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ROLE OF PARENCHYMA IN LINUM USITATISSIMUM LEAF TRACE PATTERNS

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ABSTRACT

A new notation for leaf trace patterns was developed which is consistent with contemporary contact parastichy phyllotaxis notation. New computer-aided methods for generating accurate stem tissue maps were developed. Application of these methods resulted in clarification of the role that parenchyma differentiation plays in delimiting the procambial template for Linum usitatissimum L. stem vasculature through ontogeny. Study of the tissue maps for the various leaf trace patterns exhibited by Linum stems through ontogeny generated a set of observations which permits more rigorous definition of the developmental rules for vascular pattern formation. Long-known geometric principles of phyllotaxis were found applicable to leaf trace patterns.

ESAU (1965) noted that the key to the relationship between phyllotaxis and the vascular system of the stem is the interconnections between leaf traces. Nearly every report that has addressed leaf trace pattern (LTP) includes a statement that the vascular patterns are related to the phyllotaxis of various stems. Curiously, however, with the notable exceptions of Sterling (1945) and Namboodiri and Beck (1968), no attempts have been made to rigorously define this relationship. One of the major difficulties in defining such a relationship is the disparity between the methods of notation employed by students of leaf arrangement and students of leaf trace patterns.

Classification of LTP's has usually been by a modified divergence fraction notation from phyllotaxis. Divergence fraction notation was developed by Braun and Schimper (1835) to describe the arrangement of leaves about the mature stem axis. A given pattern of leaf arrangement was specified by determining the ratio of the number of turns around the stem axis between two successive foliar members of the most apparent orthostichy to the number of leaves between these members. An orthostichy is defined as an imaginary line drawn through leaves that are vertically above one another on the stem axis. The modified divergence fraction used to designate LTP is usually formed by citing the plastochron or age difference between leaves that are interconnected by central leaf traces as the denominator of the divergence fraction, and the number of gyres the generative spiral makes about the stem between these leaves as the numerator. The generative spiral is an imaginary spiral or helix that passes through all leaves in ontogenetic sequence. Larson (1982) notes that with this notation the denominator is either equal to or a multiple of the number of main bundles that traverse the stem and also the number of orthostichies in procambial systems.

The regular organization of leaves about the plant stem has been recognized since the time of Theophrastus (Adler, 1974). Mathematical characterization of various patterns of leaf arrangement was initiated by Braun and Schimper (1835) who introduced the above mentioned divergence fraction notation, which was based on the assumption that the generative spiral could adequately be modeled as a Spiral of Archimedes. Church (1901) cogently argued that the generative spiral is better defined as an equiangular or logarithmic spiral when one considers the arrangement of leaf primordia at the level of the shoot apex where phyllotactic pattern originates, since this spiral approximates active growth processes of the shoot apex more closely than does a Spiral of Archimedes. Furthermore, in spiral systems of phyllotaxis, parastichies, but not orthostichies, can be recognized at the level of the shoot apex. Parastichies are imaginary spirals that pass through series of leaves that differ by a constant number of plastochrons (e.g., passing through the 1st, 4th, 7th, 10th, . . . leaves in sequence in the case of the 3-parastichy). Church (1901, 1904, 1968) introduced parastichy notation for phyllostactic description, which has been used in various modified forms by subsequent work-

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ers. Most contemporary students of phyllotaxis employ some form of intersecting parastichy notation to identify two sets of leaves that occur in specific relationships to one another at the level of the shoot apex when specifying patterns of leaf arrangement. A synthesis of the geometry of systems of intersecting parastichies has been recently provided by Erickson (1983).

A new notation for LTP designation is proposed, herein, which is consistent with contemporary parastichy phyllotaxis notation and adequately reflects the interconnections among leaf traces within stems. This is the first step toward clarifying the relationship between LTP and phyllotaxis.

Plant stems grow by the repeated processes of cell partitioning and cell expansion within the terminal shoot apical meristems. Overlaid on these growth processes are differentiation processes that ultimately produce recognizable patterns of tissues in the stem. As noted by Esau (1965) the interpretation of meristematic tissue that constitutes the precursor of the vascular region is not settled, but the best supported view is that a part of this tissue is procambial and the rest is less determined meristematic tissue. She proposed that "residual meristem" be used in reference to the precursor tissue of procambium and other tissue that exhibits little vacuolation at a particular level below the shoot apical meristem. She also noted that the delimitation of the future vascular tissue first becomes apparent through expansion and increased vacuolation of the ground tissue, including pith, cortex and interfascicular rays.

At present there is no histological basis for distinguishing residual meristem cells from procambial cells since these two cell types intergrade with one another in both position and dimension. Distinguishing between densely protoplasmic cells of residual meristem or procambium tissue and the highly vacuolated cells of parenchyma tissue, however, is easily accomplished. Understanding the relationship between leaf trace pattern formation and phyllotaxis becomes more manageable when these two histologically distinct cell types are used for tissue classification and all tissues of the entire stem are examined as a unit. Such an approach has rarely been attempted—most reports on LTP present these patterns in the form of diagrammatic line drawings representing procambium or vasculature tissue only. The inconsistencies among the line drawings of LTP of various workers have been cogently addressed by Beck, Schmid and Rothwell, 1982.

A new method is introduced, herein, which employs computer-aided generation of accurate tissue maps from serial transverse stem sections. This method not only provides a means of depicting procambial and/or residual meristem tissue, but the various parenchyma tissues of the stem as well. The findings made from the application of this method to tissue differentiation within flax (Linum usitatissimum L.) stems throughout ontogeny clarify the role that parenchyma tissues play in LTP formation of this species.

