A STUDY OF THE
ORIGIN AND EMERGENCE OF THE
FLOWER OF *CUPHEA VISCOSISSIMA* JACQ.

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James Francis Merritt
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by

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RESPECTFULLY DEDICATED TO

MY PARENTS,

MR. & MRS. CLIFTON MERRITT

and

AUNT NANNIE & UNCLE HAL (MR. & MRS. F. H. BADDERS)

ACKNOWLEDGMENTS

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ABSTRACT

James Francis Merritt. A STUDY OF THE ORIGIN AND EMERGENCE OF THE FLOWER OF *Cuphea viscosissima* Jacq. (Under the direction of O. Christine Wilton) Department of Biology, June, 1968.

The anatomical origin and emergence of the interaxillary flower of *Cuphea viscosissima* Jacq., were studied. Nodal and apical samples were cut in both cross and longitudinal sections and the vascular trace system defined. On the basis of both mature traces and provascular traces a hypothesis is suggested to explain the interaxillary location of the flower of *C. viscosissima*.

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INTRODUCTION

The plant, *Cuphea viscosissima* Jacq., has a feature unique to the genus *Cuphea* in the Lythraceae. The flowers are interaxillary or internodal rather than axillary or terminal. This characteristic as reported by Boubier (1896) is also found in various *Solanaceae*, such as *Solanum guineense* Lamk., and certain members of the genus *Asclepias*.

Cuphea viscosissima is one of four species of the genus *Cuphea* endemic to North America. Both common names, tarweed or blue waxweed, and the specific epithet, *viscosissima* are descriptive of its characteristics. There are many glandular hairs, especially on the stems, which secrete a sticky substance. Foreign objects such as small insects and seeds adhere to the plant. *Cuphea viscosissima* is common in old pastures and waste lands from Alabama to Massachusetts and from the Carolinas to central Arkansas (Graham 1964). In many locations it is a pest and avoided by grazing animals. It is an herbaceous annual with an indeterminant type of growth. The leaf arrangement is decussate with a vegetative bud maturing in the axil of only one leaf per node. In late stages of growth the long slender stem does not continue to grow upright, but along with the branches forms a fan-like tangle.

The vegetative phase of growth is short, the inter-axillary flowers appearing sometimes at the level of the third or fourth nodes and not later than the level of the sixth or seventh nodes. After initiation of flowering every node possesses a flower, and in late stages of growth some of the branches remain reduced, (Graham 1964) and become inflorescence-like.

The literature contains little on anatomical studies of the vascular trace system of Cuphea to explain the location of the flower. Graham (1964) states that the peduncle of alternate flowered species of Cuphea arises at a node, remains fused with the internode above and emerges at or just below the next higher node. Boubier (1896), who did original work with this characteristic using Cuphea ignea Alph., concluded that the peduncle is fused with the internode between the node of origin and the node of emergence. He also observed that the stele of the plant (C. ignea) has eight fascicles or bundles and showed that certain ones formed the floral traces and others the leaf traces. Cuphea viscosissima varies somewhat in stelar pattern from C. ignea in that the vascular cylinder is not broken into distinct bundles, but in general characteristics they are the same.

The object of this study was to describe the vascular trace system and give a pictorial series to show the anatomical origin and emergence of the flower. Also, it was desirable to determine if any differences in the vascular trace system occurred in the strictly vegetative node as compared with the floral node.

MATERIALS AND METHODS

Seeds and plants of *Cuphea viscosissima* were collected from a pasture one-half mile southeast of Greenville, N. C., Pitt County Road # 1706. Seeds were planted in a greenhouse and maintained for sampling. Nodal and apical samples were collected from plants of various developmental stages: (1) those with development of one or two nodes; (2) those with development of five to seven nodes; and (3) those with development of as many as fifteen to twenty nodes. The samples consisted of vegetative nodes, floral nodes with both primordial and mature flowers, and the cotyledonary node. The samples were preserved and fixed in formalin-acetic-alcohol, Ladawsky's Solution, and dehydrated using both ethyl and tertiary butyl alcohols. Fisher "Tissuemat" was used for embedding the material and serial sections were cut eight to ten microns thick. The sections were stained with basic safranin and fast green, cleared in xylol and mounted in "Kleermount." In addition, some sections were stained using tannic acid and iron alum with safranin and orange G. as described by Sharman (1943). Photographs were produced by exposing print enlarging paper with the use of a Bioscope with a modified light source. The light source was an instant-on American Optical microscope lamp (number B-735-3). The initial exposures, after development, yielded a negative on positive print enlarging paper. From these negative prints, positive prints were produced utilizing the contact exposure technique.

OBSERVATIONS

Cuphea viscosissima, for the short time it is vegetative, possesses a bud in the axil of each leaf. At each node, one of these buds is suppressed or fails to develop past the primordial stage while the other begins to develop into a branch. This vegetative phase lasts only until the plant has reached the fourth or fifth nodal stage. Usually at the level of the fifth node a solitary flower appears in an interaxillary or sometimes internodal position (Fig. 1). According to Boubier (1896) this flower develops at the subtending node of the node at which it appears, and the vascular tissue of the peduncle remains fused with the internode between the two nodes. After the appearance of the first flower the general morphology begins to change. It seems that the further development of the axillary buds below the level of the first flower is suppressed. However, the axillary buds at and above the level of the first flower begin to develop into axillary branches. As the plant develops further, one flower and one axillary bud are produced at each node. The resultant growth habit is an upright plant with the axillary branches forming two longitudinal lines at right angles to each other. The interaxillary flowers also form two longitudinal lines at right angles. Once initiated, this arrangement is found consistently until the end of the growing season. It appears that the axillary bud and the flower never alternate in the axils of the leaves. If the cylindrical axis of the plant is divided into equal halves, in one half (180°) vegetative buds are produced and in the other half (180°) floral buds are produced (Fig. 2). This results in the unusual growth habit of the axillary branches developing on only one side of the plant (Fig. 3). As the plant ages the axillary branches spread at greater than ninety degree angles (Fig. 4). The slender stem is unable to support the weight of the plant, and it bends producing the fan-like tangle mentioned earlier.

After a period of this initial floral-vegetative growth the axillary buds produced by the branches usually remain reduced with short internodes and become inflorescence-like. At this time the growth rate shows a marked decrease, but usually the plant continues limited growth throughout the remainder of the season.

