
IA4	IDb4	IDc4	IA4	IA4
IA5	IDb5	IDc5		IA5
	IDb6	IDc6		IDe6
	IDb7	IDc7		IDe7
		IDc8		IDe8
			IDd9	IDe9
				ID310

No collection after one week of heating. Previous data indicates negative results.

PHASE II		PHASE III
IA1	2 weeks heat	IA1
IA2		IA2
IA3		IA3
IDc4	3 weeks heat	IDc4
IDc5		IDc5
IDc6		IDc6
IDc7		IDc7
IDc8		IDc8
II9		II9 through II19
II10	4 weeks heat	III20 through III23
II11		
II12		
II13 through II 72	5 weeks heat & 1 week growth	

IAO TREATMENTS (EXPERIMENTAL)

Several apices were collected 12/2/71 in these vegetative conditions without treatment of any kind.

They were placed in FAA and CRAFS for comparative purposes, to determine the best killing-fixing agent to be used.

FAA used		CRAFS V used		
formaldehyde	10 cc	1% chromic	50 cc	Stock 1
glacetic	5 cc	10% acetic	35 cc	
95% ELOH	50 cc	formaldehyde	15 cc	Stock 2
H_2D	35 cc	Mix just before using		

The apices were aspirated and, after 12 hours, cycled through the following dehydrating-embedding process (4 hours each, minimum)

50% TBA	70%	80%	95	100#1	100#2	50-50 tBuOH-PO
H_2O 50 ml	30 ml	15 ml	5 ml	0 ml	0 ml	
EtOH 40 ml	50 ml	50 ml	40 ml	25 ml	0 ml	50% tBuOH
BuOH 10 ml	20 ml	35 ml	55 ml	75 ml	100 ml	50% paraffin oil
					eosin .1g	

1. Prepare vial with hot hard paraffin.
2. Add 50-50 paraffin oil - butyl alcohol
3. Cork and place in oven one hour or longer
4. Pour off and replace with fresh melted paraffin at least one hour after tissue reaches bottom. Don't replace cork.
5. Change paraffin 3-4 times or until odor of BuOH is gone. For delicate tissues this should all be done the same day. Don't allow tissues to cool between stages.
6. Embed in boats and thrust in ice water or freezer.

DEFINITIONS OF CODE

PHASE I; In phase I1 heat treatment varied from 0 - 3 weeks.

Ind plants 1-9 & controls	IA0 = no heat vege condition	IA2 = heat 2 weeks - collected
	IA1 = heat 1 week - collected	IA3 = heat 3 weeks - collected

In phase I2 heat treatment varied from 4 - 5 weeks.

Plants 10-15	IA4 = heat 4 weeks - collected	IA5 = heat 5 weeks - collected

In phase I3-4 heat treatment was 2 weeks through 5 weeks with collections being made at several intervals thereafter.
*No collection after 1 week heating. Earlier data indicates negative results.

Plants 16-18	IDb-3 = heated 2 weeks - transferred to growth table - collected after 1 week.
19-21	IDb-4 = heated 2 weeks - transferred to growth table - collected after 2 weeks.
22-24	IDb-5 = heated 2 weeks - transferred to growth table - collected after 3 weeks.
25-27	IDb-6 = heated 2 weeks - transferred to growth table - collected after 4 weeks.
28-30	IDb-7 = heated 2 weeks - transferred to growth table - collected after 5 weeks.
31-33	IDc-4 = heated 3 weeks - transferred to growth table - collected after 1 week.
34-36	IDc-5 = heated 3 weeks - transferred to growth table - collected after 2 weeks.
37-39	IDc-6 = heated 3 weeks - transferred to growth table - collected after 3 weeks.
40-42	IDc-7 = heated 3 weeks - transferred to growth table - collected after 4 weeks.
43-45	IDc-8 = heated 3 weeks - transferred to growth table - collected after 5 weeks.
46-48	IDd-9 - heated 4 weeks - transferred to growth table - collected after 5 weeks.
49-57	IDe-6 = heated 5 weeks - transferred to growth table - collected after 1 week.
52-54	IDe-7 = heated 5 weeks - transferred to growth table - collected after 2 weeks.
55-57	IDe-8 = heated 5 weeks - transferred to growth table - collected after 3 weeks.
48-60	IDe-9 = heated 5 weeks - transferred to growth table - collected after 4 weeks.
61-63	IDe-10= heated 5 weeks - transferred to growth table - collected after 5 weeks.

