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ORIGIN AND DEVELOPMENT OF THE NON-ARTICULATED LATICIFERS OF *JATROPHA DIOICA*

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DAVID DALMAN CASS

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ORIGIN AND DEVELOPMENT OF THE NON-ARTICULATED

LATICIFERS OF *JATROPHA DIOICA*

APPROVED BY

DISSERTATION COMMITTEE

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ORIGIN AND DEVELOPMENT OF THE NON-ARTICULATED LATICIFERS OF *JATROPHA DIOICA*

CHAPTER I

INTRODUCTION

Current information regarding the origin and development of non-articulated laticifers is limited. Blaser (1945) described the distribution of laticifers in embryos and mature plants of *Cryptostegia grandiflora*. Mahlberg (1961) found 28 laticifer initials in early embryos of *Nerium oleander*. Other reports (Esau, 1965) indicate that the number and location of laticifer initials is quite variable, even within the same genus. Karyokinesis in non-articulated laticifers is incompletely documented at present. Mahlberg (1959a) described karyokinesis in laticifers of *Nerium*. Mahlberg and Sabharwal (1966) reported progressions of mitotic activity in laticifers of *Euphorbia marginata*.

The development of laticifers has been reported in one other species of *Jatropha*. Popham (1947) observed laticifers in the embryo, stem, and root of *Jatropha cordata*. He indicated that laticifers in embryos of this species differentiate successively in cortical parenchyma, phloem, and xylem. His description did not make clear whether crosswalls and branching occur in laticifers of

J. cordata.

An initial histological survey of mature specimens of Jatropha dioica collected in the state of San Luis Potosi, Mexico served as the background for the present investigation. The decision to conduct an embryological study of laticifers in this species stemmed from that survey.

CHAPTER II

MATERIALS AND METHODS

Specimens of Jatropha dioica Sessé used in this study were collected along Texas Farm Highway 170 west of Big Bend National Park and along U.S. Highway 83 between Webb, Texas and Carrizo Springs, Texas. Mature vegetative tissues for clearing or histological examination were fixed in the field in formalin-acetic acid-50% ethyl alcohol (Johansen, 1940). Developing and mature embryos were dissected from seeds before fixation. Mature embryos were fixed in FAE. Developing embryos were fixed in either Craf III or Allen-Bouin II (Sass, 1958). Early embryonic stages were left in their embryo sacs during the entire preparation. Germination stages were obtained by planting scarified seeds in greenhouse flats. Seedlings were then fixed in FAE at 2-day intervals for 14 days. After fixation the tissues were dehydrated in tertiary butyl alcohol-ethyl alcohol (Johansen, 1940) and embedded in paraffin (Paraplast, 56-57°C). Longitudinal and transverse sections were cut at 8 or 10 microns and stained with safranin and fast green (Johansen, 1940). Clearing of aerial stems, rhizomes, and leaves was done with lactic acid by a modification of the technique of Debenham (1939).

CHAPTER III

OBSERVATIONS

The non-articulated, branched laticifers of Jatropha dioica become recognizable as a distinct cell type when the embryo is approximately 0.3 mm long. This represents the period just following cotyledon initiation. Transections near the cotyledonary node at this stage show the laticifer initials as a ring of 5-7 cells with conspicuous nuclei. Two such cells are shown in Fig. 1. Serial tracings have shown that some of these cells are up to 24 microns long. These initials were observed outside of the procambium wherever the latter was visible. Soon after its formation, each laticifer extends bidirectionally along the procambium as the procambium develops in the hypocotyl and cotyledons (Fig. 2). At the cotyledonary node, centripetal growth of each laticifer results in a group of slender laticifer tips surrounding the base of the embryonic apical meristem. No subsequent formation of laticifers or anastomoses between adjacent laticifers have been observed in this species.

Although the basic pattern of 5-7 laticifers is simple, this arrangement is soon complicated in the hypocotyl by three types of laticifer branching. This branching occurs as the laticifers grow downward into the hypocotyl. Repeated branching in the original

ring of cells (Fig. 3) produces a larger ring of up to 70 laticifer branches by the time the embryo is mature. Transections show that this branching occurs randomly in the ring. Figure 4 shows a prominent laticifer peripheral to a developing procambial strand. This laticifer is one of a ring of 20 laticifer branches present at this stage. Inward branching of this ring results in the formation of an inner ring, separated from the outer ring by developing phloem (Fig. 5,6). The inner ring occurs just outside the immature xylem. Inward branching of this type is restricted to the hypocotyl. There is an approximate one to one correspondence between outer and inner ring branches in the hypocotyl of the mature embryo. Inner and outer ring branches may subdivide and extend into the cortex during embryonic development (Fig. 7), but have not been observed to branch into the pith.