Materials and Methods—Terminology—The terminology used in this report as applied to Linum is that defined by Beck et al. (1982) with the following exceptions. The term axial bundle is not used since, as Allsopp (1964) pointed out, discussions as to which part of the vascular system is cauline and which is of foliar origin are futile, the distinction being manifestly artificial in a continuous system. Symposium is thus taken to mean a group of interconnected leaf traces. The term leaf gap is used in reference to parenchyma within the vascular cylinder in acropetal association with a leaf trace that is diverging from the vascular cylinder. The term interfascicular ray is used in reference to parenchyma within the vascular cylinder in tangential association with a leaf trace. Parenchyma comprising leaf gaps and interfascicular rays form a continuous region of tissue at the nodal region of divergence of any particular leaf trace, hence these terms refer to the spatial positions of this tissue relative to a leaf trace. The terms anodic, meaning same direction as, and cathodic, meaning opposite direction to the generative spiral (Girolami, 1953) are used instead of sinistrose and dextrrose in describing spatial relationships between leaf traces. These terms alleviate the necessity of stating the chirality of the generative spiral, which appeared to be right or left with equal frequency in Linum.

Two broad tissue categories were recognized in the initial data collection phase of this research: 1) Residual meristem/procambium (RMPC), defined by cells that had densely stained protoplasm with no noticeable vacuolation. The term "unclaimed RMPC" was later used in reference to portions of the RMPC which had no clear longitudinal association with existing leaf primordia. The term "leaf trace" was later used in reference to portions of the RMPC that had longitudinal association with leaf primordia. 2) Parenchyma (P) was defined by cells that exhibited vacuolation of at least one quarter of their cross sectional areas. The terms "pith," "interfascicular rays," "leaf gaps" and "cortex" were later used in reference to different spatial regions of the P tissue.
Esau (1965) states that the determination of the longitudinal course of procambial differentiation is technically difficult because change of the derivatives of the shoot apical meristem (SAM) into procambial cells is gradual and various observers differ in their interpretations of when procambium is actually present. To circumvent these difficulties the RMPC and P tissue categories were used as focal points for the analysis and the method described below was developed for generation of maps of these tissues from successive transverse sections that were accurately aligned with one another on the basis of morphological stem features that were independent of these tissues.

Plant material—Linum usitatissimum cv. Culbert seed was obtained from Dr. J. Miller, USDA, Dept. of Agronomy, North Dakota Univ. Plants were grown under a 16/8L/D photoperiod and a temperature regime of 20/15 C:L/D in a walk-in growth chamber. Plants were watered with distilled water daily and with full-strength Hoagland’s solution weekly. Random samples of 10 plants each were made from large populations of plants at 24, 48, 72, 96 and 120 hr after seed sowing and when leaves at nodes 1, 2, 3, 4, 5, 10, 15 and 30 were visible at the terminal shoot apex. Nodes were numbered in their order of appearance in ontogeny with number 1 being assigned to the first node above the cotyledonal node. Nodes were counted for each plant before fixation so that the sequence number of the basal-most node was known for each specimen.

All plant material was fixed with Formalin-Acetic Acid-Ethanol and processed via standard paraffin technique for serial transverse sectioning. Entire stems including hypocotyls were transversely sectioned at 8 μm for seedling material, whereas only the terminal 3.0 cm of the shoot apices were transversely sectioned at 10 μm for plants within the last three sample groups. The sectioned material was stained via Foster’s tannic acid iron chloride method (Johansen, 1940). Sectioned material was carefully screened for orthogonal orientation of the longitudinal stem axis to the plane of sectioning and three specimens exhibiting little or no skewness were analyzed at each sample time.

Data collection—As described by Girolami (1953) and confirmed by my preliminary observations, L. usitatissimum leaves have three major procambial bundles which converge to one in the basal portion of the lamina distal to the leaves’ point of attachment to the stem. A nodal plane was defined as that section in which a particular leaf primordium was at least 85% attached with the stem axis and the three procambial bundles within the leaf lamina had converged. Internode lengths between all nodes in a specimen were determined by section counts between successive nodal planes on a stem.

Photomicrographs were made at mid-inter-node levels, defined as the section midway between two successive nodal planes, for all nodes on a given specimen. In early seedling specimens, additional photomicrographs were made at periodic intervals throughout the hypocotyl. Coordinates for data points marked on each micrograph, as described below, were collected via a HIPAD digitizing tablet interfaced with a Microtechnology 130-2D computer. Careful records were kept of objective-optovar combinations for each photomicrograph generated on a Zeiss Photomicroscope III so that the magnification for each image, printed to a standard 8 × 10 format, was known and incorporated into the analyses. BASIC computer programs entitled UNROLLSTELE and BIUNRLSTELE were written to generate the quantitative data used in the mapping procedure for stems with spiral and bijugate leaf arrangement, respectively. The only difference in these two programs is the algorithm used in aligning successive sections to coincidence, as described below. Listings of these programs will be made available upon request.

Section alignment—The centers of the central procambial bundle of five successive leaf primordia which had diverged from the vascular cylinder were marked on specimens with spiral leaf arrangement as shown for primordia 33–37 in Fig. 1. These primordia were specifically selected to avoid alignment bias due to displacement of procambial regions within the vascular cylinder at positions of leaf trace divergences. These points were digitized and the sequence number of these primordia was entered into the computer for each section level.

The radii between these five points and the center of the stem, and the divergence angles between successive points were calculated via the technique developed by Maksymowych and Erickson (1977). While this technique provides good estimates of radii and angles, it does not permit estimation of the coordinates of the center of the stem in digitizer space. The digitizer coordinates for the stem center were determined by solving for the common point of intersection of circles centered about each primordium center point and with radii equal to the distance between primordium center points and the stem center as shown in Fig. 2.