The plant seems not to be photoperiod sensitive because it will produce flowers if seeds are germinated and maintained in the greenhouse in mid-winter as well as in mid-summer. No experiments concerning nutrition, growth hormones, or light quality were undertaken to determine factors affecting flowering. However, the germination of the seeds seems to be affected by light. Graham (1967) states that seeds will germinate in two to three months at 40° C. if kept in moist peat. In the research here reported seeds placed on moist filter paper in petri plates were found to germinate in light with no germination during an equivalent period in seeds maintained in darkness. The time period was determined to be about three days.

STEM ANATOMY OF *Cuphea viscosissima*

The epidermis consists of a single layer of cells with a thin layer of cutin. This is likely related to the fact that the plant wilts readily. The epidermis contains two main types of hairs or trichomes. One type is the unicellular, elongated hair which appears to be slightly lignified at the tip. The other type, the multicellular, glandular hairs secrete a sticky substance which gives the plant its "clammy" nature. The cortex is made up of thin walled parenchyma containing numerous druces. The stele is an amphiphloic siphonostele. The pith, composed of thin-walled parenchyma, also contains many druces. There are four fascicles of vascular tissue connected by interfascicular arches of undifferentiated tissue.

The vascular cylinder of the mature internode is almost circular in shape. However, the primary vascular tissue is more nearly square and only assumes the circular shape as a result of secondary growth. In association with the shape of the stele, the stem also is almost circular in shape.

The first series of vegetative nodal sections (Figs. 5-14) shows the transition from the normal internodal pattern through the nodal pattern of two successive nodes. These figures were taken from the internode below, the node, and the internode above the third node of a plant still in the initial vegetative phase of growth. Figure 5 shows the normal internodal pattern of the stem. The stele can be divided into four quadrants which will be designated as A, B, C, and D. In a typical internodal section the quadrants appear to be equal in development. As the node is approached, (Fig. 6) two opposite quadrants show greater development and move toward the outside of the stem giving the stele an oval shape.

As these two traces move out into the base of the petiole, a unilacunar gap is formed (Fig. 7). As the traces move out into the leaves the vascular tissue on either side of the gaps becomes smaller and eventually severs connection with the traces (Fig. 8). This vascular tissue then becomes the connecting link between the vascular tissue of the stele (by way of the leaf trace) and the axillary bud (Fig. 9). One of the axillary buds develops to a greater degree than the other which is apparent in Fig. 9. The vascular tissue of each axillary bud soon rounds up as a result of the growth of the axillary meristems (Fig. 10). The leaf gaps are very quickly filled by interfascicular meristematic activity produced in the shoot apical meristem. The normal internodal pattern is then restored and secondary vascular tissue is laid down which completely fills in the leaf gap (Fig. 10). Throughout the internode above the node just described the remaining quadrants (B and D) develop; and, it becomes apparent they will form the leaf traces of the next node, which is observed in figures 12-14.

The fourth node, which usually gives rise to the first flower, varies in development somewhat from the vegetative node just described. Figures 15-26 were taken from a plant which possessed an interaxillary flower at the fifth node. Figure 15 shows an internode section beneath the fourth node. Again, four quadrants are evident (A, B, C and D) two of which have developed to a greater degree than the others and these two quadrants (A and C) will form the leaf traces of the fourth node. Figure 16 shows a change in the shape of the stem at the base of the node. Enlargments appear on two sides corresponding to the enlarged quadrants A and C (Fig. 16). These enlargments are due to the presence of the bases of the petioles of the leaves. The stele loses its almost circular shape and becomes more oval as a result of the beginning divergence of quadrants A and C. Figure 17 shows the development of the leaf gaps and traces. The traces A and C have separated from the stele but connections between them and the stele continue to exist. The trace-system is the same as occurs in the vegetative nodes, a one trace unilacunar type. There is evidence that the connections between the stele and trace A shows less activity and differentiation than that of trace C (Fig. 17).

Figure 18 shows an even greater difference between the surrounding tissue of gaps A and C. The gap in the stele at point C begins to close at a lower level than that surrounding gap A. Also, the vascular trace of the axillary bud in gap C is evident. It appears first as two parallel groups of meristematic type tissue. Gap A shows little evidence of closing at this level but in Fig. 19, gap A appears to be closing. In Fig. 20, gap C is almost completely closed and the formation of the circular stele within the axillary bud is apparent. Figure 20 shows that the two opposite leaves do not separate completely from the stem at exactly the same level. In observing the external morphology of many nodes, it is obvious that this character is quite variable. At some nodes, the leaf which possesses an axillary bud may separate from the stem two to five millimeters below the opposite leaf whereas at other nodes the opposite condition exists.

Leaf A in this series shows no evidence of an axillary bud, and it seems to separate from the stem at a higher level than leaf C. Gaps A and C are completely closed (Fig. 20). However, at gap A an arrested trace remains while at gap C the normal circular pattern of the stele is formed.

Figures 21 and 22 show that the arrested trace is retained throughout the internode and that fascicles B and D which form traces for the leaves of the fifth node show progressive development. Also, they show that the external shape of the stem is modified. One side (C) is slightly depressed, and side A shows a slight swelling or protrusion. The enlargment of the stem at B and D and the change in the shape of the stele indicate that the traces of the leaves of node five are beginning to separate from the stele. Also, side A shows that the arrested trace is now beginning to emerge (Fig. 23). Figure 24 shows that the vasculature of the flower has separated from the vascular cylinder, but the peduncle is still partially fused with the stem. The leaf gaps of node five are obvious in figures 25 and 26. There is some indication that they are being closed in the same manner as occurred at node four. Figure 26 shows that the floral primordium has completely separated from the stem and the pattern of the vascular tissue seen in Fig. 19 of node four is repeated.

GENERAL ANATOMY OF THE APICAL MERISTEMS

There appear to be three types of meristems in the shoot system of Cuphea viscosissima. One is strictly vegetative producing only vegetative structures such as stem, leaves and axillary buds (Fig. 30). A second type, the floral-vegetative, produces stem, leaves, one axillary vegetative bud and one axillary floral bud (Figs. 27 & 28). The third type is the floral meristem which produces or develops into one flower (Fig. 29).

The vegetative meristem shows the typical tunica-corpus arrangement of Schmidt (Esau 1965). It appears to be relatively inactive and less organized than the floral-vegetative type meristem. The vegetative shoot meristem, a dome-shaped structure, possesses one distinct tunica layer and a second layer which appears to undergo divisions both periclinally and anticlinally. However, the anticlinal divisions seem to predominate. The corpus, composed of about five layers, appears to be small and disorganized. The cells of the outer tunica layer are larger than the remainder of the cells of the meristem. It appears that the two axillary meristems are cut off from the apical meristem by differentiation of a shell zone (Esau 1965). One of these axillary buds is cut off farther from the apex than the other (Fig. 30). The axillary meristem nearer the apex (Fig. 30,A) is the larger of the two and maintains this dominance throughout the vegetative life of the plant.