The intrusive and symplastic nature of laticifer development is evident during embryonic development. No cell disruption or tissue distortion was observed as the laticifers penetrate new tissues (Fig. 7). The lack of observable cell wall separation in advance of intruding laticifer tips suggests that such separation is due to mechanical rather than chemical means. Symplastic growth of laticifers occurs whenever laticifers are elongation concomitantly with surrounding tissues. This occurs primarily along the longitudinal axis of a developing organ, but also may occur with lateral expansion.

The distribution of laticifers in cotyledons and the embryonic leaf differs from that in the hypocotyl. Figure 8 shows the arrangement of laticifers in a transection through the cotyledonary

node of a developing embryo. Laticifers shown in contact with the abaxial surfaces of the double cotyledonary trace are continuous with outer ring laticifers in the hypocotyl. Laticifers adaxial to the cotyledonary traces are derived from laticifer tips surrounding the apical meristem (Fig. 2,8). Thus, each cotyledonary trace is in close association with abaxial and adaxial laticifer extensions. This association is maintained in the mature cotyledon (Fig. 9). Branching of cotyledonary laticifers parallels that of the cotyledonary vascular bundles (Fig. 12). A similar pattern of development of laticifers in the embryonic leaf is shown in Fig. 10.

An extensive system of laticifers is present in mature embryos of *J. dioica*. Two concentric rings of laticifers, separated by immature phloem (Fig. 11), extend most of the length of the hypocotyl, which attains a length of 2.5 mm. They appear to end blindly near the bases of the 4 adventitious root primordia. Laticifer extensions from the nodal region of the outer ring enter the cotyledons and embryonic leaf. Laticifers in the cotyledons undergo extensive branching. Centripetal extensions of the outer ring form a complex of laticifers surrounding the apical meristem. Branches of these laticifers enter the cotyledons and embryonic leaf adaxial to the procambium. Laticifer tips remaining adjacent to the meristematic tissue at the apex become incorporated in new tissues produced as the epicotyl elongates.

Under greenhouse conditions, the primary root of *J. dioica* emerges from the seed 1½-2 days after the scarified seeds are planted. Emergence of the primary root is followed by a period of

rapid elongation of the hypocotyl and its included laticifers. Mitotic activity in the seedling appears to be restricted to cells in meristematic tissues until the fourth day. Mitotic activity becomes conspicuous in elongating laticifers on the fourth day of seedling development. Isolated mitotic figures and groups of figures appear in various regions of elongating laticifers. Progressions of mitotic activity in single laticifers were observed in some slides. Whether such mitotic progressions are a frequent occurrence in laticifers of this species is not clear at present. Interphase nuclei (Fig. 13) are elongate and contain 2 nucleoli. Mitotic spindles (Fig. 14,15,16) are more elongate than those in other cells of the seedling, but mitosis is otherwise normal. The absence of a cell plate in telophase (Fig. 17) precludes the formation of crosswalls. After telophase, the daughter nuclei (Fig. 18) enlarge and are distributed along the length of the laticifer. Nuclear division was not observed in tips of laticifers or in regions no longer undergoing elongation.

Preliminary observations of cleared tissues of J. dioica suggest that the distribution of laticifers in the mature plant may be similar to that in the embryo. Cleared transections of the aerial stem and rhizome show an inner ring of laticifers just outside the vascular cambium. The outer ring is less regular than in the embryo, but is still discernible. Branching into the cortex is more extensive in the aerial stem than in the rhizome. The close relationship between laticifer and vascular bundle branching is demonstrated in cleared leaves.

ILLUSTRATIONS 1-6

1 Transection near cotyledonary node of 0.3 mm embryo, showing two laticifer initials outside the procambium. X1100

2 Median longisection of older embryo, showing laticifer extending vertically along the procambium. Arrow indicates centripetal extension toward the apical meristem. X250