With reference to Fig. 2 the mathematics
involved in this method follows: Given that the coordinates X2, Y2 and X3, Y3 and distances R2, R3 are known, solve for the coordinates XC, YC.

If we let

\[ X = XC - X3; \quad Y = YC - Y3 \]

and

\[ H = X2 - X3; \quad K = Y2 - Y3 \]

then solve the quadratic

\[ 4(H^2 + K^2)X^2 - 4ZH X + Z^2 - 4K^2R^2 = 0 \]

where \( Z = R^2 + H^2 + K^2 - R^2; \) then \( XC = X3 + X.\) Furthermore, \( Y = (Z - 2HX)/2K\) by substitution, and \( YC = Y3 + Y.\)

Since only XC and YC are points common to intersections of the circles centered about all five primordia, the other possible solution coordinates for the above quadratic could be eliminated by selection of the coordinates with the least differences between them. The above mathematics provide the first objective definition of the stem center in geometric terms.

Once the central stem coordinates \((XC, YC)\) were known for a given section, all data of that section were translated such that \((XC, YC)\) corresponded with the digitizer origin \((0, 0).\) All data for a given section were then converted from rectangular coordinates \((X, Y)\) to polar coordinates \((\rho, \phi).\)

Data from successively basal sections of a specimen were rotated to coincidence by a counterclockwise rotation through an angle equal to the mean difference between the angular coordinates of each primordium seen in both sections. On average, this mean angle of rotation was determined on the basis of four primordia that were visible at two successive section levels. Repetition of these steps for each section of a specimen resulted in a precise alignment of successively basal sections to coincidence on the basis of morphological features that were independent of the positions of internal stem tissues.

The alignment algorithm for stems exhibiting bijugate leaf arrangement with divergence angle = 90° between successive leaf pairs (decussate phyllotaxis) was slightly different. Members of each leaf pair were always 180° opposed to one another. In such plants, the center coordinate \((XC, YC)\) was calculated as the mean of half the distance in digitizer space between center coordinates of uninserted leaf primordia pairs from two successive nodes. The data for a section were then translated such that \((XC, YC) = (0, 0),\) followed by polar conversion and subsequent rotation of the data as described above. This alignment algorithm was unbiased for all sections above the cotyledon-
ary node. However, since there were no bundles external to the vascular cylinder in the hypocotyl region of the plants, the central cotyledonary procambial bundle and the procambial bundles for the first node within the vascular cylinder were used as alignment points in this region of young seedling specimens. This procedure introduced alignment bias that masked any tangential displacement of the procambial bundles in the hypocotyl regions of the plants but did not obscure the pattern of confluence between these bundles.

**Tissue analysis**—Five points in association with each RMPC sector visible in a particular section were digitized in the sequence illustrated in sector 39 of Fig. 1. Points 1 and 2 were located at the tangential boundaries of RMPC and P regions. Points 3 and 4 were located on the radial boundaries of RMPC and P regions midway between points 1 and 2. Point 5 was located on the surface of the epidermis and in radial alignment with points 3 and 4.

All points 1 and 2 within a particular section level subdivided the stem cross section into RMPC and P angular sectors. Some of these sectors could initially be readily identified as leaf traces (e.g., 38–42 in Fig. 1) while others could not. These angular data for each section level were split along an arbitrary generator, and arc sectors vs. distance below the shoot apical summit were plotted (Fig. 3) to give a preliminary map of the stem RMPC (lines) and P (spaces) tissues. RMPC sectors which were readily identifiable as leaf traces were interconnected between successive section levels.

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**Fig. 3.** Preliminary residual meristem/procambium (RMPC) and parenchyma (P) map for *Linum* vascular cylinder. Lines represent the RMPC tissue and spaces the P tissue in the vascular cylinder expressed as arc sectors of the unrolled cylinder as a function of distance below the SAM summit. RMPC sectors interconnected vertically were readily identified leaf traces. **Fig. 4.** Final RMPC and P map of *Linum* vascular cylinder. All RMPC arc sectors interconnected vertically share the same ontogenetic sequence number.
These preliminary maps were used in conjunction with the photomicrographs and microscope observations to assign numerical sequence numbers to all unnumbered RMPC arc sectors to generate the finished maps as shown in Fig. 4. In the maps presented in the results section of this paper the RMPC sectors are shaded and the P regions of the maps are white to emphasize the overall interrelationships of these tissues. The numbers that appear in association with each leaf trace of those maps are the nodal sequence numbers of the leaves each trace entered. Numbers in parentheses are predicted sites of leaf primordia which were not yet formed at the time of sampling. These sites were predicted by successive additions of 137° or 90° to existing spiral or decussate leaf trace positions, respectively.

Methods of analytic geometry permitted calculation of the stem tissue dimensions from the groups of five points in association with the RMPC sectors as follows.