The floral-vegetative meristem (Fig. 27) is also dome-shaped and possesses an outer tunica layer of cells which are larger than the inner cells. The second layer is more typical of a tunica layer than that of the vegetative meristem because there is no evidence of periclinal divisions in this layer. The cells of this type of meristem appear to be slightly larger and slightly denser in staining which indicates a greater activity. The differentiation of the axillary meristems seems to be the same as previously described (Fig. 28). However, the dominant axillary meristem becomes the axillary flower. The opposite meristem does not remain suppressed as it does in the vegetative state, but develops into a branch. On the other hand the axillary floral meristem does not differentiate at the node where it first appears but remains attached to the stem, thus losing its axillary position (Fig. 27). As elongation of the cells of the stem occurs, the flower is shifted farther from the axil of its origin until it is located in its final interaxillary or even internodal position.

The floral meristem (Fig. 29) appears to possess one tunica layer and the rest of the meristematic tissue is unorganized. All cells of the meristem are nearly equal in size and, except for the outer layer, appear to undergo cell divisions in different planes.

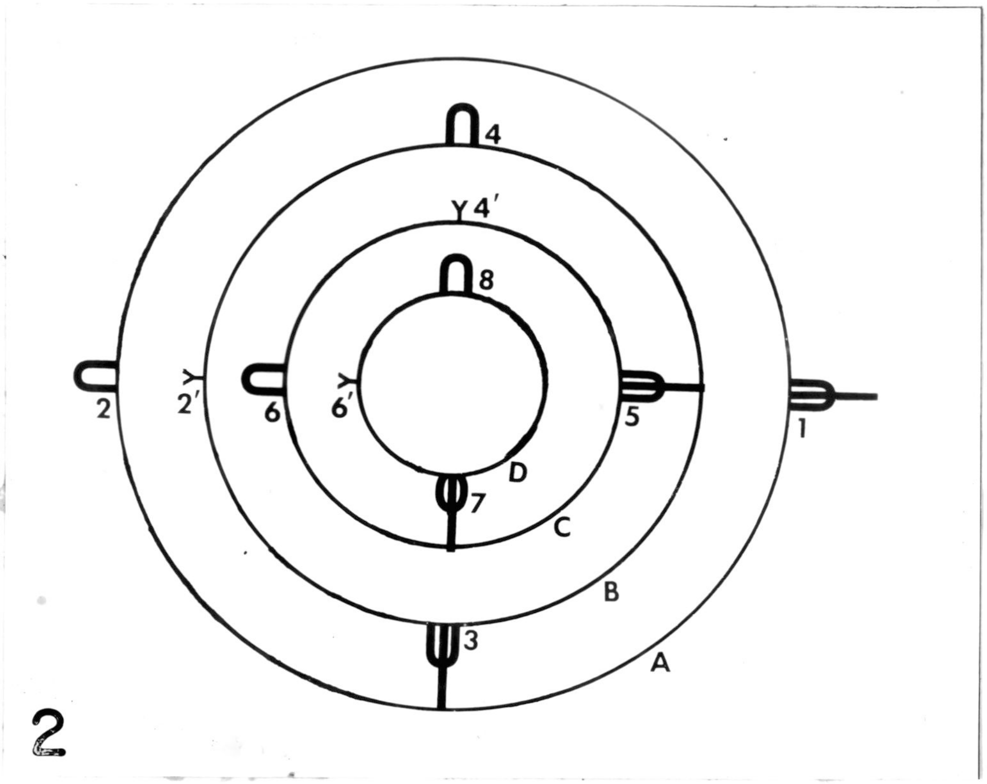
Figure 1A. Eight-week old plant with development of about nine nodes. Arrows indicate flowers in inter-axillary positions. Two-thirds normal size.

Figure 1B. Eight-week old plant with development of about ten nodes. Arrow indicates flower in internodal position. One-half normal size.

1A

1B

Figure 2. Diagramatic representation of the stem of <u>Cuphea viscosissima</u> Jacq., top view. A, B, C, and D represent four nodes. 1, 3, 5, and 7 indicate axillary branches at right angles on one side of the stem. 2, 4, 6, and 8 indicate leaves opposite to 1, 3, 5, and 7 which produce axillary flowers 2', 4' and 6' which are in an interaxillary position.



2

U= LEAF

Ψ= LEAF WITH AXILLARY BRANCH

Y=FLOWER

Figure 3. Three month old plant showing that one-half of the plant possesses axillary branches. One-fifth normal size.

Figure 4. Four month old plant which shows fan-like growth habit. One-sixth normal size.

3

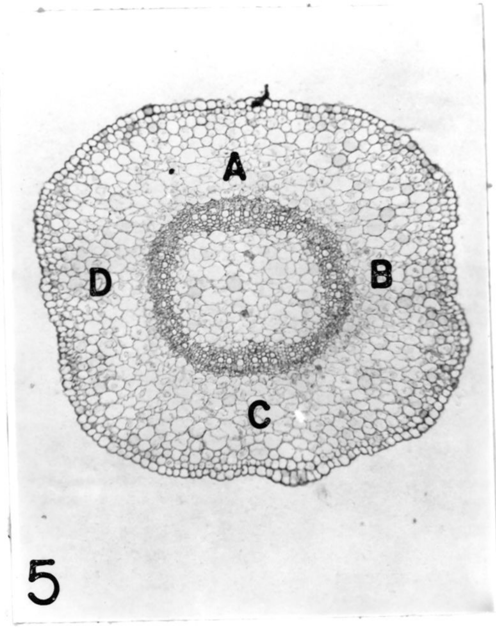
4

Figures 5 through 14. Cross sections through two successive vegetative nodes and internodes of a plant with development of five nodes. Magnification: X65.

Figure 5. Cross section of the second internode showing the four quadrants A, B, C, and D of the stele.

Figure 6. Cross section at the base of the third node showing the leaf traces A and C.