3 Tangential section of embryo. Arrow indicates origin of a branch in the outer ring of laticifers. X290

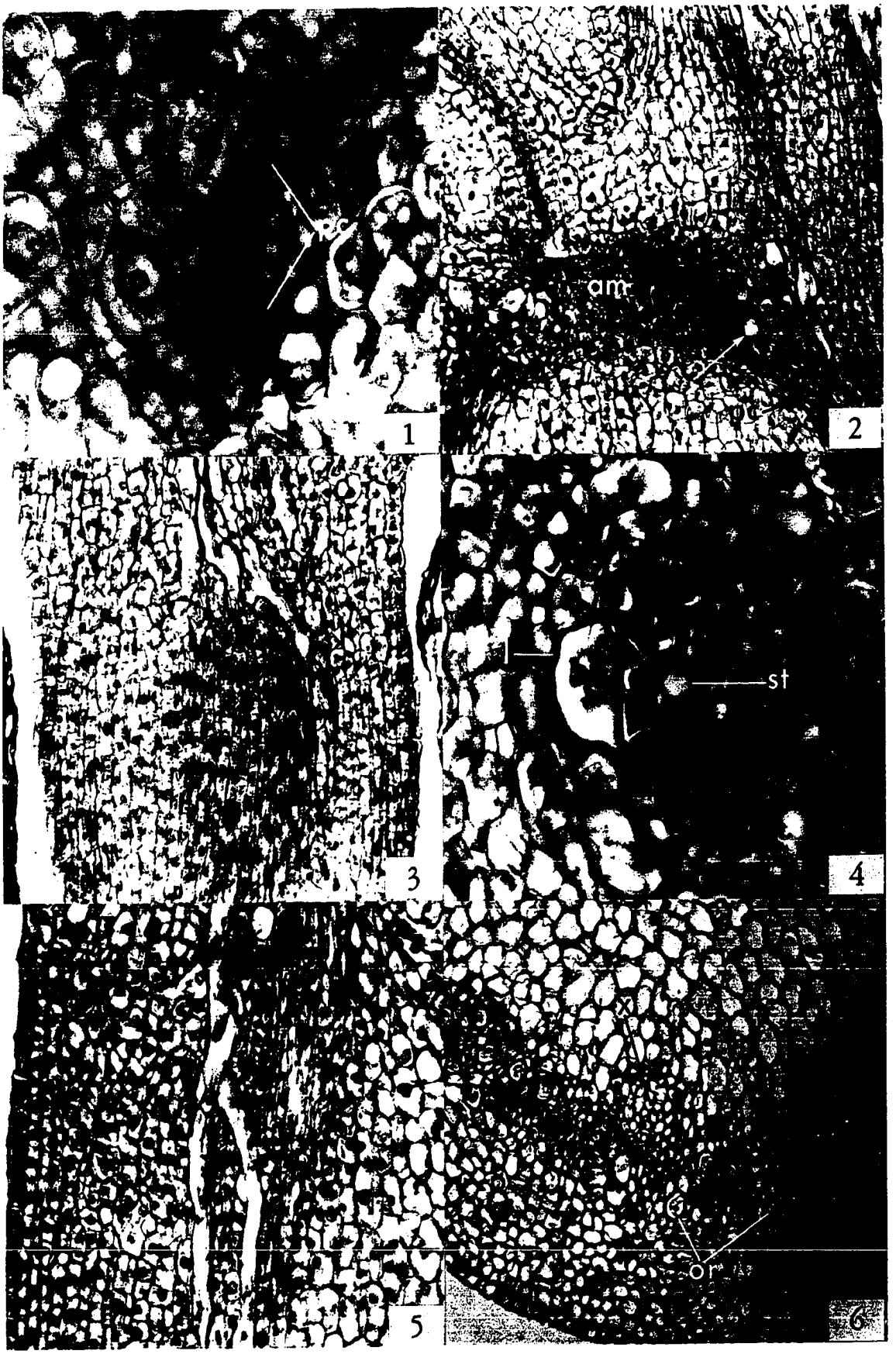
4 Transection below cotyledonary node of embryo, showing relationship of laticifer to developing vascular tissues. One sieve tube is recognizable. X690

5 Near median longisection through hypocotyl. Arrow indicates origin of the inner ring. X330

6 Transection through hypocotyl of developing embryo after formation of the inner ring of laticifers. X340

Key to abbreviations:

am	apical meristem	or	outer ring laticifer
cot	cotyledon	p	phloem
ct	cotyledonary trace	pc	procambium
ir	inner ring laticifer	st	sieve tube
l	laticifer	x	xylem
lp	leaf primordium		


pc
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am
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or
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6

ILLUSTRATIONS 7-12

7 Near median longisection through hypocotyl of developing embryo, showing laticifer branching into cortex. Arrow indicates formation of an additional branch. Note the absence of tissue distortion adjacent to laticifer. X302

8 Transection through cotyledonary node of older embryo, showing relationship of laticifers to cotyledonary traces and apical meristem. Arrow indicates a centripetal laticifer extension. Note the double cotyledonary trace. X268