1. RMPC Arc = φ1 - φ2
2. P Arc within Vascular Cylinder = φ2 - φ6
3. Pith Radius = ρ3
4. RMPC Radius = ρ4 - ρ3
5. Cortex Radius = ρ5 - ρ4
6. Intra-RMPC Sector Length = [ρ3 + ((ρ4 - ρ3)/2)(φ1 - φ2)]
7. Inter-RMPC Sector Length = \sqrt{ρ6^2 + ρ^2 - 2ρ6ρ2COS(φ2 - φ6)}
8. Pith Sector Area = [ρ3(ρ4 + ρ3)](φ1 - φ2)]/2
9. RMPC Sector Area = 0.5(φ1 - φ2)(ρ4 + ρ3)(ρ4 - ρ3)
10. Cortical Sector Area = 0.5(φ1 - φ2)(ρ5 + ρ4)(ρ5 - ρ4)

As shown in Fig. 5 these methods subdivide the stem tissue into a cylindrical P pith region and two annuli, the innermost representing RMPC and P tissue of the vascular cylinder, the outermost representing P tissue of the cortex and the epidermis. The parenchyma of the vascular cylinder annulus was either ray parenchyma (R) on either side of a leaf trace, or leaf gap parenchyma (G), acropetally above a leaf trace. The relationship between the stem anatomy and the tissue maps can be clearly visualized by comparing the micrograph of Fig. 5 to the 18 (×10⁻¹ μm) level of the Fig. 4 map. Since the section level and the sequence number of each arc sector were known, these data could be further examined either on the basis of individual tissue sectors of known plastochnic age, or integrated at each section level to provide information on all the tissue of one type at a particular section level.

**LTP notation** — The new notation J(m; n)/D was developed to describe leaf trace patterns in flax stems, where: J = jugularity, or number of leaves per node; (m; n) = the plastochnic age differences between leaves that are interconnected by juncture of their traces. It should be noted here that some traces only interconnected with one other trace, while others interconnected to three; in these cases the notation (m) and (m, n, o) were employed. D = the plastochnic age difference between a bifurcated leaf trace and the leaf subtending the bifurcating leaf gap. In cases where bifurcation does not occur, /D is simply omitted.

**RESULTS** — The following tissue maps were found to be common to all three stem samples at each stage in ontogeny. Representative maps from one of the samples taken at each stage is presented to illustrate the pertinent features. Multiple maps made from specimens sampled at the same stage of ontogeny are presented where variation in LTP was observed.

The 24 hr seedlings had one pair of decussate leaf primordia each with a single leaf trace (1, 1'; Fig. 6). The cotyledons, positioned 90° rel-
Fig. 6, 7. Vascular cylinder tissue maps for *Linum* stems sampled through ontogeny. Shaded regions represent RMPC tissue, open regions P tissue. Ontogenetic sequence numbers are shown at mid-internode levels for all leaf traces. Numbers in parentheses identify unclaimed RMPC sectors, for which the expected ontogenetic sequence numbers are predicted by extension of the divergence angle. 6. 24 hr seedling. 7. 48 hr seedling.
ative to these leaf primordia and 180° opposed to one another, have three leaf traces (C, R, L, C', R', L'; Fig. 6). All eight of these leaf traces extended through the acropetal portion of the hypocotyl. The pith had almost completely disappeared at the level at which the lateral trace of one cotyledon (R'; Fig. 6) was confluent with the adjacent lateral of the opposed cotyledon (L; Fig. 6). The individual leaf traces of each leaf primordium (1, 1'; Fig. 6) were confluent with an adjacent cotyledon trace (L, L'; Fig. 6) in the hypocotyl. In terms of vascular trace interconnections between cotyledons and leaf primordia there were thus two separate sym-podia, designated in Fig. 6 by prime and un-prime numbers. These sympodia were, however, interconnected via the confluent lateral cotyledon traces in the hypocotyl. There was no unclaimed RMPC tissue between the 1 primordia at the mid-internode level of the 1 primordia in the 24 hr seedlings; rather, the cells in this region of the stem were all highly vacuolated.

The 48 hr seedlings were essentially like the 24 hr seedlings except that growth of the stem had resulted in an apparent displacement of the primordia away from the shoot apical meristem summit and the leaf traces of the 1 primordia were now bridged by unclaimed RMPC tissue ([2], [2']; Fig. 7). At this stage in development, there were leaf gaps acropetal to the cotyledon traces, and the interfascicular ray parenchyma of the 1 traces had become apparent. These regions of P tissue divided the unclaimed RMPC into four discrete sectors at 160 µm below the SAM summit, which converged to two RMPC sectors 80 µm below the SAM summit.

The 72 hr seedlings exhibited variation with regard to both leaf arrangement and leaf trace patterns. Either the decussate phyllotaxis was perpetuated (Fig. 8) or there was a spiral transformation at node two (Fig. 9). Although all plants eventually underwent spiral transformation of phyllotaxis, some plants were observed to produce as many as twelve decussate nodes prior to this event.

In the 72 hr seedling illustrated in the map of Fig. 8 the decussate pattern was perpetuated by the initiation of two additional leaf pairs which were 180° opposed to one another and alternated 90° between successive nodes. The leaf traces associated with these primordia (2, 2', 3, 3'; Fig. 8) appeared to be derived from the unclaimed residual meristem visible in the 48 hr seedling (Fig. 7). There were still two very symmetrical separate sympodia (primed and unprimed in Fig. 8) at this stage of development with leaf traces 2 and 3 interconnected with leaf trace 1 in each sympodium. Additional unclaimed RMPC sectors ([4], [4'], [5], [5']; Fig. 8) were apparent. These sectors of residual meristem were of different lengths and were continuous with existing leaf traces. Between 240 and 80 µm below the SAM summit these sectors were tangentially isolated via the parenchyma associated with the development of the rays and leaf gaps of existing traces. At 80 µm below the SAM summit these four sectors converged to two unclaimed RMPC sectors acropetal to the 2-primordia leaf gaps. The leaf gap parenchyma associated with the cotyledons was confluent with the interfascicular ray parenchyma associated with the 2-primordia. This stem exhibited a 2(1; 2) LTP according to the proposed notation.