Figure 7 and 8. Serial cross sections of the third node. A and C show the one trace unilacunar leaf trace system. The formation of the vascular tissue of the axillary buds is also apparent.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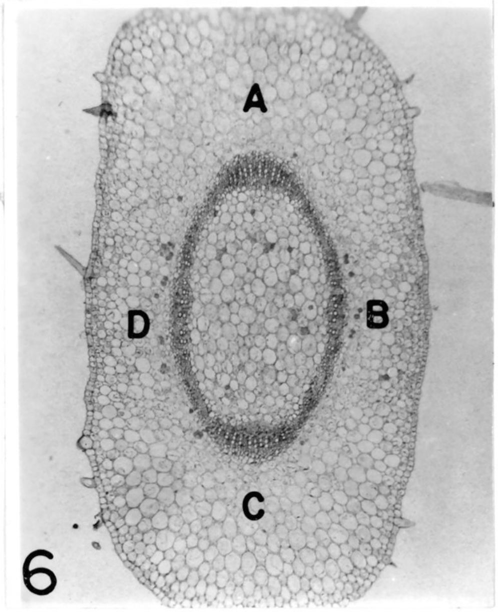
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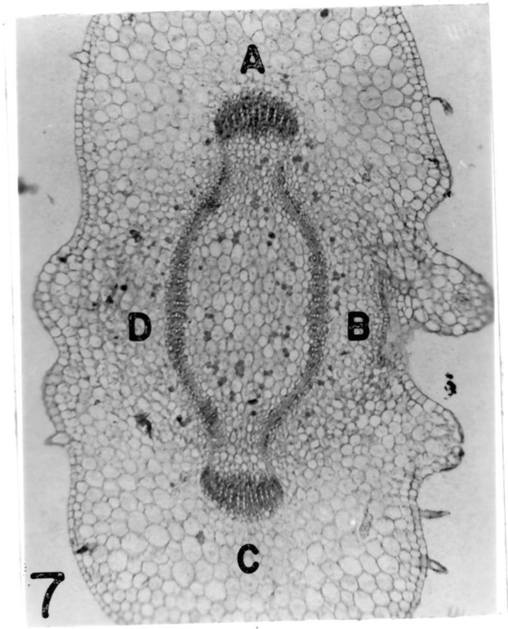
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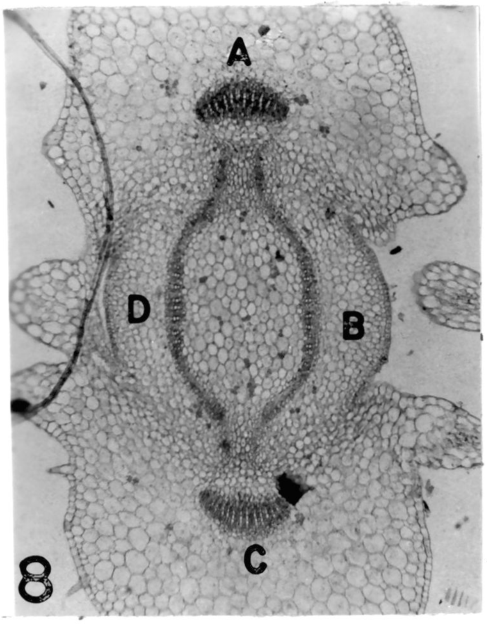
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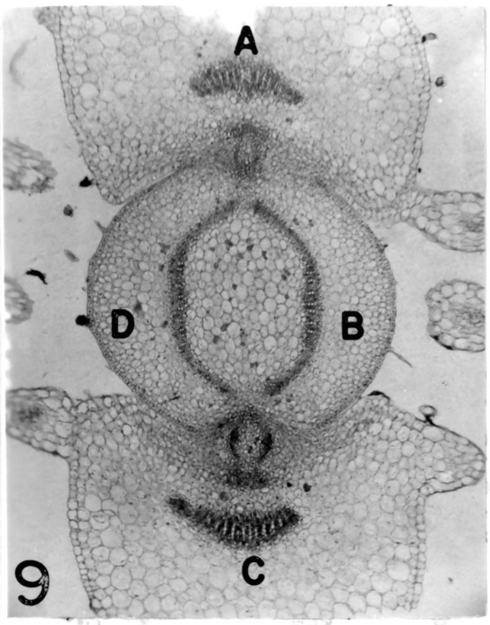
Figure 9. Cross section of the upper part of the third node. A and C show the development of the axillary meristem. The axillary bud at position C is dominant.

Figures 10 and 11. Serial cross sections of the third internode. B and D show development of the leaf traces of the fourth node.

Figure 12. Cross section of the base of the fourth node. Bases of the petioles are responsible for the enlargements at B and D.



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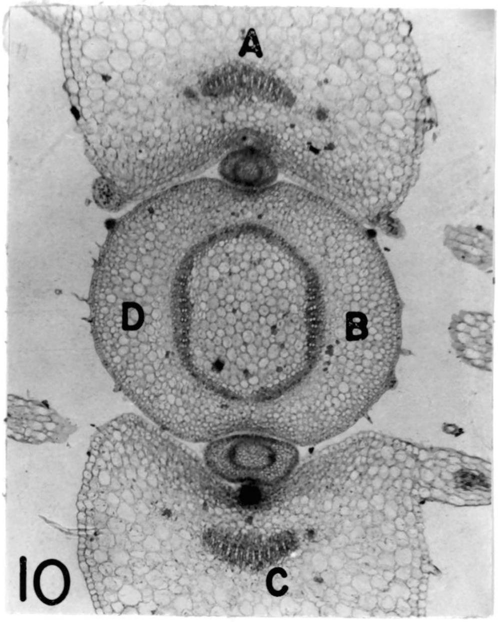

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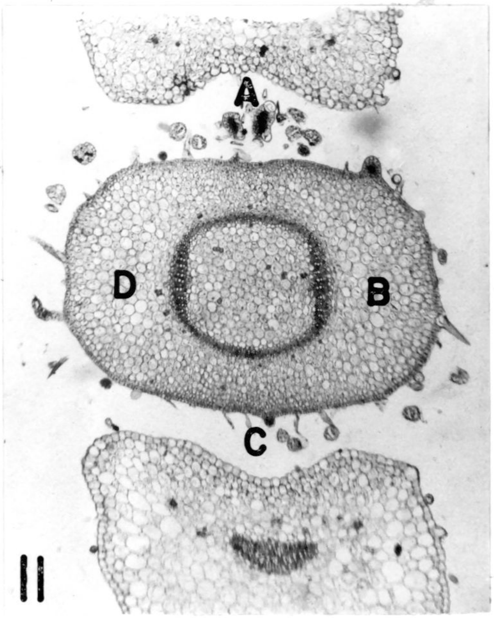