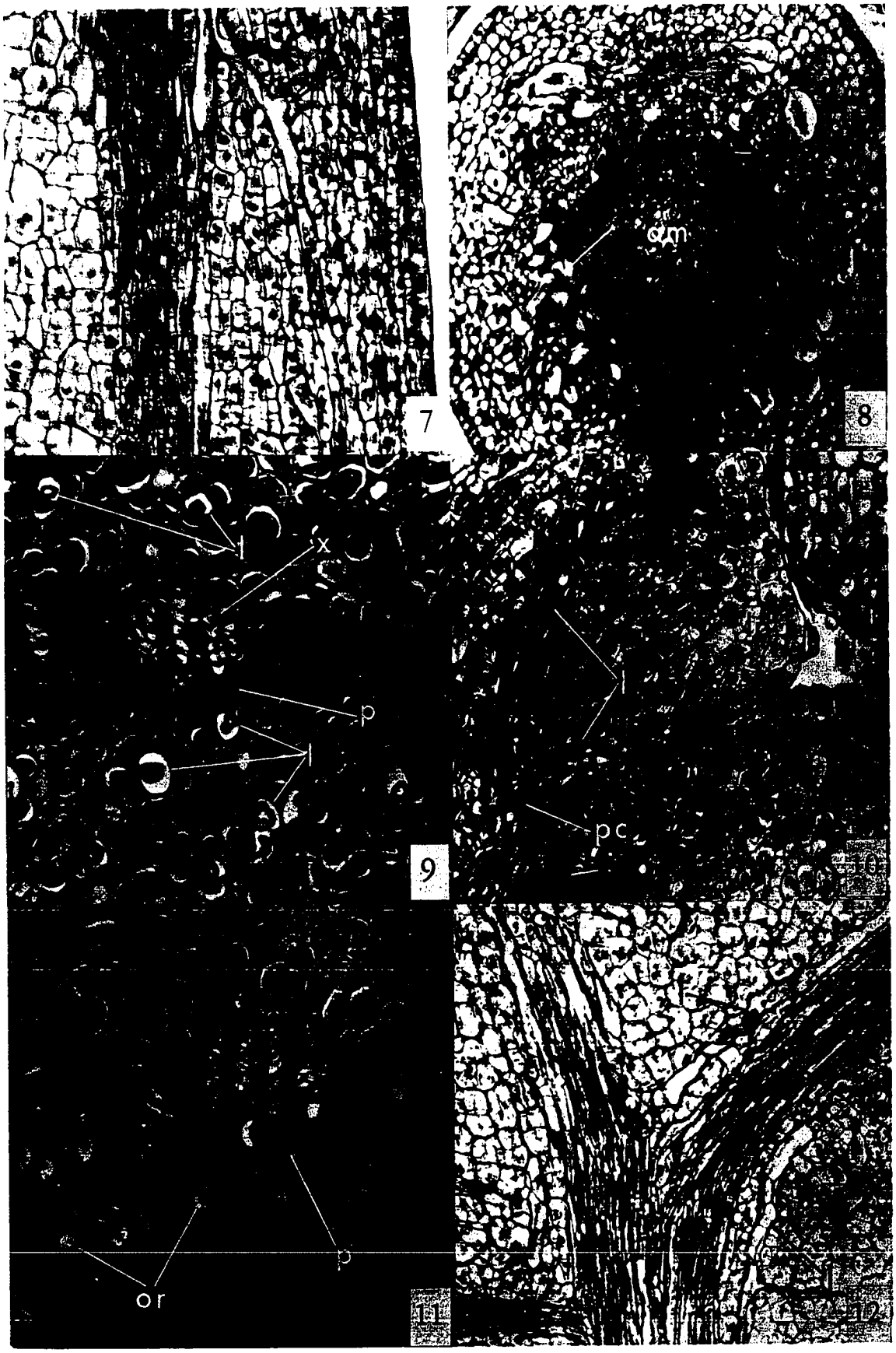
9 Transection of cotyledon of mature embryo, showing laticifers lying abaxial and adaxial to vascular bundle. X340

10 Median longisection through embryonic leaf primordium, showing laticifers abaxial and adaxial to procambium. X327

11 Transection through hypocotyl of mature embryo, showing increased separation of outer and inner ring laticifers. X300

12 Longisection of cotyledon of immature embryo, showing laticifers paralleling vascular tissues. X296

Key to abbreviations on page 8


7
8
x
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pc
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or
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ILLUSTRATIONS 13-18