The 72 hr seedling illustrated by the map in Fig. 9 underwent spiral transformation at node 2 and had the following divergence angles between successive primordia: 1–2 = 130°; 1–2 = 45°; 2–3 = 115°; 3–4 = 165°; 4–5 = 140°; 5–6 = 140°. The leaf traces associated with primordia 2, 3, 4 and 6 appeared to be derived from the unclaimed residual meristem area apparent in the 48 hr seedling ([2], [2']; Fig. 7). There were still two separate but asymmetrical sympodia consisting of traces 1', 2, 3, 5 and traces 1, 4, 6 (Fig. 9). The leaf gap parenchyma associated with the cotyledons was confluent with interfascicular ray parenchyma associated with the 2 and 3 primordia. Note the similarity between leaf trace interconnections, irrespective of sequence numbers, in the sympodia of the 72 hr decussate stem (Fig. 8) and the sympodia of this stem. Leaf traces associated with the 1-primordia were confluent with leaf traces in the cistolic and anodic positions in both stems. The obvious differences between the sympodia of these two 72 hr seedlings were the vertical levels of primordia insertion and the divergence angles between primordia. Leaf primordium 5 was also larger than leaf primordium 6 in the seedling undergoing spiral transformation at node 2 (Fig. 9). It therefore appeared that leaf trace 5 was produced before the unclaimed RMPC to the cistolic side of trace 1 was organized into leaf trace 6 in the stem with spiral phyllotaxis portrayed in Fig. 9. The leaf gap acropetal to trace 1' divided trace 5 into two sectors, thereby leading to interconnections between trace 5 and traces 3 and 2, or a (2; 3)/4 LTP in the proposed notation. The two-plastochron interconnection occurred in the cistolic position relative to the older trace. The three-plastochron interconnection occurred in the anodic position relative to the older trace. The more basipetal pattern in this sympodium is a (1; 2) LTP. The one-plastochron interconnection was cistolic, whereas the two-plastochron interconnection
Fig. 8, 9. 72 hr seedling vascular cylinder tissue maps. 8. Stem with decussate phyllotaxis. 9. Stem that underwent spiral transformation of phyllotaxis at node two.
was anodic relative to the older leaf trace. The opposing symposium of this stem exhibits a (3; 5) LTP. The three-plastochnon interconnection was anodic, whereas the five-plastochnon interconnection was cathodic relative to the older leaf trace. There were six discrete unclaimed RMPC sectors between 160 and 80 \( \mu \)m below the SAM summit which were tangentially isolated by P tissue of the leaf gaps and interfascicular rays associated with existing leaf primordia. At 80 \( \mu \)m below the SAM summit these sectors converged to two unclaimed RMPC sectors, represented by (10) + (7) + (9) and (11) + (8) in Fig. 9.

The 96 hr seedlings with spiral transformation at node 2 had 7 or 8 leaf primordia with an average divergence angle of 135° between primordia 5–8. Leaf trace 7 (Fig. 10) was interconnected with leaf trace 4 of one symposium, whereas leaf trace 8 was interconnected to leaf trace 5 of the other symposium. These new leaf trace interconnections occurred on the anodic sides of traces 4 and 5. Prospective traces 9 and 10 were interconnected with traces 4, 6 and 5, 7, and were bifurcated by leaf gaps associated with traces 1 and 2, respectively, thus producing a (3; 5) LTP. The three-plastochnon interconnection occurred in anodic positions, the five-plastochnon interconnection in the cathodic positions relative to the older leaf trace. The division of prospective trace 10 by the leaf gap of trace 2 and its juncture with traces 5 and 7 provided the first effective bridge between the hitherto separate symodia of the stem. There was no confluence between the leaf gap associated with the 2-primordium and the interfascicular ray parenchyma associated with the primordia on the anodic (5) and cathodic (7) side of the 2-primordium (Fig. 10). This effectively “closed” the hitherto “open” two-sympodial system of the flax stem. There were five discrete unclaimed RMPC sectors between 120 and 60 \( \mu \)m below the SAM summit which were delimited by P tissue of leaf gaps and interfascicular rays associated with existing leaf primordia. These strands were of different longitudinal extents and all ultimately converged to two sectors of unclaimed RMPC ([11] + [8] + [10] and [12] + [9]; Fig. 10) at 50 \( \mu \)m below the SAM summit.

Following the above described developments the flax stem vasculature entered a temporary phase of (3; 5)/8 LTP, the characteristics of which can be seen with reference to trace 19 of the map in Fig. 11. The largest vertical extent of this trace was interconnected with trace 14, that was 5 plastochnons older. The shorter vertical extent of this trace was interconnected with trace 16, that was 3 plastochnons older. This trace was subdivided by the leaf gap associated with trace 11, that was 8
plastochrons older. The longer 5-plastochron trace interconnection in this pattern was always in the cathodic position of the older trace, whereas the shorter 3-plastochron trace interconnection was always in the anodic position of the older trace. There were no confluent regions between leaf gap parenchyma and interfascicular ray parenchyma associated with different leaf traces. Therefore, at this stage and throughout the rest of flax ontogeny, there was only one sympodium—the system effectively being a “closed” type of vascular cylinder. There were five discrete sectors of unclaimed RMPC between 150 and 95 μm below the SAM summit ([21]-[25]; Fig. 11) which were partitioned by the parenchyma associated with rays and leaf gaps of existing leaf traces. These sectors were confluent at 80 μm below the SAM summit.