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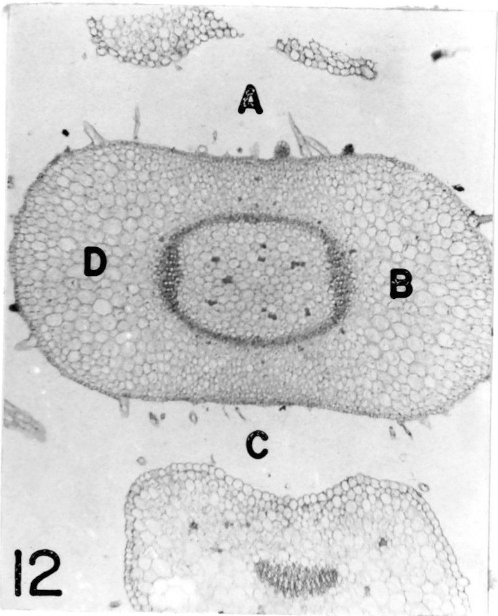

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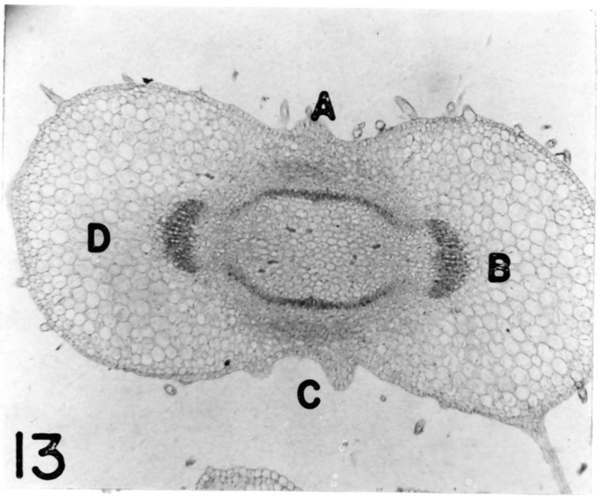
Figure 13 and 14. Serial cross sections through the fourth node. The leaf trace system and vascular tissue are to be seen in quadrants B and D.



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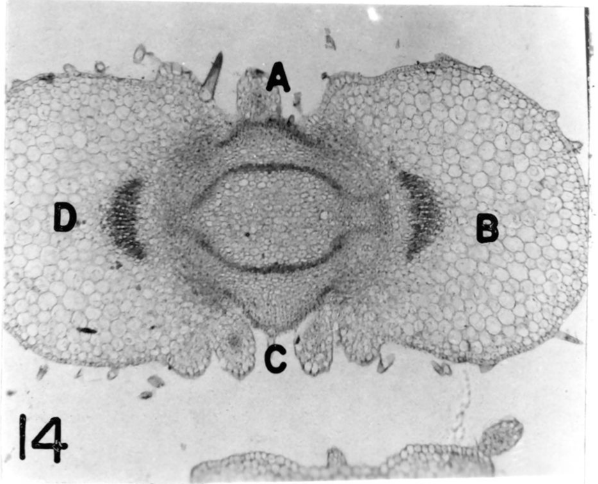

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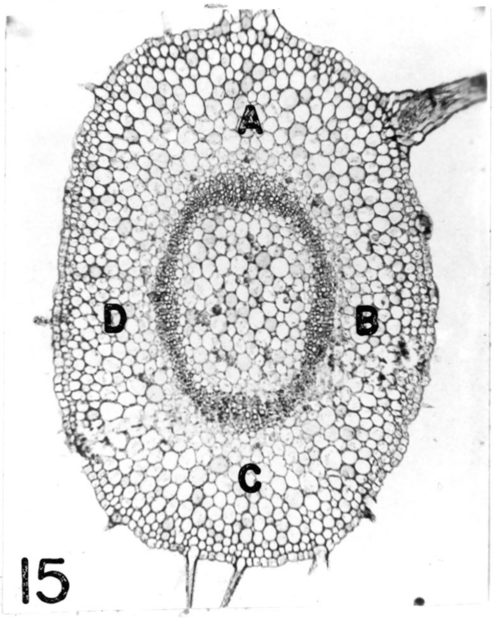
Figures 15 through 26. Cross sections through two successive floral nodes of a plant with development of about six nodes. Magnification: X55.

Figure 15. Cross section of the third internode of a plant in flower. A, B, C, and D show the quadrants of vascular tissue.

Figure 16. Cross section of the base of the fourth node of a plant in flower. A and C indicate diverging leaf traces.

Figure 17 and 18. Serial cross sections through the fourth node. C indicates developing vascular tissue of the axillary bud. A indicates arrested development of floral trace.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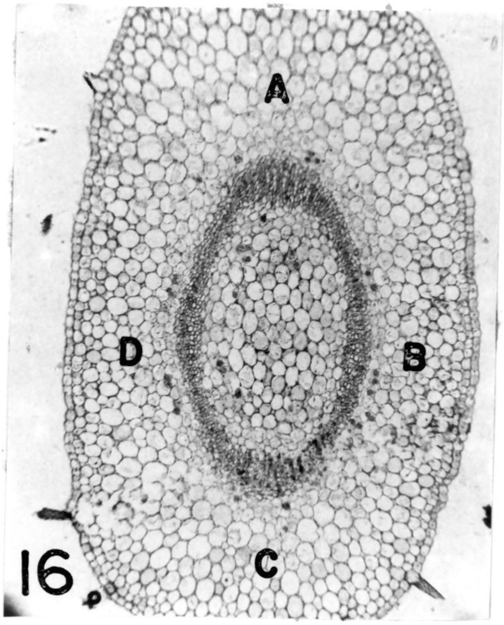
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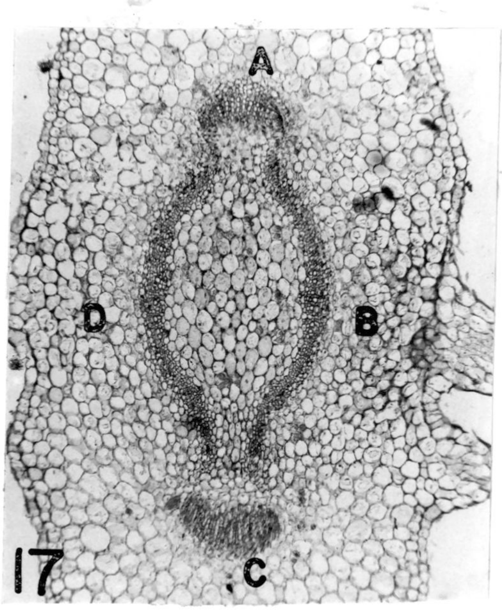
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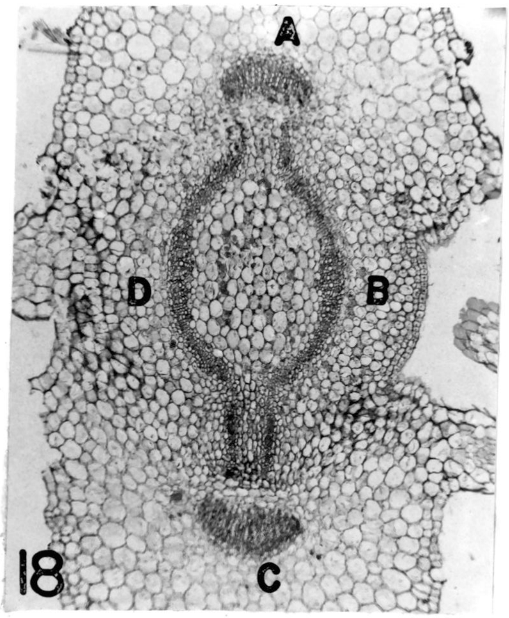
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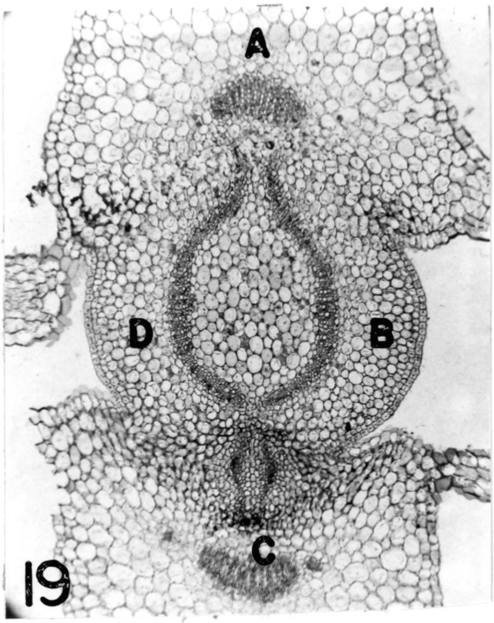
Figure 19. Cross section through the upper part of the fourth node of a plant in flower. C indicates vascular tissue of axillary bud. A indicates closing of leaf gap.