13 Interphase nucleus in laticifer.

14 Arrows indicate two metaphase figures in branched cortical laticifer.

15 Early anaphase in laticifer. Arrow indicates laticifer cell wall.

16 Late anaphase in laticifer.

17 Telophase in cortical laticifer. Arrow indicates newly-formed nuclear membrane. No cell plate is visible.

18 Post-division nuclei in laticifer.

These illustrations were photographed from slides of a 4-day old seedling. All X1360


13
14
15
17

CHAPTER IV

DISCUSSION

Laticifers of Jatropha dioica arise from a small number of cells early in embryonic development. These cells are interpreted as specialized parenchyma cells derived from the inner layer of the embryonic cortex. Their origin is obscured in later stages as they penetrate other tissues. The approximate stage in which laticifers are formed in this species corresponds fairly well to that described in embryos of Nerium oleander (Mahlberg, 1961), although the site of initiation appears to differ. Mahlberg showed laticifer initials within the ring of procambial tissue in a transection through the cotyledonary node of an embryo 200 microns long. He suggested that the initials may have been derived from the procambium. The size, shape, and position of laticifer initials of J. dioica make such an interpretation difficult in this species. Blaser (1945) described concentric rows of latex tubes in the cortex of embryos of Cryptostegia grandiflora. He suggested that the innermost row of tubes, bordering on the immature phloem, represented the basic ring of latex tubes and that all others were branches. Although embryos of Cryptostegia lack a ring of laticifers inside the phloem, the interpretation of Blaser seems more nearly in agreement with my findings in J. dioica.

The close relationship between the penetration of laticifers into new tissues and the pattern of vascular tissue development has been described in embryos and mature plants of several species. Blaser (1945) showed latex tubes on both surfaces of veins of leaves of Cryptostegia and indicated that vein branching was paralleled by laticifer branching. Mahlberg (1959b) showed elongating laticifers of Euphorbia marginata embryos along the periphery of the provascular cylinder. Mahlberg (1961) observed developing laticifers of Nerium embryos in association with the abaxial surface of the single procambial trace which extended into each cotyledon. In all of these species, subdivisions of laticifers along procambial strands were reported to extend into the cotyledonary mesophyll and cortex of the embryonic hypocotyl. My findings are in agreement with those mentioned above.

The absence of observable anastomoses and the occurrence of free-nuclear division in laticifers of J. dioica support the classical concept of the origin of the multinucleate protoplast of non-articulated laticifers. Mahlberg (1959a) described free-nuclear division in the non-articulated laticifers of Nerium and indicated that mitotic figures were more common in regions of elongation than in laticifer tips. In a more recent article, Mahlberg and Sabharwal (1966) reported progressions of mitotic activity in non-articulated laticifers of developing embryos of E. marginata. The occasional mitotic progressions observed in laticifers of J. dioica may correspond to those described in E. marginata. Their nature and possible physiological significance require additional clarification.

The close developmental and distributional relationship between laticifers of *J. dioica* and phloem suggests a possible functional relationship. Initial physiological experiments on living seedlings of this species are now being conducted to examine this possibility.

CHAPTER V

SUMMARY

Laticifers of *J. dioica* arise early in embryonic development as a ring of 5-7 cells with conspicuous nuclei. These cells lie peripheral to the procambium near the cotyledonary node. Early laticifer development includes vertical extension along the procambium and centripetal growth toward the base of the apical meristem. Development of laticifers in this species may be described as a combination of intrusive and symplastic growth. The mature hypocotyl contains two concentric rings of laticifers derived from branches of the original cells. The inner ring lies just outside the immature xylem and is separated from the outer ring by developing phloem. The inner ring is produced by inward branching of the outer ring as the latter grows downward into the hypocotyl. Cortical ramifications are produced by both outer and inner rings, but no branches have been observed in the pith. This basic pattern appears to be repeated in all portions of the stem. Laticifers surrounding cotyledonary and leaf vascular bundles are derived from branches lying adaxial and abaxial to the procambium as it develops in these organs. Laticifer branching in cotyledons and leaves closely follows that of veins. No fusions between adjacent laticifers were observed.

The multinucleate condition of laticifers of this species results from free-nuclear division, occurring primarily in regions of laticifer elongation. Progressions of nuclear division were observed in a few laticifers.

Laticifers of J. dioica are interpreted as specialized cortical parenchyma cells developing in the embryo. The close developmental and distributional relationship between laticifers and phloem suggests a possible functional relationship. This possibility is now being examined.

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