The transition from a (3; 5)/8 LTP to a (5; 8)/13 LTP via a (3; 5)/8/13 LTP can be seen with reference to trace 27 of the map in Fig. 12. This transition was not abrupt but rather occurred via a gradual separation of the 3-plastochron interconnection by the differentiation of parenchyma associated with the leaf gap 8 plastochrons older, cf. traces 27 and 24 in relation to the gap associated with trace 19; while new 8-plastochron interconnections were established between trace 27 and 19 by the differentiation of the leaf gap of trace 14 which was 13 plastochrons older than trace 27, cf. traces 27, 22, 19 in relation to the gap associated with trace 14. The 5-plastochron interconnection was shortened as compared to the (3; 5)/8 LTP via the differentiation of parenchyma associated with gaps 13 plastochrons older (cf. 14; Fig. 12) and interfascicular ray parenchyma of primordia that differ by 8 plastochrons in age, while the new longer 8-plastochron interconnection is established in the anodic position relative to the older leaf trace by this same overlap. There were no confluent regions of leaf gap and interfascicular ray parenchyma associated with leaf traces of different ages during this transition. The perforation, or region of parenchyma not associated with any leaf trace, that is visible in the anodic position relative to trace 19 and cathodic position relative to the leaf gap of 14 of Fig. 12 was unique to this particular stem. Similar perforations were not visible in the other stems which included the (3; 5)/8/13 LTP; rather, the tissue was entirely RMPC in nature.

The transition described above appeared consistently in association with the leaf trace of the 27th- or 28th-plastochron-old leaf primordia in the stems that were analyzed. There were six discrete sectors of unclaimed RMPC between 170 and 80 μm below the SAM summit which were confluent at 60 μm below the SAM summit. These sectors were delimited by P tissue of leaf gaps and interfascicular rays associated with existing leaf traces.

The (5; 8)/13 LTP illustrated in the map of Fig. 13 appeared to be stable up to at least the 80th plastochron, as defined by total nodes, on the flax stem. The interconnections between leaf traces of this pattern can be seen with reference to trace 43 of this figure which was interconnected to a trace 8 plastochrons older (35; Fig. 13), and a trace 5 plastochrons older (38; Fig. 13). Trace 43 was subdivided by the
parenchyma differentiated in association with the leaf gap for a trace 13 plastochrons older (30; Fig. 13). The longer eight-plastochron trace interconnection was always located in the anodic position and the shorter five-plastochron trace interconnection in the cathodic position relative to the older leaf trace in stems exhibiting this LTP. Note the subdivision of unclaimed residual meristem ([48]–[55]; Fig. 13) into discrete sectors between 140 and 80 μm below the shoot apical meristem summit by differentiation of parenchyma associated with the leaf gaps and interfascicular rays of leaf traces for existing leaf primordia. These sectors were confluent at 50 μm below the SAM summit.

Flax stem ontogeny was subdivided a posteriori into five stages on the basis of total number of nodes. These stages correspond to periods in which *Linum* stems exhibited different patterns of leaf trace interconnections. Fig. 14–18 illustrate the longitudinal functions for the mean radii of pith, RMPC, and cortex plus epidermis tissue of flax stems sampled at these five stages of ontogeny. The captions for these figures summarize the limits of these stages and the allometric coefficients, obtained by regression analysis, which were used to generate these longitudinal maps. The maps were generated by successive addition of mean radii for pith, RMPC, and cortex plus epidermis calculated via the regression lines.

Two-way analysis of variance on these data revealed that there were highly significant differences between the allometric constants for the three tissue categories ($F_{2,57} = 52.1, P < 0.01$). In general the RMPC tissue radius expanded at the slowest relative rate, the pith at twice the RMPC rate, and the cortex at three times the RMPC rate (cf. captions for Fig. 14–18). The analysis also revealed a highly significant difference in allometric constants through time ($F_{4,57} = 25.9, P < 0.01$). In general there was a progressive decrease in the allometric constants through ontogeny, meaning that the relative contribution of all tissues to stem radius per unit vertical distance decreased through time. That is, the overall conic form of the stem became steeper through ontogeny. The tissue-time interaction was also revealed to be highly significant ($F_{8,57} = 3.1, P < 0.01$). During the first two stages of ontogeny the allometric constant decreased for all tissue types (cf. Fig. 14–18). This decrease continued for cortex tissue but the allometric constant for RMPC tissue appeared to level off at stage three for the duration of ontogeny, whereas the constant for pith appeared to increase at stage three, then level off at this higher value.

**Discussion and Conclusions**—**General conclusions**—Examination of the details of all these maps reveals that the following characteristics are common to the delimitation of all leaf traces regardless of the LTP: 1) Within the shoot apical meristem there are large angular sectors of RMPC underlying presumptive sites of primordial initiation; a fraction of one of these sectors becomes separated tangentially from the remainder via appearance of vacuolated P cells of the interfascicular rays on either side of the newly formed leaf trace and acropetally from the remainder via appearance of vacuolated P cells comprising the associated leaf gap. 2) Further delimitation of any given leaf trace is associated with simultaneous lon-
Fig. 14–18. Longitudinal profiles of pith, RMPC of vascular cylinder, and cortex tissue of *Linum* stems at increasing stages of ontogeny. K1, K2, K3 = Allometric constants describing relationships between stem tissue radii and stem length for pith, RMPC, and cortex, respectively. 14. Stems with 1–4 visible leaf primordia. K1 = 0.898, K2 = 1.044, K3 = 1.772. 15. Stems with 5–9 visible leaf primordia. K1 = 0.470, K2 = 0.446, K3 = 1.114. 16. Stems with 15–30 visible leaf primordia. K1 = 0.600, K2 = 0.233, K3 = 0.904. 17. Stems with 32–34 visible leaf primordia. K1 = 0.533, K2 = 0.192, K3 = 0.732. 18. Stems with 35–80 visible leaf primordia. K1 = 0.576, K2 = 0.197, K3 = 0.640.