Figure 20 and 21. Serial cross sections through the fourth internode. A indicates arrested floral trace.

Figure 22. Cross section through the base of the fifth node of the plant. A indicates beginning emergence of peduncle trace. B and D indicate enlargments of base of the petioles.



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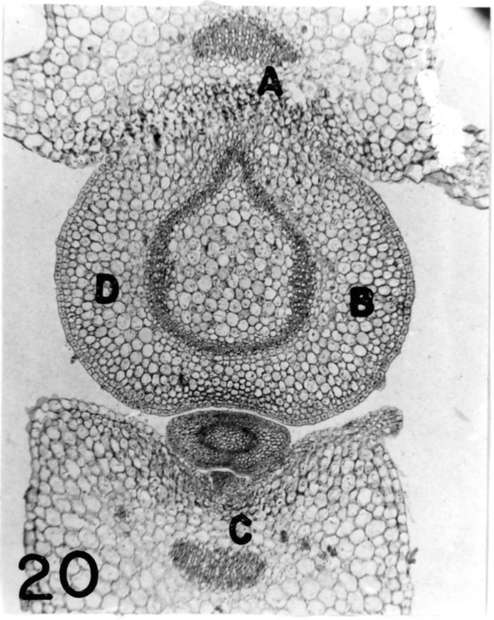

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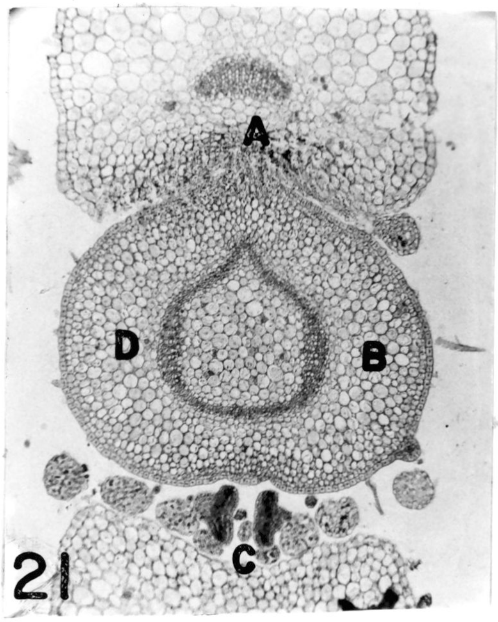

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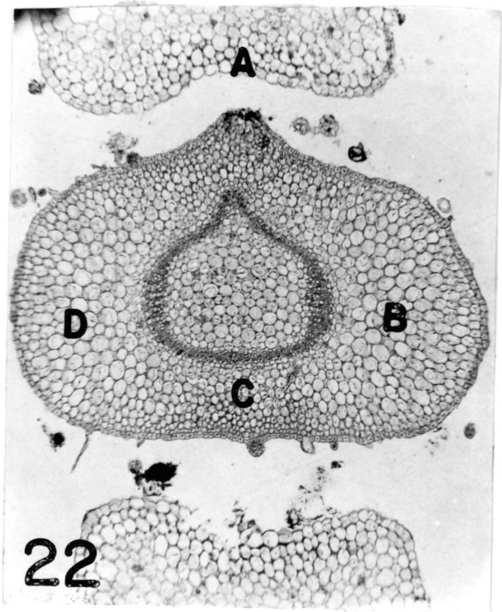

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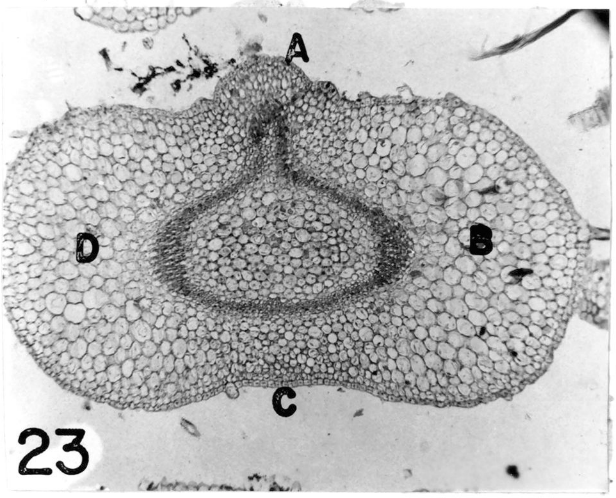
Figure 23 and 24. Serial cross sections of the base of the fourth node. A indicates the emergence of the vascular tissue of the peduncle.

Figure 25 and 26. Serial cross sections through the fifth node of the plant. B and D indicate repetition of the leaf trace system observed in figures 17 and 18.