In Phase II heating is optimum (3 weeks - 25° C). Plants are transferred to growth table and collections are made weekly until 19 weeks (anthesis). Phase I data is used for first 8 weeks since treatment would be identical. 64-96 II9 through II19 = 9th through 19th week.

In Phase III (P1) heating is optimum. Plants are used from Phases I and II through week 19 since treatment for this segment of Phase III would be identical. Since the early development of the lateral bud (which eventually becomes the next bulb) requires development past week 19, it will be collected through the 23rd week.

The following page is a layout of coding for each plant. A legend is at the bottom of the page.

HEAT BENCH

IDc	IA1	IDc	IDe	IA3	IDE	IDE	IA5	II	II	IDb	II	II	IDb	II	III	IDc	III
6		8	6		8	10		10	12	4	14	16	6	18	20	4	22
12/22	12/8	12/22	1/5	12/22	1/5	1/5	1/5	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
37	1	43	49	7	55	61	13	67	73	19	79	85	25	91	97	31	103
IDc	IA1	IDc	IDe	IA3	IDE	IDE	IA5	II	II	IDb	II	II	IDb	II	III	IDc	III
6		8	6		8	10		10	12	4	14	16	6	18	20	4	22
12/22	12/8	12/22	1/5	12/22	1/2	1/2	1/2	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
38	2	44	50	8	56	62	14	68	74	20	80	86	26	92	98	32	104
IDc	IA1	IDc	IDe	IA3	IDE	IDE	IA5	II	II	IDb	II	II	IDb	II	III	IDc	III
6		8	6		8	10		10	12	4	14	16	6	18	20	4	22
12/22	12/8	12/22	1/5	12/22	1/5	1/5	1/5	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
39	3	45	51	9	57	63	15	69	75	21	81	87	27	93	99	33	105
IDc	IA2	IDd	IDe	IA4	IDE	II	IDb	II	II	IDb	II	II	IDb	II	III	IDc	III
7		9	7		9	9	3	11	13	5	15	17	7	19	21	5	23
12/22	12/15	12/29	1/5	12/29	1/5	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
40	4	46	52	10	58	64	16	70	76	22	82	88	28	94	100	34	106
IDc	IA2	IDd	IDe	IA4	IDE	II	IDb	II	II	IDb	II	II	IDb	II	III	IDc	III
7		9	7		9	9	3	11	13	5	15	17	7	19	21	5	23
12/22	12/15	12/29	1/5	12/29	1/5	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
41	5	47	53	11	59	65	17	71	77	23	83	89	29	95	101	35	107
IDc	IA2	IDd	IDe	IA4	IDE	II	IDb	II	II	IDb	II	II	IDb	II	III	IDc	III
7		9	7		9	9	3	11	13	5	15	17	7	19	21	5	23
12/22	12/15	12/29	1/5	12/29	1/5	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/15	12/22	12/22	12/22	12/22
42	6	48	54	12	60	66	18	72	78	24	84	90	30	96	102	36	108

GROWTH BENCH

IDe	IDb	IDe	II9	IDb	III1	III3	IDb	III5	III7	IDc	III9	III21	IDc	III23	IDd
7	3	9		5			7			5			7		9
1/19	12/22	2/2	2/2	1/5	2/16	3/1	1/15	3/15	3/29	1/5	4/12	4/26	1/19	5/10	2/2
52	16	58	64	22	70	76	28	82	88	34	94	100	40	106	46
53	17	59	65	23	71	77	29	83	89	35	95	101	41	107	47
54	18	60	66	24	72	78	30	84	90	36	96	102	42	108	48
IDe	IDb	IDe	III0	IDb	III2	III4	IDc	III6	III8	IDc	III20	III22	IDc		IDe
8	4	10		6			4			6			8		6
1/26	12/29	2/9	2/9	1/12	2/23	3/8	12/29	3/22	4/5	1/12	4/19	5/3	1/26		1/12
55	19	61	67	25	73	79	31	85	91	37	97	103	43	109	49
56	20	62	68	26	74	80	32	86	92	38	98	104	44	110	50
57	21	63	69	27	75	81	33	87	93	39	99	105	45	111	51

I-III: Phases of study
A,D: A = controls; D = experimental
a-e: Corresponds to 1-5 weeks of heating

IDc
6 ——— Nurber of weeks total growth from induction
12/22 ——— Date collected
37 ——— Specimen number

IDc = IDD
4 weeks

See pg 27