Gitudinal expansion of the population of parenchyma comprising the leaf gap and that comprising the interfascicular rays in opposite directions relative to the nodal region where these two parenchyma populations are spatially continuous. 3) Interrelationships between the interfascicular rays and leaf gaps associated with existing leaf traces result in isolated sectors of unclaimed RMPC within the shoot apex. 4) The pattern of leaf trace interconnections at any stage of ontogeny is delimited by the interrelationships existing between the parenchyma of leaf gaps and interfascicular rays associated with leaf traces of existing leaf primordia.

The anatomical characterization of leaf gap and interfascicular rays are quite well known. Nonetheless, this appears to be the first report in which the role that these tissues play in delimiting leaf trace patterns can be clearly visualized. Previous students of leaf trace patterns have been intent upon presenting the interconnections among residual meristem and procambium within plants while virtually ignoring the role that parenchyma differentiation plays in delimiting those interconnections. This may be related to the difficulties hitherto encountered in the laborious reconstructions of stem tissues.

At present we have no good histological basis for distinguishing residual meristem tissue from procambial tissue, but we can distinguish these
tissues from vacuolated parenchyma tissue. The current analytic procedure takes advantage of that fact and provides quantitatively accurate maps of the relative positions of RMPC and P tissue within the stem. Currently it is not known to what extent the cell population comprising the RMPC contributes to the proliferation of parenchyma tissue comprising pith, cortex, interfascicular rays and leaf gaps; to what extent these tissues proliferate by cell division and expansion of like cells within these regions; nor to what extent redifferentiation of cells of parenchyma tissue contributes to proliferation of RMPC. The relative role each of these tenable possibilities plays in tissue pattern formation awaits a higher resolution study of these tissues at the cellular level.

Jean (1982) discusses vascular trace patterns in terms of hierarchical control of phyllotaxis, by which he means those rules or constants which arise within a collection of elements, but which affect individual elements of the collection (Pattee, 1970). In the broad sense of this definition, the phenomena described in the points 1–4 above support Jean’s interpretation. The vascular trace maps presented by Jean (1982) in support of hierarchical control, however, are not consistent with the maps and rules discovered for Linum LTP. For example, the bifurcation induction lines originally proposed by Bolle (1939) and expounded upon by Jean (1982) appear to have no counterpart in the flax vascular system. Rather, the phenomena described in points 1–4 above appear to explain the observed patterns of leaf trace interconnections.

Changes in LTP through ontogeny—Consideration of the flax LTP’s throughout ontogeny as summarized via these vascular tissue maps reveals: 5) The flax leaf trace vasculature initially consists of two separate sympodia or an open vascular system which becomes a closed single sympodium system with the differentiation of leaf trace 10. The open condition of the initial LTP results from the existence of confluent regions between the leaf gap and interfascicular ray parenchyma associated with different leaf traces. The closed condition arises when this region of confluence is interrupted by the apparent failure of the RMPC to differentiate into parenchyma, thereby resulting in an asymmetric bifurcation of developing leaf traces. 6) In 72 hr seedlings with stems exhibiting decussate or spiral phyllotaxis, the initial 1-traces are contiguous with traces in the anodic and cathodic locations. The longitudinal extent of the parenchyma associated with these traces varies depending on the phyllotaxis. This presumably is due to the difference in the initial timing of the events described in point 1 above. Therefore, the variation in early 72 hr seedling LTP reflects the differences in the number of leaves per node and the angular relationships between these leaves. 7) In accordance with the proposed LTP notation, the flax stem undergoes a progressive change from a (1; 2) → (2; 3)/4 → (3; 5) → (3; 5)/8 → (3; 5; 8)/13 → (5; 8)/13 leaf trace pattern. The last LTP appears to be stable from ca. 28th plastochron up to at least the 80th plastochron of ontogeny. 8) The number of discrete unclaimed RMPC sectors increases from four in the early seedling stages to five in the intermediate stages to six in the older stages of flax ontogeny. These discrete sectors always form confluent regions of RMPC tissue in the more acropetal regions of the SAM and are always confluent with leaf traces in the basipetal region of the SAM. It should be stressed that the increase in the number of discrete unclaimed RMPC sectors observed through ontogeny is the result of interaction between the phenomena described under points 3 and 7 above.

There are few reports in the literature that describe LTP changes through ontogeny in sufficient detail to permit comparison with those changes observed in flax. Balfour and Philipson (1962) described the vascular arrangement during ontogeny for Godetia whitneyi and for Iberis amara, which both have single leaf traces associated with each leaf on the stem. The early LTP for these species which produce four decussate leaf pairs before undergoing spiral transformation of phyllotaxis is a 2(1; 1)/2 LTP according to the notation proposed herein. That is, an individual leaf trace is bifurcated by the leaf gap associated with leaves two plastochrons older and both members of each leaf pair are interconnected with the leaf traces associated with leaf pairs one plastochron older. These species have, therefore, initially closed vascular systems. According to Balfour’s and Philipson’s description, the early leaf trace connections in Godetia and Iberis after spiral transformation of phyllotaxis can be characterized as initially a (2; 3) LTP, then subsequently a (5) LTP with no bifurcated leaf traces. The transitions in these species’ vasculature, therefore, appear to be from closed systems to open systems, or the exact opposite to that which was observed in flax stems. This difference could presumably be explained by the absence or presence of confluence between leaf gaps and interfascicular rays associated with different leaf traces as described in point 5 above.