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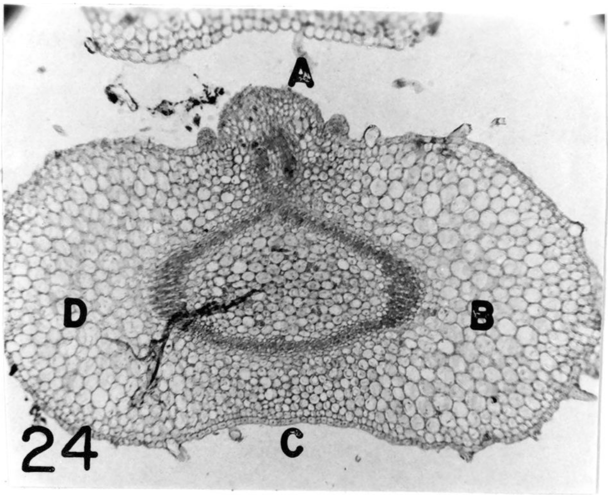

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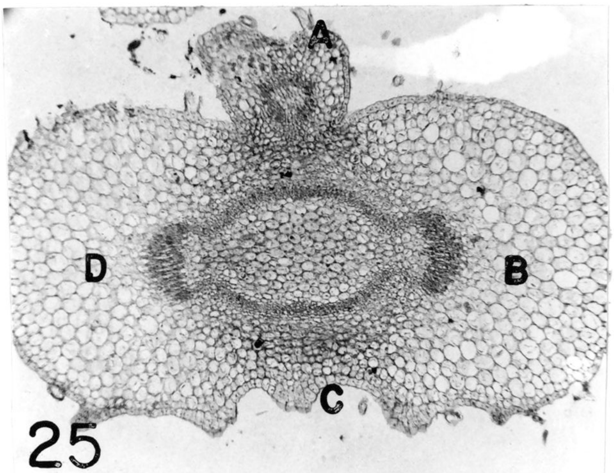

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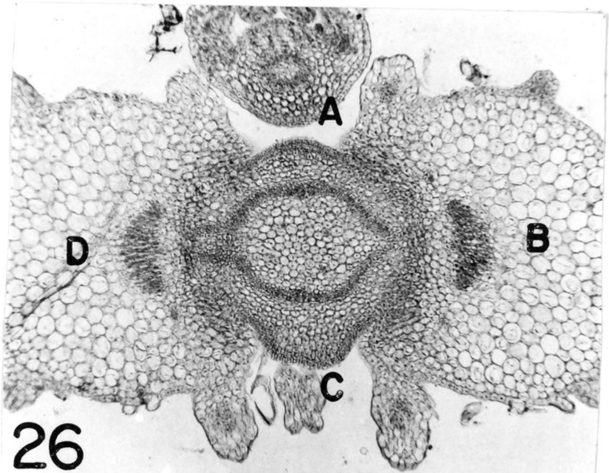

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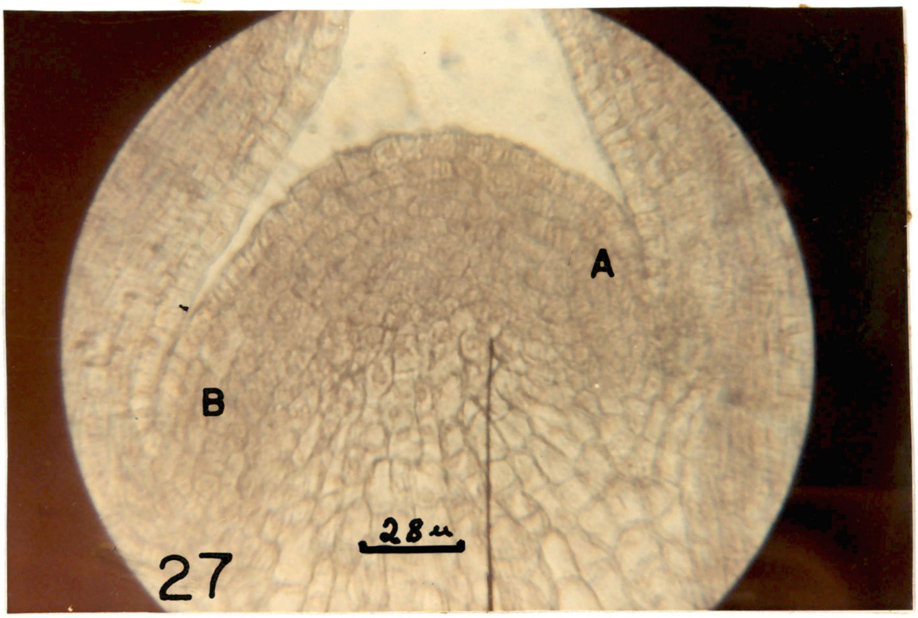
Figure 27. Longitudinal section of a floral-vegetative meristem showing unequal development of the axillary meristems. A indicates early differentiation of the floral meristem. B indicates early differentiation of the vegetative meristem. Magnification: X970.

Figure 28. Longitudinal section of a floral-vegetative meristem showing later development of the axillary meristems. A indicates the axillary floral meristem. B indicates the axillary vegetative meristem. C indicates developing flower and shows that it is displaced from the axil of its origin (D). E indicates leaf axil in which a vegetative bud was produced. Magnification: X100.



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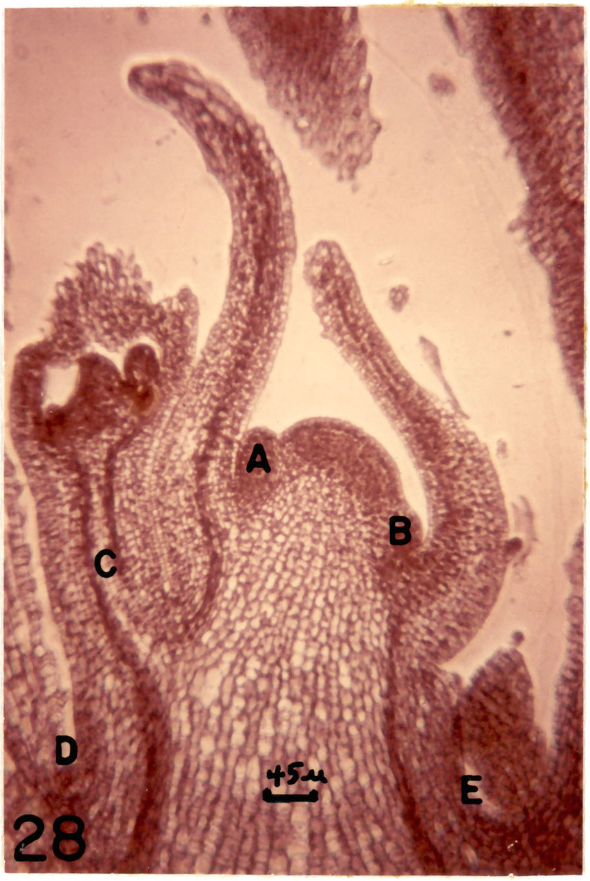