Jensen (1968) described changes in vascu-
lature patterns observed during *Kalanchoe mangini* ontogeny. Up to the second node *Kalanchoe* exhibits a 2(1; 1)/2 LTP and de-
cussate phyllotaxis. The unilacunar system is thereaf-ter transformed into a trilacunar sys-
tem. The proposed LTP notation must be elab-
orated to encompass such three-leaf trace sys-
tems. This is easily done by including the symbols M, A, and C for the median, anodic lateral, and cathodic lateral leaf traces, respec-
tively, into the notation. Jensen’s (1968) pattern 4 would thus be expressed as 2(M1A’1C/ 2M; A1M; C1A’) LTP. This notation implies that the system is bijugate; that median traces are bifurcated by median leaf gaps two plas-
tochrons older, and are confluent with the an-
odic and cathodic lateral traces of opposite leaf pairs one plastochron older; the anodic laterals are confluent with the median traces of the leaf pairs one plastochron older and the cathodic laterals are confluent with the anodic lateral traces of the opposing leaves one plastochron older. Allsopp (1964) notes that it is evident that the development of the vascular system is greatly influenced by the relative sizes and growth activities of the leaf primordia and the apical part of the stem axis. The relationships between the relative size of primordia and the number of unclaimed RMPC sectors which ultimately become associated with a leaf need to be investigated further.

**LTP notation**—The new notation proposed for LTP designation accurately conveys all leaf trace relationships and is consistent with the contact parasiticly notation for phyllotaxis. This consistency permits application of mathematical facts known from phyllotaxis to be applied to problems of leaf trace patterns. This notation should also prove useful to considerations of functional relationships between leaves on plant stems since more complete information on potential translocation pathways is con-
voyed with the proposed notation. For ex-
ample, plants with (5; 8)/13 LTP, leaves differ-
ing in age by multiples of 5 and 8 plastochrons are directly interconnected and leaves differing by 13 = (5 + 8), 18 = (5 + 8 + 5), and 21 = (5 + 8 + 8) plastochrons are indirectly inter-
connected via their leaf traces. The divergence fraction notation for this LTP would be 5/13 (cf. Girolami, 1953) which conveys little about the actual vascular interconnections.

**LTP and phyllotaxis relationship**—Nam- boodiri and Beck (1968) following Sterling (1945) attempted to define the relationship be-
tween the vascular system and phyllotaxis by

the following three criteria: 1) Direction of the generative spiral. 2) Angle of divergence. 3) Number of sympodia. These workers correctly pointed out that if the above three criteria were known, then Sterling’s (1945) fourth suggested criterion, the direction of trace linkages, was redundant. They presented a diagrammatic arg-
ument that illustrates that the direction of trace linkages can be predicted on the basis of the above three criteria. These workers ex-
pressed both LTP and phyllotaxis in diver-
gence fraction notation. Utilizing contact pa-
rasiticly notation for phyllotaxis and the proposed LTP notation it is possible to further generalize the above criteria as follows.

Van Iterson (1907) formally developed the geometric relationships between members of regular points on cylinders, planes, and cones. He established that the angular relationships between successive members of a m-parasiticly in such systems with a divergence angle equal to \(\alpha\) can be expressed by: \(m\alpha = \Delta m 2\pi + \delta m\). In this equation, the secondary divergence (\(\delta m\)) is an angular correction between an artificial orthostichy (expressed by \(\Delta m 2\pi\)) imposed be-
tween each successive \(m\)th member of the sys-
tem and the actual positions of members reg-
ularly arranged along a \(m\)-parastichy. The sign of \(\delta m\) determines whether the \(m\)th member along the parasiticly will be anodic (+) or cathodic (−) relative to the 0 or older member of the parasiticly. This relationship is com-
pletely independent of the chirality of the generative spiral. Since it has long been recognized (cf. Sinnott, 1960) that the chirality of the gen-
erative spiral for a given plant occurs with equal frequency in both directions and that generative spiral chirality is not an inherited quality, it seems desirable to exclude this parameter from considerations of LTP-phyllotaxis relationships. The above equation effectively does this. In order to test the accuracy of the equa-
tion a divergence angle of 137.5° was assumed and the secondary divergence calculated via equation (1) for sequential members of the Fi-
bonacci series. These calculations result in \(\delta 1 = -85^\circ, \delta 3 = 52.5^\circ, \delta 5 = -32.5^\circ; and \delta 8 = 20.00^\circ\). The alter-
ation cathodic, anodic, cathodic, an-
odic is exactly the relationship that was ob-
served in the flax LTP’s.

From these considerations, it is proposed that the criteria for establishing the relation-
ship between LTP and phyllotaxis suggested by Namboodiri and Beck (1968) can be re-
duced to: 1) knowledge of the divergence angle and 2) knowledge of whether leaf gaps and interfascicular ray parenchyma associated with different leaf traces are contiguous (open sys-

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confluent leaf traces can be determined on the basis of the sign of the secondary divergence angle from the above equation.

In conclusion, this report presents the initial steps taken toward a more rigorous definition of the relationships between the tissue differentiation processes that generate leaf trace patterns and the stem growth processes that generate patterns of phyllotaxis. The development of a new notation for describing LTP’s brings this aspect of the problem into line with contemporary contact parastichy notation for phyllotaxis, whereby the mathematical concepts of phyllotaxis can be brought to bear on leaf trace interconnections. In addition, the new notation provides convenient summaries of all the potential vascular pathways among leaves in plant stems. The development of new quantitative methods of mapping all the histologically distinct tissues of the stem has permitted a more definitive assessment of the role that differentiation of vacuolated parenchyma plays in the delimitation of leaf trace patterns. Since these differentiation events are reiterated in association with initiation of leaf primordia during every plastochron, a complete understanding of how LTP’s change requires an understanding of how relative positions of leaf primordia change on the stem axis through ontogeny (Meicenheimer, 1987).

LITERATURE CITED