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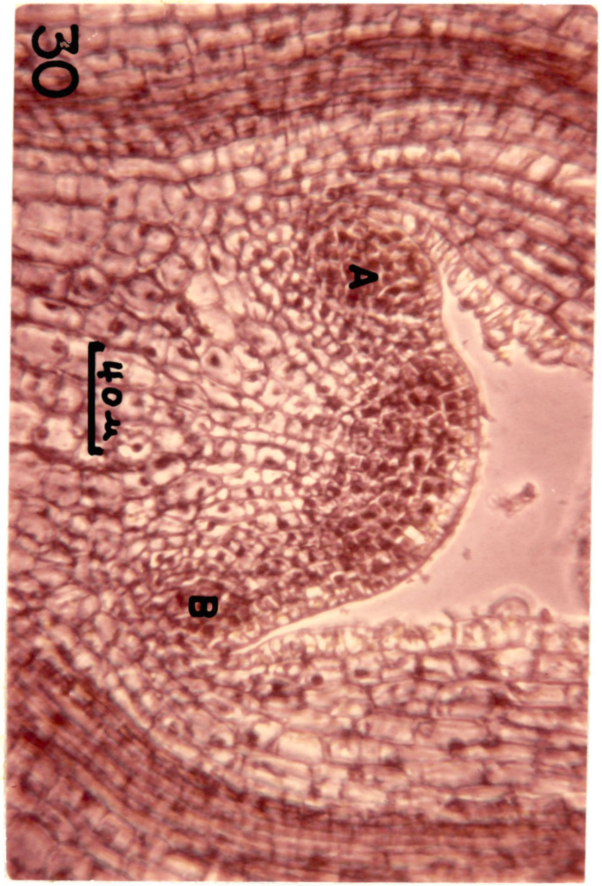
Figure 29. Longitudinal section of a floral-vegetative meristem showing a floral primordium (A). Magnification: X430.

Figure 30. Longitudinal section of the vegetative meristem. The pattern is similar to that of the floral-vegetative meristem. A indicates dominant axillary bud. B indicates suppressed axillary bud primordium. Magnification: X430.


29
40 µ
A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40 µ
A
B

DISCUSSION

These observations are in line with Boubier's (1896) conclusions that in alternate flowered Cuphea the floral trace is initiated in an axillary position at a node but does not emerge until the next higher node. As a result, its position of emergence is interaxillary and sometimes even internodal. The floral trace is obvious in the internode between the node of origin and the node of emergence. The actual gap in the vascular cylinder caused by the floral trace remains throughout the internode. However, the vascular tissue forms an unbroken cylinder due to the fusion of the peduncle with it. The peduncle is not in the same circumference as the vascular cylinder but is displaced slightly to the exterior forming what appears to be a ridge on the vascular cylinder.

The origin and emergence of the flower in the meristem appears to be the same. The floral primordium is produced in an axillary position in place of a vegetative branch. The displacement of the floral primordia from the axillary position to the interaxillary position occurs soon after the formation of the floral primordia. Anatomical evidence indicates that the axillary position of the floral primordia is lost before major differention within the floral meristem occurs. This condition appears to be influenced by cell elongation of the stem tissue between the leaf and the flower since the vascular tissue of the peduncle and the stem are in close association. It seems that, if the tissue of the peduncle were produced by the floral meristem the vascular tissue of the peduncle and that of the stem would be separate although within the stem. Since this is not the case it is concluded that unequal elongation of cellular differentiation of the tissues between the leaf and the floral primordium occurs. Evidence for this is that in all longitudinal sections of the floral-vegetative apical meristems, the tip appears to be curved away from the side of the plant upon which flowers are produced. The curving of the tip is thought to be due to unequal elongation because in the strictly vegetative meristem the tip appears to be straight.

In conclusion, the interaxillary position of the flower of *Cuphea viscosissima* is probably due to unequal cell division and elongation in the apical meristem. One hypothesis to explain this characteristic is that during differentiation of the floral meristem a growth substance may be produced which increases cell division or elongation of the cells adjacent to and beneath the floral meristem.

Most flowering plants have a period of vegetative development before floral initiation. However, *Cuphea viscosissima*, under favorable conditions, shows vigorous vegetative growth on one side and floral development on the other side of the stem. Because of this phenomenon it seems feasible to consider this plant in relation to possible use in research pertaining to hormonal effects.

A characteristic of the species of *Cuphea* which may make it a convenient experimental plant is its prolific production of seeds and the ease with which they can be germinated. The seeds are easily collected after ripening on the parent plant and they will germinate in about three days on moist filter paper in light whereas they require two to three months in the dark. After germination, seedlings in a greenhouse produce flowers in five to seven weeks.

Another characteristic of this plant which favors its use for physiological study is its lack of sensitivity to photoperiods. The plant appears to be day-neutral and would require no special light conditions for flowering. However, like many other plants, it grows faster under long summer days than under short winter days. Where growth chambers are available *Cuphea viscosissima* would be quite acceptable for a study of flowering in culture because of its height (about four inches) when the first flower appears. Through manipulation of the culture medium the effect of various nutrient media on flowering could be studied. Also, if the plant could be grown from callus, the nutritional and hormonal effects on flowering could be determined. The apical meristem with its unusual localization of floral and vegetative areas suggests another possibility for tissue culture study.

SUMMARY

The location of origin, emergence and maturation of the flowers of *Cuphea viscosissima* Jacq., were studied. The origin and emergence of the flower is axillary in the meristematic stage but, during maturation its position becomes interaxillary. The flower arises in the axil of one leaf per node. The vascular tissue of the peduncle remains fused with the vascular cylinder of the internode above and emerges in the mature state in an interaxillary position at the next higher node. Evidence indicates that the displacement of the flower from its axillary position of origin is due to differential elongation of the cells between the floral primordia and the leaf axil.

It was also determined that the vegetative meristem which produces stem, leaves, and axillary branches is smaller and less active than the floral-vegetative meristem which produces stem, leaves, one axillary branch and one axillary flower. The dominant axillary structure whether flower or branch seems to differentiate nearer the apical tip than the suppressed axillary branch or the branch opposite a flower.

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